



Effect of actinidin from kiwifruit (*Actinidia deliciosa* cv. Hayward) on the digestion of food proteins determined in the growing rat

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ABSTRACT

This study aimed to determine the effect of dietary actinidin (provided as Hayward kiwifruit) on the gastric and small intestine digestion of six food protein sources in rats. For each protein source, two semi-synthetic test diets were formulated containing either freeze-dried Hayward kiwifruit (actinidin present) or freeze-dried Hort16A kiwifruit (actinidin absent). Actinidin activity is extremely low in Hort16A kiwifruit. Titanium dioxide was also included as an indigestible marker. Rats were fed freshly-prepared diets, euthanised and the gastric and ileal contents collected. The chyme and digesta samples were subjected to electrophoresis (SDS–PAGE), densitometry and titanium analysis and the degradability of individual proteins calculated. Dietary actinidin had no ($p > 0.05$) effect on the gastric degradability of zein and whey protein isolate but increased gastric degradability of beef muscle protein, gelatin, soy protein isolate and gluten by 40%, 60%, 27% and 29% units, respectively. Dietary actinidin had little or no effect on ileal protein degradability. Overall, dietary actinidin enhanced the gastric digestion of some food proteins.

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1. Introduction

The digestion of dietary protein begins in the stomach where it undergoes initial breakdown through the action of pepsin and hydrochloric acid. The resulting peptides and protein fragments then enter the small intestine where digestion continues through the action of pancreatic and intestinal proteases, resulting in amino acids and small peptides, which are then absorbed. Some food proteins are resistant to either gastric digestion, small intestinal digestion or both, while others are readily digested. The presence of large amounts of poorly digested dietary proteins in the stomach may lead to a reduction in the stomach emptying rate (Porter & Rolls, 1971; Zebrowska, 1968) and result in feelings of overfullness. Improving the digestion of dietary proteins through means of dietary intervention may reduce the negative impact associated with consuming diets high in protein, particularly those that are not easily digested.

Actinidin (EC 3.4.22.14) is a cysteine protease present in green (Hayward) kiwifruit (*Actinidia deliciosa* cv. Hayward). It has long been popularly assumed that actinidin aids the digestion of proteins when kiwifruit is consumed with a meal containing protein, but there is little evidence in the literature to support this contention. Recent *in vitro* studies using simulated gastric and small

intestinal digestion examined the effect of a kiwifruit extract containing actinidin on the hydrolysis of food proteins (Kaur, Rutherford, Moughan, Drummond, & Boland, 2010a, 2010b). This work found that actinidin enhanced the simulated gastric digestion of sodium caseinate, soy protein isolate, beef muscle protein, gluten and gliadin (Kaur et al., 2010a). Furthermore, the digestion of whey protein isolate, zein, collagen, gluten and gliadin by actinidin was enhanced under conditions that simulated both gastric and small intestinal digestion (Kaur et al., 2010b).

This study extends the work of Kaur et al. (2010a, 2010b) by examining the effect of a freeze-dried Hayward kiwifruit preparation containing actinidin on protein digestion in the stomach and small intestine of the growing rat. The proteins examined in this study were whey protein isolate (WPI), zein, soy protein isolate (SPI), beef muscle protein, gluten and gelatin, and constituted a subset of the food proteins previously examined by Kaur et al. (2010a, 2010b).

2. Materials and methods

2.1. Materials

Fresh green (*A. deliciosa* cv. Hayward) and ZESPRI® Gold (*Actinidia chinensis* cv. Hort16A) kiwifruit, pre-ripened to a similar firmness (firmness RTE [ready to eat] of 0.5–0.8 and 0.5–1.0 kgf, respectively), were prepared for inclusion into the rat diets as

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follows: the skins of the Hayward and Hort16A kiwifruit were removed and the fruit crushed by hand. The pulp was collected under carbon dioxide (to reduce the potential for actinidin oxidation) into plastic bags, frozen and freeze dried. The freeze-dried material was then blended under nitrogen using a kitchen food processor. The freeze-dried kiwifruit pulp was then stored at 4 °C prior to inclusion into the rat diets. In a similar *in vitro* based study (Kaur et al., 2010a) metabisulfite was used to preserve actinidin activity during the preparation of the kiwifruit extract. In this study, metabisulfite was not used, since it is toxic to rats.

WPI (Alacen 895) was obtained from Fonterra Co-Operative Group Ltd., Auckland, New Zealand. Gelatin was supplied by Gelita New Zealand Ltd., Christchurch. SPI (SUPRO® 670) was obtained from Columbit NZ (Auckland, New Zealand), and the wheat gluten (Amygluten 110) was obtained from Tate & Lyle, Amylum group, Aalst, Belgium. Zein was obtained from Sigma-Aldrich (St. Louis, MO). Beef muscle protein was prepared by freeze drying and grinding high quality low fat beef steak. Purified actinidin (50,000 units/g) was obtained from New Zealand Pharmaceuticals Ltd., Palmerston North.

2.2. Methods

2.2.1. Actinidin activity assay

The enzyme activity of the fresh kiwifruit and freeze dried kiwifruit was determined as described by Boland and Hardman (1972) and Kaur et al. (2010a).

