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Research article

Determination of biological safety profile and nutritional composition of cattle small intestinal digesta ("Pia"), a traditional food ingredient in Northeastern Thailand

Chuenjit Chancharoonpong[a], Chuleeporn Chumnanka[a], Kannika Wongpanit[b], Sathaporn Jittapalapong[c], Rungnapa Boonpawa[a,*]

[a] *Department of Food Technology and Nutrition, Faculty of Natural Resources and Agro-industry, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon 47000, Thailand*

[b] *Department of Agriculture and Resources, Faculty of Natural Resources and Agro-industry, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon 47000, Thailand*

[c] *Faculty of Veterinary Technology, Kasetsart University, Bangkok 10900, Thailand*

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Abstract

Importance of the work: Cattle small intestinal digesta (CSID), locally known as "Pia", has been used as a traditional food ingredient in Northeastern Thailand. However, the information on its biological safety and nutritional value is lacking.

Objectives: To determine the biological safety profile and nutritional composition of CSID.

Materials & Methods: CSID samples from nine individual cattle were collected from a local slaughterhouse in Sakon Nakhon province and divided into duodenum, jejunum and ileum samples. All samples were subjected to microbial analysis, parasite egg determination and nutritional composition analysis.

Results: The microbial loads (total plate count, *Escherichia coli*, coliform and *Staphylococcus aureus*) of most samples were within the Thai legal limits and *Salmonella* spp. and *Listeria monocytogenes* were not present in 25 g samples. In contrast, 66% of samples were single or double infected with parasite eggs identified as rumen fluke eggs, strongylid eggs and capillaria eggs. The CSID samples on average (± SD) wet weight basis contained 92.74±1.61%, 0.92±0.90%, 1.46±0.42%, 2.24±0.96%, 0.17±0.06% and 3.14±1.76% for moisture, crude fiber, ash, protein, fat and carbohydrate contents, respectively. The CSID samples contained all essential amino acids that accounted for 34.39 mg/g wet weight, of which lysine and leucine were the major essential amino acids. The CSID comprised 7.70 mg/g wet weight, 3.10 mg/g wet weight and 2.00 mg/g wet weight of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids, respectively.

Main finding: The CSID samples contained substantial amounts of nutrients, illustrating their nutritional value; however, dishes containing CSID must be thoroughly cooked. This information will contribute greatly to the safer use and further development of CSID as a traditional food ingredient.

* Corresponding author.
E-mail address: rungnapa.boon@ku.th (R. Boonpawa)

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Introduction

Cattle small intestinal digesta (CSID) is the partially digested materials in the small intestinal lumen, with dark greenish color (Fig. 1) that is considered waste from beef production in most parts of the world (Cherdthong, 2020). However, CSID, locally known as "Pia", has a long history of use as a traditional food ingredient in Northeastern Thailand, adding a unique, bitter taste and strong odor to local dishes (Yeemin et al., 2022). As this region has an erratic rainfall, poor soil and is susceptible to drought, the local people rely on a wide variety of food sources, including those commonly considered as food waste, such as CSID (Asavarut et al., 2016). Such traditional consumption has been passed from generation to generation; however, despite a long history of CSID use as a food ingredient, several concerns have been raised on its suitability for human consumption due to the lack of information on its safety and nutritional value (Yeemin et al., 2022).

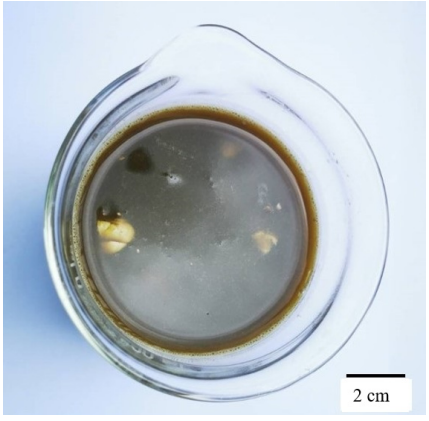

Fig. 1 Cattle small intestinal digesta (Pia) samples

The distinctive eating behavior of local people in Northeastern Thailand by consuming raw or undercooked dishes has resulted in a high prevalence of parasitic infection in this region (Asavarut et al., 2016). The most serious underlying health problem in this region is the incidence of cholangiocarcinoma or bile duct cancer, caused by prolong infection by the liver fluke, *Opisthorchis viverrini* (Sripa and Pairojkul, 2008; Asavarut et al., 2016). Based on recent studies, there is still a high prevalence of gastrointestinal parasitic infections in cattle raised in Thailand (Laoraksawong et al., 2018; Adisakwattana et al., 2020; Thanasuwan, 2021). Besides parasitic infections, consumption of raw or undercooked dishes also poses the health problem on food poisoning caused by foodborne pathogens, such *Salmonella* spp. and *Streptococcus* spp. (Vaeteewootacharn et al., 2005; Noppon et al., 2014).

The cattle digestive system contains complex microbiota, which play an important role in the digestion of plant materials (Sutton and Reynolds, 2011; Mao et al., 2015). In ruminants including cattle, the small intestine is the major site for the absorption of most nutrients, including amino acids, long chain fatty acids, glucose and vitamins, with volatile fatty acids being absorbed in the rumen (Sutton and Reynolds, 2011). To date, there has been only one published report on using rumen digesta as alternative protein sources for ruminants feeds, resulting in an enhancement in digestibility, diet utilization and ruminal fermentation (Cherdthong, 2020). Based on available data, cattle rumen digesta contain 18.53–19.56% dry matter, 8.7–35.97% dry matter and 28.28–34.91% dry matter of crude protein, ash and crude fiber, respectively (Agbabiaka et al., 2012; Cherdthong and Wanapat, 2013; Elfaki and Abdelatti, 2015).

