



SHORT COMMUNICATION

Low Protein Digestibility of Beef Puree in Infant *In Vitro* Digestion Model

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Abstract This study investigated protein digestibility of beef puree in infant and adult *in vitro* digestion models. The simulated digestive juices for infant and adult were prepared. Protein digestibility of beef puree was calculated in the gastric and gastrointestinal compartments. The 10% trichloroacetic acid soluble nitrogen and α -amino group contents of gastric digesta were lower in the infant *in vitro* digestion model than those in the adult *in vitro* digestion model ($p < 0.05$). In addition, the gastrointestinal digesta from the infant *in vitro* digestion model had lower value of the 10% trichloroacetic acid soluble nitrogen and α -amino group contents than those of the adult *in vitro* digestion model ($p < 0.05$). The results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that the remarkable bands of actin and myosin light chain B were found in the digesta of beef puree from the infant *in vitro* digestion model. The results of this study revealed the lower protein digestibility of beef puree in infants compared to that in adults. Therefore, the development of ways to increase digestibility of meat protein can improve the nutritional quality of meat products for infants.

Keywords infant, complimentary food, beef, protein digestibility

Introduction

Infants are generally served complementary foods between 6 and 24 months of age because their nutritional needs are not fully met by breast milk (Gibson et al., 1998). The composition and bioavailability of proteins, minerals such as copper, calcium, manganese, phosphorus, zinc, and iron, and vitamins including riboflavin and thiamin have to be importantly considered in complementary foods for infant health (Gibson et al., 1998; Gibson et al., 2010).

Meat is a good source for complementary foods. It is a high-quality protein food because it contains all essential amino acids at adequate amounts and it is highly digested, absorbed, and used in the human body (Denis et al., 2016; Pereira and Vincente, 2013; Sayd et al., 2016). In addition, meat contains various minerals and vitamins, and beef, especially, contains high levels of heme iron (Lombardi-Boccia et al., 2002). Therefore, beef is generally used and recommended as complementary food for infants (Binnie et al., 2014).

The low bioavailability of food proteins in infants has been reported and is caused by the immature state of the infant digestive tract (Gan et al., 2018). Previous studies have reported the higher pH and lower protease concentration in the stomach of infants compared to adult stomach (Bourlieu et al., 2014; Gan et al., 2018). In addition, the concentrations of small intestine proteases such as trypsin and chymotrypsin are lower in infants compared to adults (Nguyen et al., 2015a). Dupont et al. (2010) have found that the digestibility of bovine β -casein and egg ovalbumin was lower in infants than in adults when proteins were digested in each *in vitro* digestion model. The low digestibility of food proteins in infant digestive tract can decrease food protein availability and have adverse consequences for infant health. The partially hydrolyzed proteins would be fermented in the colon, which generates substances such as p-cresol, phenol, sulfide and ammonia and consequently results in pain, diarrhea, allergy, and constipation (Gan et al., 2018; Sante-Lhoutellier et al., 2008; Windey et al., 2012). Thus, it is important to understand protein digestibility of foods served to infants. However, to our knowledge, no studies have been reported on meat protein digestibility in infants. Therefore, this study was conducted to investigate protein digestibility of beef puree in infant and adult *in vitro* digestion models.

Materials and Methods

Beef puree preparation

The beef (semitendinosus muscle) was purchased from local market (Daejeon, Korea) and ground by meat mincer (M-12S, Hankook Fugee Industries Co., Ltd., Hwaseong, Korea). The ground beef (50 g) was mixed with distilled water (150 mL) and heated in a water bath at 80°C for 30 min. After cooling at room temperature (20°C), the beef puree was homogenized (T25 digital, IKA GmbH & Co. KG, Staufen, Germany) at 13,000 rpm for 1 min.

The crude protein composition of beef puree was measured by the Kjeldhal method (AOAC, 2010) and it was 19.14% (data not shown).

In vitro digestion of beef puree

The protein digestibility of beef puree was investigated in gastric digesta and gastrointestinal digesta. The contents of the digestive fluids such as pepsin from porcine mucosa, gastric lipase from *Rhizopus oryzae*, bile salt, trypsin from *bovine pancreas*, chymotrypsin from *bovine pancreas*, and pancreatic lipase from *porcine pancreas* were purchased from Sigma-Aldrich (St. Louis, MO, USA). The simulated digestive fluids were prepared as previously described by Dupont et al. (2010) and Nguyen et al. (2015b). The digestive fluids of adults were simulated as follows; gastric juice (pepsin 182 unit/mg and gastric lipase 21 unit/mg in 0.15 M NaCl, pH 1.8 adjusted with 0.1 M HCl), duodenal juice (trypsin 34.5 unit/mg, chymotrypsin 0.4 unit/mg, and pancreatic lipase 2,000 unit/mg in 0.1 M NaCl, pH 7.5 adjusted with 1.0 M NaOH), and bile juice (4 mM in distilled water, pH 7.5 adjusted with 1.0 M NaOH). The digestive fluids of infants were simulated as follows; gastric juice (pepsin 22.75 unit/mg and gastric lipase 21 unit/mg in 0.15 M NaCl, pH 3.8 adjusted with 0.1 M HCl), duodenal juice (trypsin 3.45 unit/mg, chymotrypsin 0.04 unit/mg, and pancreatic lipase 200 unit/mg in 0.1 M NaCl, pH 7.5 adjusted

with 1.0 M NaOH), and bile juice (4 mM in distilled water, pH 7.5 adjusted with 1.0 M NaOH).

The *in vitro* digestion of beef puree was conducted according to the study of Kim and Hur (2018). A total of 4 mL of beef puree were mixed with 10 mL of gastric juice, and then digested for 2 h in a shaking water bath at 37°C. The gastric digesta sample was collected immediately after digestion and kept at -70°C until analysis. For gastrointestinal digestion of beef puree, the gastric digesta was mixed with the 10 mL of simulated duodenal juice and 5 mL of bile juice. The mixture was digested for 2 h in a shaking water bath at 37°C. The gastrointestinal digesta was immediately cooled on ice and kept at -70°C until analysis. Digestion was carried out three times on different days.

Trichloroacetic acid (10%) soluble nitrogen

Trichloroacetic acid (TCA) solution (20%) was added to an aliquot of digesta sample (1:1, final concentration 10%). The mixture was centrifuged (1580 R, LABOGENE Co., Ltd., Seoul, Korea) at 2,063×g for 30 min. The supernatant was collected after filtration through a filter paper (No. 4, Whatman, Madistone, UK). Duplicated supernatants and precipitations were defined as soluble and insoluble samples, respectively. The simulated gastric and gastrointestinal digestive juice were used as blank samples.

The Kjeldhal method (AOAC, 2010) was used for crude protein analysis. The soluble and insoluble nitrogen was calculated by subtracting the result of blank from that of the digested samples. The sum of soluble and insoluble nitrogen was used as total nitrogen. The percentage of TCA soluble nitrogen was expressed as follows:

$$\text{TCA soluble nitrogen (\%)} = (10\% \text{ TCA soluble nitrogen} / \text{Total nitrogen}) \times 100$$

α -Amino group contents

The quantification of α -amino groups in the digesta was carried out by the reaction of the digesta with o-phthal-dialdehyde (OPA) following the method of Church et al. (1983). The OPA solution was freshly prepared (20 min prior to use) as follows: 25 mL of 100 mM sodium tetraborate, 2.5 mL of 20% sodium dodecyl sulfate (SDS), 40 mg of OPA dissolved in 1 mL of methanol, and 100 μ L beta-mercaptoethanol. After mixing, the OPA solution was diluted to a final volume of 50 mL with distilled water.