As undercooked dishes are a distinctive characteristic dish containing CSID, knowledge on food safety, especially biological safety, is important to ensure the safe consumption of CSID. Furthermore, its nutritional value is a key factor determining the suitability of CSID as a food ingredient. Hence, the present study aimed to determine the biological safety profile of CSID, including the presence of foodborne pathogens and parasite eggs as well as the nutritional composition. The obtained information will ultimately result in the safer use of CSID and help to ascertain its suitability and nutritional value as a food ingredient.

Materials and Methods

Sample collection

The CSID samples were obtained from a local slaughterhouse in Sakon Nakhon province, Northeastern Thailand. Prior to scheduled slaughtering, the cattle were fasted for 24 h. CSID samples from nine individual cattle were collected and divided into duodenum, jejunum and ileum samples. The pH and total acidity values of the samples were determined using a pH meter (pHCore; Sartorius; Germany) and titration methods (titration against 0.1 N NaOH expressed as % lactic acid equivalent based on Association of Official Analytical Chemists (2005). All samples were stored at -18 °C until further analysis.

Microbial analysis

The enumeration of total viable count was performed based on the method described by Maturin and Peeler (2001) with some modifications. A sample (25 g) was mixed with 225 mL sterile 0.85% (weight per volume, w/v) saline and homogenized for 2 min in a stomacher machine (Seward Stomacher, 400 Circulator; UK). The homogenates were serially diluted with 0.85% (w/v) saline (dilution range of 1×10^{0}–1×10^{-5}) and spread onto the plate count agar. The plates were aerobically incubated at 35 °C for 24 h. The diluted homogenates were further used for analysis of coliform, *Escherichia coli* and *Staphylococcus aureus*.

Coliform and *E. coli* were enumerated using Compact Dry "Nissui" EC (Nissui Pharmaceutical; Japan) according to the manufacturer's instructions. Briefly, 1 mL aliquots of the diluted samples were inoculated in the middle of the Compact Dry EC and aerobically incubated at 35±2 °C for 24 h. The counts of coliforms and *E. coli* were based on color differences, where red/pink colonies were determined as coliforms and blue/blue purple colonies as *E. coli*.

The enumeration of *S. aureus* was performed using Compact Dry "Nissui" X-SA (Nissui Pharmaceutical; Japan) according to the manufacturer's instructions. Briefly, 1 mL aliquots of the diluted samples were inoculated in the middle of the Compact Dry X-SA and aerobically incubated at 37±1 °C for 24 h. The light blue/blue colonies were counted for *S. aureus*.

The analysis of *Salmonella* spp. was performed following methods described by Jacobson et al. (2012). Briefly, 25 g of sample were mixed with 225 mL sterile lactose broth and homogenized for 2 min in a stomacher machine (Seward Stomacher, 400 Circulator; UK). After incubation at 35 °C for 24±2 h, 0.1 mL and 1 mL of the pre-enrichment were transferred to the tubes containing 10 mL of Rappaport-Vassiliadis media and tetrathionate broth; subsequently, they were incubated at 42 ± 0.2 °C and 43 ± 0.2 °C, respectively, for 24 ± 2 h. Following the incubation, 10 µL of the enrichment cultures were streaked onto bismuth sulfite, xylose lysine deoxycholate and Hektoen enteric agars; these plates were incubated at 35 °C for 24 ± 2 h. *Salmonella* typically forms black colonies on xylose lysine desoxycholate and Hektoen enteric agar, while on bismuth sulfite agar *Salmonella* appear black or at times metallic (Jacobson et al., 2012). Suspected *Salmonella* colonies from each plate were inoculated into triple sugar iron agar and lysine iron agar and further incubated at 35 °C for 24 ± 2 h. If *Salmonella* is present, the triple sugar iron agar will turn black in the butt of the tube, while the butt of lysine iron agar will darken (Jacobson et al., 2012).

The presence of *Listeria monocytogenes* in the samples was analyzed following the methods described by Hitchins et al. (2017). Briefly, 25 g of sample were mixed with 250 mL sterile buffered listeria enrichment broth containing pyruvate without selective additives and homogenized for 2 min in a stomacher machine (Seward Stomacher, 400 Circulator; UK). After incubation at 30 °C for 4 h, selective enrichment agents (acriflavin, cycloheximide and sodium nalidixic acid) were added to achieve final concentrations of 10 mg/L, 40 mg/L and 50 mg/L, respectively, and then further incubated at 30 °C for 24 h. For isolation, 10 µL of the enrichment cultures were streaked onto Oxford agar and incubated at 35 °C for 24 ± 2 h. Two or more *Listeria* colonies (gray to black colonies surrounded by a black halo) were streaked onto trypticase soy agar with 0.6% yeast extract plate and incubated at 30 °C for 24 ± 2 h. Final isolate identification of *Listeria* was carried out using API *Listeria* (bioMerieux; USA).