An 1 mL of OPA reagent was added to 50 μ L aliquot of the digesta sample, and the solution was incubated for 2 min at room temperature. The absorbance was measured at 340 nm using a spectrophotometer (DU[®]530, Beckman Coulter Inc., CA, USA). The quantity of α -amino groups (μ M NH₂/g protein) produced after the digestion was calculated by subtracting the quantity of NH₂ in the simulated digestive juices (blank) from that in the digested samples. The standard curve was made by using glycine. The total amount of protein was calculated by combining the soluble and insoluble values in the TCA soluble nitrogen analysis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a 12.5% polyacrylamide gel containing 30% acrylamide solution, 1.5 M Tris-HCl (pH 8.8), 0.5 M Tris-HCl (pH 6.8), 10% ammonium persulfate, and N,N,N',N'-tetramethyl-ethylenediamine. The digesta sample was mixed with the same volume of 2× sample buffer composed of 125 mM Tris-HCl (pH 6.8), 20% glycerol, 2% SDS, 2% mercaptoethanol, and 0.02% bromophenol blue, and heated at 95°C on a heating block for 90 sec. The 10 μ L (74.7 μ g protein) of a sample and the 5 μ L of protein molecular markers (9-

200 kDa) were loaded. Electrophoretic separation was performed with the pageRun system (AE-6531 mPAGE, ATTO Co., Tokyo, Japan) by applying 40 mA for 40 min. The running buffer was composed of 25 mM Tris, 0.1% SDS, and 192 mM glycine. Proteins in the gels were stained with Coomassie Brilliant Blue and then destained in a 10% acetic acid solution. The stained gel was scanned using a GS-710 (Bio-Rad Laboratories Inc, Hercules, CA, USA) densitometer at an optical resolution of 63.5 $\mu\text{m}/\text{pixel}$.

Statistical analysis

The digestion of beef puree was performed three times at each batch, and it was conducted in three independent batches. The data were statistically analyzed by t-test. Statistical significance was assumed at $p < 0.05$. The SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA) was used for statistical analyses.

Results

The 10% TCA soluble nitrogen of gastric digesta after beef puree digestion was 35.7% and 11.59% for the adult and infant *in vitro* digestion models, respectively (Fig. 1; $p < 0.05$). The gastrointestinal digesta from the infant *in vitro* digestion model had 43.26% of the 10% TCA soluble nitrogen that was significantly lower than the 47.19% from the adult *in vitro* digestion model ($p < 0.05$).

The α -amino group contents of gastric digesta were significantly lower in the infant *in vitro* digestion model (20.68 $\mu\text{M}/\text{g}$ protein) than those in the adult *in vitro* digestion model (48.97 $\mu\text{M}/\text{g}$ protein) (Fig. 2; $p < 0.05$). The gastrointestinal digesta from the adult and infant *in vitro* digestion models contained 96.66 and 83.07 μM α -amino groups/g protein, respectively ($p < 0.05$).

The result of SDS-PAGE showed that the bands of actin, troponin T, and myosin light chains were more remarkable in the gastric digesta from the infant *in vitro* digestion model compared to those from the adult *in vitro* digestion model (Fig. 3). After gastrointestinal digestion, the intensities of actin and myosin light chain B bands were still higher in the digesta of the infant model than those in the digesta of the adult model.

Discussion

The 10% TCA soluble nitrogen of digesta contains free amino acids and small peptides consisting of 3–4 residues, which

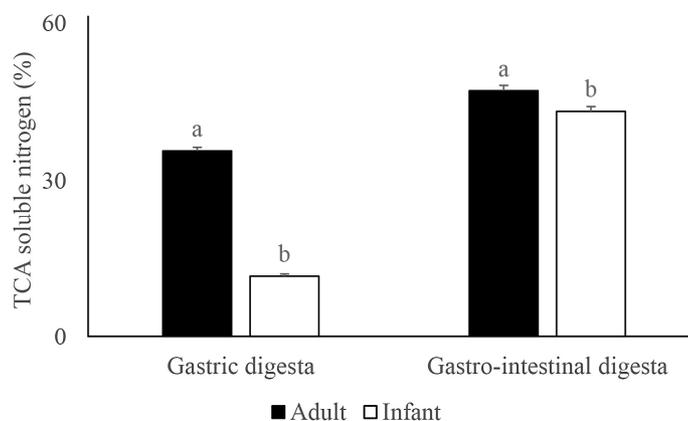


Fig. 1. TCA soluble nitrogen (%) of the digesta. ^{a,b} Different letters represent significant differences between means ($p < 0.05$).