Due to insufficient amounts of samples for *Salmonella* spp. and *L. monocytogenes* analysis, the remaining CSID samples from the same segment (duodenum, jejunum and ileum) were pooled.

Parasite eggs determination

For parasite eggs determination, the samples were prepared using a standard centrifugal sedimentation technique. Freshly obtained CSID samples were transported and stored in sealed plastic bags at 4 °C until analysis (within 1–2 wk). A sample (1 g) was mixed with 10 mL of 10% buffered formalin and poured in a 15 mL centrifuge tube. Ethyl acetate was added until one-half to three-quarters of the tube and shaken for about 50 times. After centrifugation at 500 g for 2–3 min, the top three layers were removed and the obtained sediment was suspended with a few drops of formalin. Then, 1–2 drops of the suspended sediment were placed on a glass slide and the presence of parasite egg was determined using a stereo dissecting microscope at 10× magnification.

Proximate analysis

Proximate analysis was performed to determine the nutritional composition of the CSID following the methods recommended by AOAC (2005). The moisture content was determined by oven-drying the samples at 130 °C until reaching a stable weight (AOAC 925.10). The crude protein content was determined using the copper catalyst Kjeldahl method (AOAC 984.13) and calculated using a nitrogen factor of 6.25. The crude fat content was determined using the Soxhlet extraction method

(AOAC 2003.05). The crude ash content was determined based on incineration at 600 °C (AOAC 942.05). The crude fiber content was determined using the acid-base method (AOAC 962.09). The crude carbohydrate content was estimated based on Equation 1:

$$\text{Carbohydrate} = 100 - (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash} + \text{Fiber}) \quad (1)$$

where the amounts of crude carbohydrate, protein, fat, ash and fiber were expressed in units of grams per 100 g wet weight CSID.

Amino acid composition analysis

For the amino acid composition analysis, pooled CSID samples were sent to ALS Laboratory Group (Thailand) Co., LTP, part of the ALS Group (Bangkok, Thailand) and were analyzed based on AOAC 994.12 (Association of Official Analytical Chemists, 2016). In short, homogenized samples were subjected to cool performic acid oxidation prior to hydrolysis. The resultant hydrolysate was diluted and measured using an amino acid analyzer. Twenty amino acids were analyzed and quantified using the calibration curves obtained from respective amino acid standards.

Fatty acid composition analysis

For fatty acid composition analysis, pooled CSID samples were sent to ALS Laboratory Group (Thailand) Co., LTP, part of the ALS Group (Bangkok, Thailand) and were analyzed using an in-house method (STM No. 03-010) based on AOAC 996.06 (Association of Official Analytical Chemists, 2016). Briefly, homogenized samples were extracted using a hydrolytic method prior to analysis using gas chromatography. Thirty-six fatty acids were analyzed and quantified using the calibration curves obtained from respective fatty acids standards.

Results and Discussion

Biological safety profile of cattle small intestinal digesta

To address concerns regarding the food safety of CSID consumption, the present study investigated the biological safety profiles of CSID samples purchased from a local slaughterhouse in Sakon Nakhon province, Thailand. There are no legal limits on microbial loads in CSID as it is a local food ingredient, so the obtained results were compared with related raw meat products. Based on Thai Agricultural Commodity and Food Standard and the Department of Livestock Development on microbial criteria for raw meat, the acceptable microbial loads of total plate count, *E.coli*, coliform and *S. aureus* should not exceed 5.70 log colony forming units (CFU)/g (5×10^5 CFU/g), 2.00 log MPN/g (1×10^2 MPN/g), 3.70 log MPN/g (5×10^3 CFU/g) and 2.00 log CFU/g (1×10^2 CFU/g), respectively, while *Salmonella* spp. should not be present in a 25 g sample (Thai Agricultural Commodity and Food Standard, 2004; Department of Livestock Development, 2008). The results (Table 1) show that the majority (8 out of 9) of total

Table 1 Microbiological safety profiles of cattle small intestinal digesta (CSID) samples

CSID section	Range (Log CFU/g)	Total plate count	*E. coli*	Coliform	*S. aureus*	*Salmonella* spp.	*L. mono-cytogenes*
		Positive sample/total sample (% of positive sample)[1]				Presence in 25 g sample[2]	
Duodenum	< 1.0	0/9 (0%)	9/9 (100%)	7/9 (78%)	9/9 (100%)	0/2 (0%)	0/2 (0%)
	1.0–3.0	6/9 (67%)	0/9 (0%)	2/9 (22%)	0/9 (0%)		
	3.0–5.0	2/9 (22%)	0/9 (0%)	0/9 (0%)	0/9 (0%)		
	5.0–7.0	0/9 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)		
	7.0–9.0	1/9 (11%)	0/9 (0%)	0/9 (0%)	0/9 (0%)		
Jejunum	< 1.0	0/9 (0%)	8/9 (89%)	6/9 (67%)	9/9 (100%)	0/2 (0%)	0/2 (0%)
	1.0–3.0	6/9 (67%)	1/9 (11%)	3/9 (33%)	0/9 (0%)		
	3.0–5.0	2/9 (22%)	0/9 (0%)	0/9 (0%)	0/9 (0%)		
	5.0–7.0	0/9 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)		
	7.0–9.0	1/9 (11%)	0/9 (0%)	0/9 (0%)	0/9 (0%)		
Ileum	< 1.0	0/9 (0%)	5/9 (56%)	7/9 (78%)	9/9 (100%)	0/2 (0%)	0/2 (0%)
	1.0–3.0	1/9 (11%)	2/9 (22%)	0/9 (0%)	0/9 (0%)		
	3.0–5.0	4/9 (44%)	2/9 (22%)	1/9 (11%)	0/9 (0%)		
	5.0–7.0	1/9 (11%)	0/9 (0%)	1/9 (11%)	0/9 (0%)		
	7.0–9.0	3/9 (33%)	0/9 (0%)	0/9 (0%)	0/9 (0%)		

[1] Data expressed as number of positive sample/number of total samples (% of positive sample). Data obtained from duplicated experiments.

[2] Data were expressed as number of positive samples (presence of food pathogen)/number of total samples (% of positive sample). Data obtained from duplicated experiments.

plate counts for the duodenum and jejunum samples were lower than the legal limit of 5.70 log CFU/g, while 5 out of 9 ileum samples were within the limit. The total plate counts of 1 out of 9 duodenum samples and 4 out of 9 ileum samples exceeded the legal limits (>7 log CFU/g). For the *E. coli* load, all duodenum and jejunum samples and 7 out of 9 ileum samples were within the legal limits (<2 log CFU/g), while 2 out of 9 were about 4 log CFU/g. The coliform load in all duodenum and jejunum samples and 7 out of 9 ileum samples were within the legal limits (<1 log CFU/g). Furthermore, 2 out of 9 of the ileum samples had coliform loads of 3.86 and 6.28 log CFU/g, respectively. The *S. aureus* loads for all samples were within the legal limits (<1 log CFU/g). Neither *Salmonella* spp. nor *L. monocytogenes,* were present in the 25 g of samples from all three sections of CSID samples. The duodenum samples were weakly acidic, while the jejunum and ileum samples were weakly basic. These pH values were consistent with the measured total acidity values of 0.91 ± 0.04%, 0.11 ± 0.02% and 0.21 ± 0.04% lactic acid equivalent for the duodenum, jejunum and ileum samples, respectively. Normally, acidic digesta (pH about 2.0–3.0) entering the duodenum is rapidly neutralized by pancreatic and bile secretions (Sutton and Reynolds, 2011; Mao et al., 2015). The pH values of CSID samples are in the optimal pH range (6.5–7.5) for most spoilage and pathogenic microbial growth (Tortora et al., 2010).

Besides microbial load, the present study investigated the prevalence of parasite eggs in the CSID samples (Fig. 2). The results revealed that all three sections of CSID samples had similar prevalence levels of parasite eggs. For the nine individual fattening beef cattle, there were no parasite eggs in

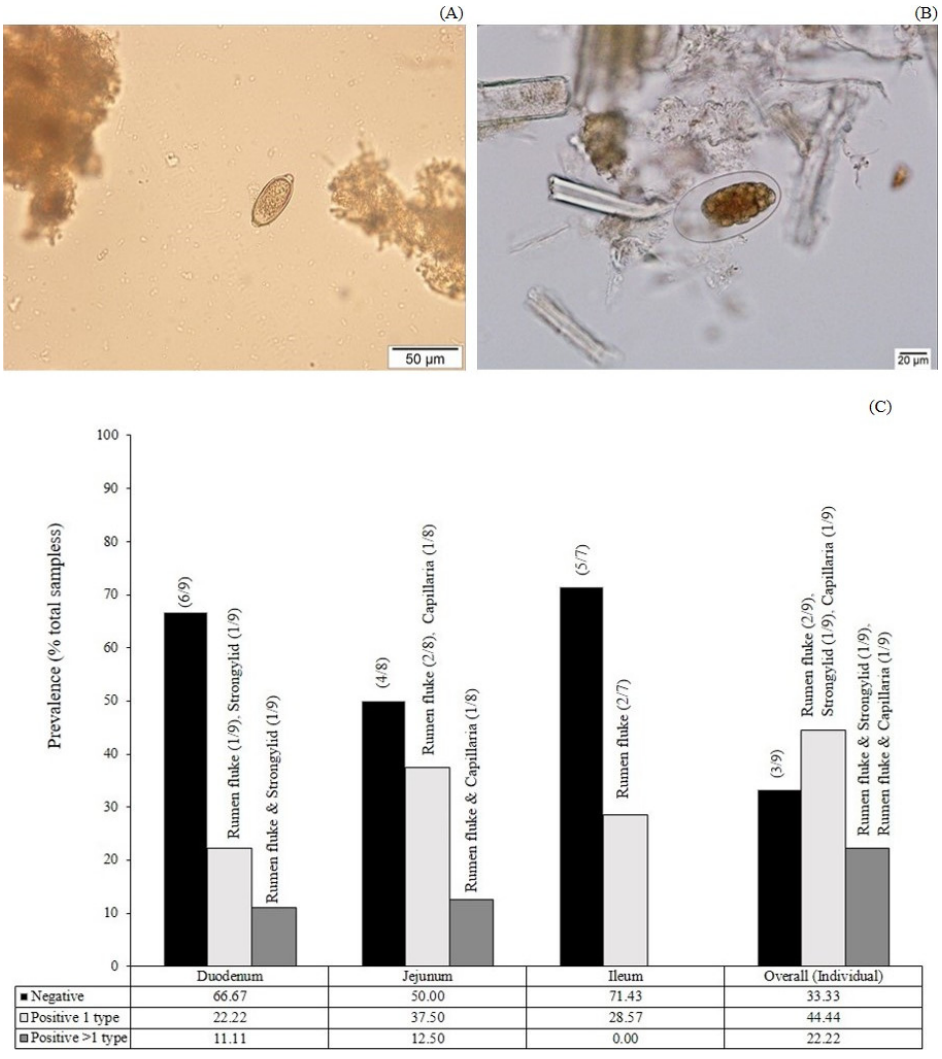

Fig. 2 Microscopic images of cattle small intestinal digesta samples: (A) capillaria eggs; (B) strongylid eggs; (C) prevalence of parasite eggs