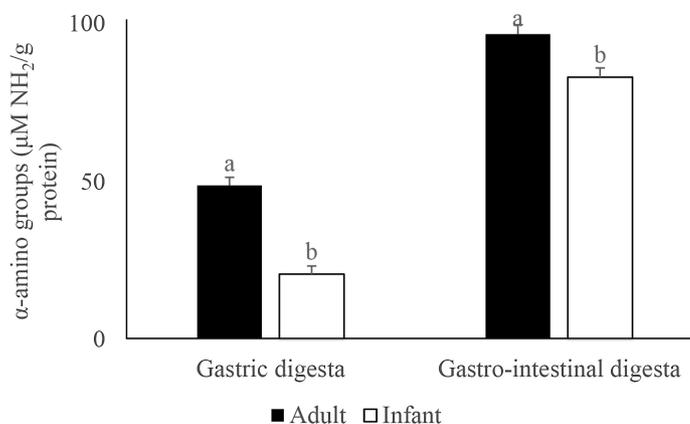


Fig. 2. α -Amino groups ($\mu\text{M NH}_2/\text{g protein}$) of the digesta. ^{a,b} Different letters represent significant differences between means ($p < 0.05$).

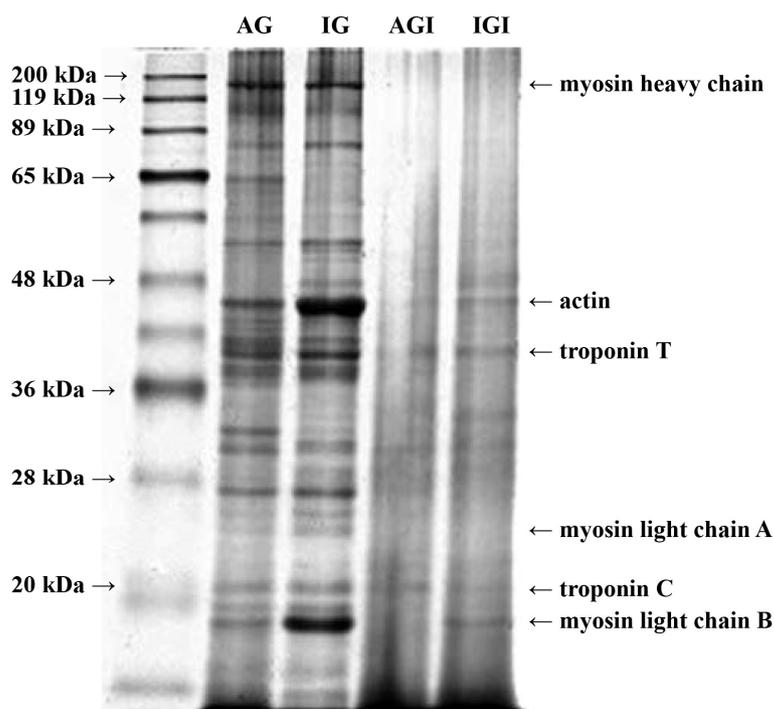


Fig. 3. SDS-PAGE electrophoretogram of proteins from adult and infant digesta. AG, adult gastric digesta; IG, infant gastric digesta; AGI, adult gastrointestinal digesta; IGI, infant gastrointestinal digesta; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

are derived by protein digestion (Greenberg and Shipe, 1979; Yvon et al., 1989). In addition, protein hydrolysis by digestion in the stomach and small intestine results in an increase in the α -amino groups via cleavage of peptide bonds (Church et al., 1983; Goodman, 2010). Therefore, the results of this study revealed that protein digestibility of beef puree was lower in the infant *in vitro* digestion model than in the adult *in vitro* digestion model. In addition, the difference in protein digestibility between infant and adult *in vitro* digestion models was higher in gastric digesta than gastrointestinal digesta.