33% of samples, 44% of samples contained one type of parasite eggs and the remaining 22% of samples were doubly infected with gastro-intestinal parasites. The parasite eggs found in the CSID samples were identified by a trained technician as rumen fluke eggs (found in 4 out of 9 individual cattle), strongylid eggs (found in 2 out of 9 individual cattle) and capillaria eggs (found in 2 out of 9 individual cattle). Other studies also reported high prevalence levels (59–96%) of gastro-intestinal parasitic infection in cattle raised in several regions of Thailand, with the most prevalent parasites being identified as strongylids, liver fluke and rumen fluke (Wongsawang et al., 2014; Pornsukarom et al., 2020; Thanasuwan, et al., 2021). Some studies also reported a still high prevalence of parasitic infection (about 18–23%) in several regions of Thailand, especially for *Strongyloides stercoralis* and *Opisthorchis viverrini* infections (Laoraksawong, et al., 2018; Adisakwattana, et al., 2020). Notably, parasitic infection with liver fluke (*Opisthorchis viverrini*) could lead to cholangiocarcinoma, a rare type of bile duct cancer (Sripa and Pairojkul, 2008). Overall, the presence of gastro-intestinal parasite eggs in CSID samples could posed a serious health problem to consumers, especially for those who enjoy eating raw or undercooked dishes containing CSID. Consequently, such dishes should be thoroughly cooked before consumption at a core temperature of 60–75 °C for 15–30 min, which can be achieved using normal household cooking methods and are sufficient to terminate most parasite stages (Franssen et al., 2019).

Nutritional composition of cattle small intestinal digesta

To ascertain the suitability and nutritional value of CSID as a food ingredient, nutritional composition was determined. The results obtained from proximate analysis (Table 2) revealed that the CSID samples were quite watery (90.90–93.90% moisture content), which was consistent with the reported dry matter content of CSID in the terminal ileum of 60–80 g/kg or about 92–94% moisture content (Sutton and Reynolds, 2011) and similar to the reported moisture content in cattle rumen digesta of 89.1–98.4% (Cherdthong, 2020). The duodenum samples contained the highest crude protein content compared to the jejunum and ileum samples, whereas the ileum samples contained the highest crude fiber, ash and carbohydrate contents. The nutritional composition levels on a wet weight basis of the CSID samples were on average (± SD) 0.92 ± 0.90%, 1.46 ± 0.42%, 2.24 ± 0.96%, 0.17 ± 0.06% and 3.14 ± 1.76% for crude fiber, ash, protein, fat, and carbohydrate contents corresponding to a dry weight basis to 11.49 ± 9.92%, 20.07 ± 3.27%, 32.61 ± 17.02%, 2.27 ± 0.52% and 45.31 ± 13.84%, respectively. Since no data were available on the nutritional composition levels of CSID, the obtained results in the present study were compared with reported data on cattle rumen digesta and raw beef. The obtained nutritional composition levels of CSID in the present study were quite similar to levels previously reported nutritional contents on a dry matter basis in cattle rumen digesta that contained 28.28–34.91%, 8.7–35.97% and 18.53–19.56% of crude fiber, ash, and crude protein, respectively (Agbabiaka et al., 2012; Cherdthong and Wanapat, 2013; Elfaki and Abdelatti, 2015). Comparing to raw beef, the crude ash and carbohydrate content of the CSID were comparable to the reported crude ash (1.07–1.11% wet weight) and crude carbohydrate (0.97–2.88% wet weight) contents in chuck, round and loin beef cuts (Wu et al., 2016). However, the chuck, round and loin beef cuts contained about 32- and 9-fold higher crude fat (4.12–6.16% wet weight) and crude protein (20.20–22.61% wet weight) contents than CSID (Wu et al., 2016).

The amino acid profiles of food ingredients are important as a key determinant of protein quality, especially the amount of essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), which are not produced by the human body but are needed for new protein synthesis (Górska-Warsewicz et al., 2018). The amino acid composition levels of the CSID samples are shown in Table 3, indicating that all three sections of the CSID

Table 2 Proximate composition and physiochemical properties of cattle small intestinal digesta

Small intestinal section	Moisture content (%)	Fiber content (%)	Ash content (%)	Protein content (%)	Fat content (%)	Carbohydrate content (%)	pH	Total acidity (%)
Duodenum	93.43±0.03	0.12±0.02	1.08±0.03	3.34±0.04	0.11±0.01	2.08±0.10	5.70±0.01	0.91±0.04
Jejunum	93.90±0.05	0.67±0.07	1.39±0.03	1.82±0.04	0.16±0.04	2.74±0.08	7.45±0.02	0.11±0.02
Ileum	90.90±0.04	1.97±0.08	1.91±0.02	1.56±0.08	0.23±0.06	5.40±0.04	7.77±0.02	0.21±0.04
Average	92.74±1.61	0.92±0.90	1.46±0.42	2.24±0.96	0.17±0.06	3.41±1.76	6.97±1.11	0.41±0.31

Data expressed as mean ± SD % wet weight of pooled samples obtaining from triplicate experiments.
Total acidity expressed in the unit of % lactic acid equivalent.

Table 3 Amino acid composition levels of cattle small intestinal digesta

Amino acid	Duodenum (mg/g wet weight)	Jejunum (mg/g wet weight)	Ileum (mg/g wet weight)	Total (mg/g wet weight)
Essential amino acids				
Histidine	1.25	0.0001	0.0001	1.25
Isoleucine	1.96	0.66	0.20	2.82
Leucine	5.20	1.67	0.59	7.46
Lysine	5.56	1.62	0.53	7.71
Methionine	2.68	0.79	0.29	3.76
Phenylalanine	3.01	0.76	0.33	4.10
Threonine	2.12	0.75	0.35	3.21
Tryptophan	0.0001	0.19	0.0001	0.19
Valine	2.64	0.94	0.31	3.89
Total	24.42	7.37	2.60	34.39
Non-essential amino acids				
Alanine	2.10	0.65	0.35	3.10
Arginine	3.93	1.53	0.61	6.07
Aspartic acid	4.72	1.48	0.67	6.87
Cystine	0.56	0.15	0.12	0.84
Glutamic acid	5.34	1.53	0.83	7.70
Glycine	1.72	1.51	0.38	3.61
Hydroxylysine	0.00	0.00	0.00	0.00
Hydroxyproline	0.04	0.05	0.07	0.15
Proline	3.31	1.18	0.61	5.10
Serine	2.19	0.52	0.33	3.04
Tyrosine	2.22	0.62	0.28	3.12
Total	26.13	9.22	4.24	39.60
Overall total	50.55	16.60	6.84	73.98

contained all 9 essential amino acids and 11 non-essential amino acids. Among the three small intestinal sections, the duodenum CSID contained the highest amino acid contents (50.55 mg/g wet weight) followed by the jejunum (16.60 mg/g wet weight) and ileum (6.84 mg/g wet weight), respectively. Altogether, CSID contained in total 73.99 mg/g wet weight of amino acids, which was about 3-fold lower than the reported total amino acids in chuck, round and loin beef cuts (221.27–248.56 mg/g wet weight), according to Wu et al. (2016). Focusing on essential amino acids, the overall CSID samples contained in total 34.39 mg/g wet weight of essential amino acids, accounting for 46% of total amino acids, of which lysine and leucine were the major essential amino acids in the CSID samples. Raw beef contains 100.75–112.33 mg/g wet weight of essential amino acids, which is about 3-fold higher that for CSID, of which lysine and leucine are the major essential amino acids, similar to CSID (Wu et al., 2016).

The fatty acid profiles of the CSID samples are shown in Table 4. Similar to the amino acid composition, the duodenum CSID contained the highest fatty acid contents followed by the jejunum and ileum, respectively. In total, 11 fatty acids were identified and quantified in the CSID, accounting for 7.70 mg/g wet weight of saturated fatty acids, 3.10 mg/g wet weight of monounsaturated fatty acids and 2.00 mg/g wet weight of polyunsaturated fatty acids. The major fatty acids in the CSID samples were mainly stearic acid (C18:0) and palmitic acid (C16:0), contributing about 50% of total fatty acids. Notably, CSID was a source of omega-6 (mainly linoleic acid, C18:2) and omega-9 fatty acids (oleic acid, C18:1), amounting to 4.90 mg/g wet weight. About one-half of total fatty acids in raw lean beef are consist mainly of palmitic acid (C16:0) and stearic acid (C18:0), whereas polyunsaturated fatty acid profiles in raw lean beef consist of C18:2 (ω-6) and C18:3 (ω-3), which differ from CSID (Williams, 2007). As observed in raw beef, CSID also contained a low amount of *trans*-fatty acid (C18:1 *trans*) with a value of 0.20 mg/g wet weight in CSID and 0.22 mg/g wet weight in raw beef (Williams, 2007). *Trans*-fatty acid is a product of rumen microbial fermentation (Williams, 2007).

Table 4 Fatty acid composition levels of cattle small intestinal digesta

Fatty acid	Duodenum (mg/g wet weight)	Jejunum (mg/g wet weight)	Ileum (mg/g wet weight)	Total (mg/g wet weight)
Saturated fatty acids				
Capric acid (C10:0)	0.10	<0.10	ND	0.20
Lauric acid (C12:0)	0.10	0.10	ND	0.20
Myristic acid (C14:0)	0.30	0.10	0.10	0.50
Palmitic acid (C16:0)	2.00	0.50	0.60	3.10
Stearic acid (C18:0)	2.60	0.60	0.50	3.70
Total	5.10	1.40	1.20	7.7
Mono unsaturated fatty acids				
Oleic acid (C18:1, ω-9)	1.90	0.50	0.50	2.90
trans-Elaidic acid (C18:1 trans)	0.20	ND	ND	0.20
Total	2.10	0.50	0.50	3.10
Poly unsaturated fatty acids				
Arachidonic acid (C20:4, ω-6)	0.30	ND	ND	0.30
Docosadienoic acid (C22:2, ω-6)	0.10	ND	ND	0.10
Eicosatrienoic acid (C20:3, ω-6)	0.40	ND	ND	0.40
Linoleic acid (C18:2, ω-6)	1.00	0.20	ND	1.20
Total	1.80	0.2	-	2.00
Overall total	9.00	2.10	1.70	12.80