The low protein digestibility of beef puree in the gastric digesta of the infant *in vitro* digestion model was caused by the higher pH of gastric juice and the lower pepsin concentration compared to adult. Pepsin is the major protease in the stomach, and its activity is greatly affected by the pH of gastric juice (Yao and Forte, 2003). Gastric digestion of food proteins begins with the secretion of hydrochloric acid from parietal cells (Yao and Forte, 2003). Pepsinogen is secreted first and deactivated

in acidic environments and then, converted to pepsin (Gritti et al., 2000; Kageyama, 2002). In addition, proteins are denatured in acidic condition, lose their secondary and tertiary structures and consequently, protease accessibility is increased (Kauzmann, 1959; Tanford, 1968). Pepsin has been reported to be optimally active in pH 1.8-2 (Rick and Fritsch, 1974). It has been reported that infant gastric juice has a higher pH than the adult. Bourlieu et al. (2014) have reported that the gastric pH of infants before feeding was between 3.2 and 3.5 and increased to 6.0 to 6.5 after feeding. Also, Li-Chan and Nakai (1989) have reported that after 2 h of feeding, the gastric pH of infants was between 4 and 5, whereas that of the adult was less than 2. Previous studies have found that pepsin activity in the infant stomach was less than 10% of the adult, and the secretion of pepsin in the infant stomach was less than that of adults (Hamosh, 1996; Nguyen et al., 2015a).

As chyme enters the small intestine, pancreatic proteases are secreted with pancreatic bicarbonate. Bicarbonate neutralizes gastric acid, increasing the pH in the active range of pancreatic enzymes (Goodman, 2010). Key proteases of small intestine are trypsin, chymotrypsin, elastase, enterokinase and carboxypeptidase B (Dallas et al., 2012). Trypsin, the main digestive enzyme in the small intestine, cleaves peptides at the carboxyl side chain of lysine and arginine. Chymotrypsin acts on the carboxyl side of tyrosine, tryptophan, phenylalanine (Appel, 1986). The optimum pH for trypsin is known to be 7–9, and that for chymotrypsin 7.8–8.0 (Tsiatsiani and Heck, 2015). Edginton and Fotaki (2010) reported that the intestinal pH of infants is similar to that of adults. However, low activities of trypsin and chymotrypsin were found in infant small intestine compared to those of adult small intestine because of the lower content of trypsin and chymotrypsin in the infant small intestine compared to the adult small intestine (Nguyen et al., 2015a). Therefore, in the gastrointestinal digesta, protein digestibility of beef puree was still lower in infant compared to adult *in vitro* digestion model although the difference was reduced compared to that of gastric digesta.

Sayd et al. (2016) reported that the muscle contractile and structural proteins were preferentially hydrolyzed in the small intestine while the hydrolysis of muscle proteins began in the stomach. In the present study, myosin heavy and light chains, actin, and troponin T and C were identified by SDS-PAGE of gastric digesta from the infant and adult *in vitro* digestion models. However, the gastric digesta of the infant *in vitro* digestion model contained higher levels of unhydrolyzed actin, troponin T, and myosin light chains than that of the adult *in vitro* digestion model. In addition, actin and myosin light chain B were hydrolyzed at slower rates in the gastrointestinal digesta of the infant *in vitro* digestion model compared to those in the adult infant *in vitro* digestion model. Similar results were obtained in the study of Denis et al. (2016). They found low hydrolysis of some muscle proteins containing actin in the duodenal compartment after cooked beef digestion at elderly digestive conditions, which are characterized by a slower decline in pH and a lower pepsin content in the gastric juice compared to adult conditions.

The digestibility of meat proteins is undoubtedly outstanding compared to that of plant proteins. However, the decrease in meat protein digestibility results in reduced nutritional quality of meat. Denis et al. (2016) reported that the delay in the hydrolysis of meat protein during digestion in the gastrointestinal tract could decrease protein gain by the body, although the final protein digestibility was not different. Digestibility of meat protein is affected by intrinsic and extrinsic factors such as pH, oxidation, denaturation etc. (Liu and Xiong, 2000; Nguyen et al., 2015a). Based on the results of this study, infants have lower protein digestibility of beef puree than that observed in adults. Therefore, the development of ways to increase digestibility of meat protein can improve the nutritional quality of complimentary foods including meat products for infants.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Author Contributions

Conceptualization: Hur SJ, Choi YS, Jung S. Data curation: Lee S, Jo K, Kim HJ, Jung S. Formal analysis: Lee S, Jo K. Writing-original draft: Lee S, Jung S. Writing - review & editing: Lee S, Jo K, Hur SJ, Choi YS, Kim HJ, Jung S.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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