Total of 36 fatty acids analyzed and only detected fatty acids presented here;
ND = not detected at limit of quantification of 0.08 mg/g wet weight

All biological organisms identified in the present study were analyzed in raw CSID. Most spoilage microbial and food pathogens of the tested raw CSID were within the legal limits for raw meat; however, there were still high prevalence levels of parasite eggs present in the tested samples. To control the microbial loads and to prevent cross contamination in raw CSID, special attention should be focused on proper handling, sanitation of handlers and the working environment, proper storage conditions of CSID and strictly following the Good Manufacturing Practice standards. It is recommended CSID food handlers wear gloves during handling of raw CSID, especially by those having a wound on their hand to prevent infection by the parasite eggs. Most food pathogens and non-pathogens can be killed at temperatures of 70 °C for at least 2 min (James et al., 2021), while a core temperature of 60–75°C for 15–30 min is sufficient to kill most parasites at all stages (Franssen et al., 2019). This information indicates that a normal cooking temperature should be effective in neutralizing food pathogens, non-food pathogens and parasites. In addition, providing dishes containing CSID that are thoroughly cooked, they should be safe for consumption. From a nutritional point of view, raw CSID contained all the essential amino acids and other nutrients in substantial amounts, indicating the benefit of adding CSID to dishes. During cooking (heating), the protein in CSID will be degraded as is the protein from other sources, with the cooking loss being greatly influenced by the temperature (Huang et al., 2011). However, CSID contains other nutrients, such as fiber, that is not present in other raw food materials from animal origins. Subject to taking into account the proper handling and cooking of CSID, the benefits from using CSID as a food ingredient should outweigh the risk associated with the consumption of CSID. However, it is important to note that the CSID samples in the present study were only collected from nine individual cattle due to financial constraints, which is a limitation of the present study. Even though the results obtained in the present study cannot be extrapolated to represent the whole situation, it still provides valuable knowledge as this was the first attempt to investigate the biological safety profile and nutritional composition of CSID, a traditional food ingredient.

In conclusion, CSID, or dishes containing CSID, should not be consumed raw as there was still a high prevalence of parasite egg present in the raw CSID. The CSID contained substantial levels of nutrients, confirming its suitability as a food ingredient. This report was the first to investigate the biological safety profile and nutritional composition of CSID. The present results could be used as fundamental knowledge for the safer use and further development of CSID as a traditional food ingredient. For further consideration, CSID has potential to be a functional ingredient considering its

location in the small intestinal lumen where there are various beneficial microbes and digested active compounds such as fibers.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Adisakwattana, P., Yoonuan, T., Phuphisut, O., et al. 2020. Clinical helminthiases in Thailand border regions show elevated prevalence levels using qPCR diagnostics combined with traditional microscopic methods. Parasit. Vectors 13: 416. doi.org/10.1186/s13071-020-04290-0

Agbabiaka, L.A., Madubuike, F.N., Amadi, S.A. 2012. Studies on nutrients and anti-nutrients of rumen digesta from three most domesticated ruminants in Nigeria. Pak. J. Nutr. 11: 678–680. doi: 10.3923/pjn.2012.678.680

Association of Official Analytical Chemists. 2005. Official Methods of Analysis of AOAC International, 18th ed. AOAC International. Gaithersburg, MD, USA.

Association of Official Analytical Chemists. 2016. Official Methods of Analysis of AOAC International, 20th ed. AOAC International. Rockville, MD, USA.

Asavarut, P., Norsworthy, P., Cook, J., Taylor-Robinson, S., Harrison, R. 2016. Diet and disease: Transgressing boundaries between science and society-understanding neglected diseases through the lens of cultural studies and anthropology. Med. Humanit. 42: 181–183. dx.doi.org/10.1136/medhum-2016-010893

Cherdthong, A. 2020. Potential use of rumen digesta as ruminant diet—A review. Trop. Anim. Health Prod. 52: 1–6. doi.org/10.1007/s11250-019-02018-6

Cherdthong, A., Wanapat, M. 2013. Manipulation of *in vitro* ruminal fermentation and digestibility by dried rumen digesta. Livest. Sci. 153: 94–100. doi.org/10.1016/j.livsci.2013.02.008

Department of Livestock Development. 2008. Microbiological guideline for chilled/frozen meat and poultry meat for export. Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

Elfaki, M.O.A., Abdelatti, K.A. 2015. Nutritive evaluation of rumen content from cattle, camel, sheep and goat. Glob. J. Anim. Sci. 3: 617–621.

Franssen, F., Gerard, C., Cozma-Petruţ, A., et al. 2019. Inactivation of parasite transmission stages: Efficacy of treatments on food of animal origin. Trends Food Sci. Technol. 83: 114–128. doi.org/10.1016/j.tifs.2018.11.009

Górska-Warsewicz, H., Laskowski, W., Kulykovets, O., Kudlińska-Chylak, A., Czeczotko, M., Rejman, K. 2018. Food products as sources of protein and amino acids-The case of Poland. Nutrients 10: 1977. doi.org/10.3390/nu10121977

Hitchins, A.D., Jinneman, K., Chen, Y. 2017. Detection of *Listeria monocytogenes* in foods and environmental samples, and enumeration of *Listeria monocytogenes* in foods. In: Bacteriological Analytical Manual. U.S. Food & Drug Administration. MD, USA. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration, 31 October 2017.

Huang, F., Huang, M., Xu, X., Zhou, G. 2011. Influence of heat on protein degradation, ultrastructure and eating quality indicators of pork. J. Sci. Food Agric. 91: 443–448. doi.org/10.1002/jsfa.4204

Jacobson, A.P., Gill, V.S., Irvin, K.A., Wang, H., Hammack, T.S. 2012. Evaluation of methods to prepare samples of leafy green vegetables for preenrichment with the bacteriological analytical manual salmonella culture method. J. Food Prot. 75: 400–404. doi.org/10.4315/0362-028X.JFP-11-196

James, C., Dixon, R., Talbot, L., James, S.J., Williams, N., Onarinde, B.A. 2021. Assessing the impact of heat treatment of food on antimicrobial resistance genes and their potential uptake by other bacteria–A critical review. Antibiotics 10: 1440. doi.org/10.3390/antibiotics10121440

Laoraksawong, P., Sanpool, O., Rodpai, R., Thanchomnang, T., Kanarkard, W., Maleewong, W., Kraiklang, R., Intapan, P.M. 2018. Current high prevalences of *Strongyloides stercoralis* and *Opisthorchis viverrini* infections in rural communities in northeast Thailand and associated risk factors. BMC Public Health 18: 940. doi.org/10.1186/s12889-018-5871-1

Mao, S., Zhang, M., Liu, J., Zhu, W. 2015. Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: Membership and potential function. Sci. Rep. 5: 16116. doi.org/10.1038/srep16116

Maturin, L., Peeler, J.T. 2001. Aerobic plate count. In: Bacteriological Analytical Manual. U.S. Food & Drug Administration. MD, USA. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count, 27 September 2019.

Noppon, B., Khaeng-air, S., Sopa, A., Phuaram, P., Wongsan, R., Laohasinnurak, T. 2014. *Streptococcus suis* serotype 2 in uncooked pork meat products in Khon Kaen, Northeastern Thailand, and their antimicrobial profiles. Int. J. Sci. Eng. Res. 5: 1130–1133.

Pornsukarom, S., Sanguanprasit, B., Sangwittayanon, K., Chan-on, M., Tyobamrung, P., Singasa, K., Udomying, C. 2020. Prevalence and analysis of risk factors for gastrointestinal parasites in beef cattle herds in Aranyaprathet district, Sa Kaeo province, Thailand. Jpn. J. Vet. Res. 68: 69–76. doi.org/10.14943/jjvr.68.2.69

Sripa, B., Pairojkul, C. 2008. Cholangiocarcinoma: Lessons from Thailand. Curr. Opin. Gastroenterol. 24: 349–356. doi: 10.1097/MOG.0b013e3282fbf9b3

Sutton, J.D., Reynolds, C.K. 2011. Nutrients, digestion and absorption: Small intestine of lactating ruminants. In: Fuquay, J.W. (Ed.). Encyclopedia of Dairy Sciences, 2nd ed. Academic Press. San Diego, CA, USA, pp. 989–995.

Thai Agricultural Commodity and Food Standard. 2004. Thai Agricultural Community and Food Standard for Beef (TACFS 6001-2004). Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

Thanasuwan, S., Piratae, S., Tankrathok, A. 2021. Prevalence of gastrointestinal parasites in cattle in Kalasin province, Thailand. Vet. World 14: 2091–2096. doi.org/10.14202/vetworld.2021.2091-2096

Tortora, G.J., Funke, B.R., Case, C.L. 2010. Microbiology: An Introduction-Pearson International edition, 10th ed. Pearson Benjamin Cummings. San Francisco, CA, USA.

Vaeteewootacharn, K., Sutra, S., Vaeteewootacharn, S., et al. 2005. Salmonellosis and the food chain in Khon Kaen, northeastern Thailand. Southeast Asian J. Trop. Med. Public Health. 36: 123–129.

Williams, P. 2007. Nutritional composition of red meat. J. Acad. Nutr. Diet. 64: 113–119. doi.org/10.1111/j.1747-0080.2007.00197.x

Wongsawang, W., Sanyutitham, S., Nakthong, C. 2014. The survey of gastro-intestinal parasites in beef, Sai-Yok district, Kanchanaburi province. J. Appl. Anim. Sci. 7: 33–42.

Wu, G., Cross, H.R., Gehring, K.B., Savell, J.W., Arnold, A.N., McNeill, S.H. 2016. Composition of free and peptide-bound amino acids in beef chuck, loin, and round cuts. J. Anim. Sci. 94: 2603–2613. doi.org/10.2527/jas.2016-0478

Yeemin, P., Sumangka, J., Sotthisawad, K. 2022. Nutrition and some pathogenic bacteria contamination of cow's intestinal contents. UDRU Sci. Tech. J. 10: 17–29.