2.2.2. Diet formulation

The study took advantage of the fact that while Hayward kiwifruit contain substantial amounts of actinidin, the levels in Hort16A are extremely low (Bublin et al., 2004). Two experimental diets for each protein source were formulated and the composition of these is shown in Table 1. The diets were formulated to have a similar nutrient composition across diets. Basal diets were formulated to be identical to the experimental diets, except the freeze

dried kiwifruit were replaced by wheat starch. All diets met the nutrient requirements of the growing rat except protein (NRC, 1995). The actinidin activity of Hort16A kiwifruit and Hayward kiwifruit used in this study was determined to be 0 and 300 units g⁻¹ kiwifruit dry matter, respectively. A small amount of purified actinidin (1 g kg⁻¹ diet) was also added to the Hayward kiwifruit test diets to compensate for the loss of actinidin activity in the Hayward kiwifruit that occurred during freeze drying; diets were supplemented with purified actinidin to provide an actinidin activity similar to that of the fresh Hayward kiwifruit. In addition a protein-free diet containing soybean oil (50 g kg⁻¹), purified cellulose (50 g kg⁻¹), vitamin mix (50 g kg⁻¹) and mineral mix (50 g kg⁻¹), sucrose (100 g kg⁻¹), titanium dioxide (3 g kg⁻¹) and wheat starch (697 g kg⁻¹) was also prepared and used to determine the molecular weights of endogenous proteins present in the stomach and terminal ileum of the rats.

The inclusion rate for the freeze-dried kiwifruit into the experimental diets was calculated, assuming that a person may consume two kiwifruit with an average sized meal as follows:

$$\text{Inclusion rate(\%)} = [(2KW \times DM)/(DI/3)] \times 100$$

where *KW* is the kiwifruit weight (120 g per kiwifruit), *DM* is the kiwifruit dry matter (14% of the fresh weight) and *DI* is the estimated average daily dry matter intake for an adult human (500 g).

2.2.3. In vivo digestibility trial

Ethics approval for the animal trial was obtained from the Animal Ethics Committee, Massey University, Palmerston North, New Zealand. One hundred and two Sprague–Dawley male rats, of 197 ± 2.9 g (mean ± SE) bodyweight, were housed individually in stainless steel wire-bottomed cages in a room maintained at 22 ± 2 °C, with a 12 h light/dark cycle. The diets were randomly allocated to the rats, such that there were eight rats on each test diet and six rats on the protein-free diet. The rats received their respective basal diet for a 13-day period. On each day each rat received its respective diet as nine meals given hourly between

Table 1
Ingredient composition (g kg⁻¹ air dry weight) and determined nutrient composition (g kg⁻¹ DM) of the experimental diets with the presence [Hayward kiwifruit, (+) A] and absence [Hort16A kiwifruit, (–) A] of actinidin.

	WPI		Beef muscle		Gelatin		SPI		Gluten		Zein	
	(+) A	(–) A	(+) A	(–) A	(+) A	(–) A	(+) A	(–) A	(+) A	(–) A	(+) A	(–) A
<i>Diet composition</i>												
Wheat cornflour	360	364	356	360	370	374	350	354	338	342	356	360
Soybean oil	50	50	50	50	50	50	50	50	50	50	50	50
Cellulose	50	50	50	50	50	50	50	50	50	50	50	50
Sucrose	100	100	100	100	100	100	100	100	100	100	100	100
Vitamin mix ^a	50	50	50	50	50	50	50	50	50	50	50	50
Mineral mix ^a	50	50	50	50	50	50	50	50	50	50	50	50
WPI	133	133	–	–	–	–	–	–	–	–	–	–
Meat protein	–	–	137	137	–	–	–	–	–	–	–	–
Gelatin	–	–	–	–	122	122	–	–	–	–	–	–
SPI	–	–	–	–	–	–	143	143	–	–	–	–
Wheat gluten	–	–	–	–	–	–	–	–	155	155	–	–
Zein	–	–	–	–	–	–	–	–	–	–	137	137
Hayward Kiwifruit	200	–	200	–	200	–	200	–	200	–	200	–
HortA Kiwifruit	–	200	–	200	–	200	–	200	–	200	–	200
Purified actinidin ^b	4	–	4	–	4	–	4	–	4	–	4	–
Titanium dioxide	3	3	3	3	3	3	3	3	3	3	3	3
<i>Diet analysis (g kg⁻¹ DM)</i>												
Dry matter	928	924	931	932	917	919	923	922	920	921	930	930
Crude protein	138	136	141	132	165	142	139	140	145	142	145	158
Ash	56	54	55	59	53	54	55	55	51	51	50	52
Ether extract	60	64	57	62	58	63	57	63	61	67	66	74
NDF ^c	39	25	40	27	42	40	39	29	40	28	34	26

^a Vitamin/mineral mix was formulated to meet the requirements for vitamins and minerals as described by the National Research Council (1995).

^b The purified actinidin was diluted with wheat starch prior to addition to the experimental diets.

^c NDF, neutral detergent fibre.

08:30 and 16:30 h. At each meal time the diets were freely available for a 10-min period. Water was available at all times. On the 14th day of the study, the rats received their respective freshly prepared test diet. Approximately 5.5–7 h after the start of feeding, the rats were euthanised by asphyxiation in carbon dioxide gas and then decapitation. The body cavity was opened and the stomach and small intestine were removed. Twenty centimetres of ileum immediately anterior to the ileo-caecal junction (ileal sample) were dissected out. In addition, it was the intention to collect duodenal samples but upon examining the rat duodenal sections it was obvious that for all the rats there was no digesta in the duodenum. Consequently, jejunal and gastric samples were taken instead. For the jejunal samples, 30 cm of intestine distal from the pylorus were dissected out. The dissected stomach and intestine sections were washed with distilled deionised water to remove any blood and hair and carefully dried on an absorbent paper towel. The stomach chyme and digesta were then gently flushed from the jejunal and ileal sections with distilled deionised water. The chyme and digesta from the rats were immediately frozen at -20°C to quench enzyme activity before being freeze dried ready for analysis.

2.3. Chemical analysis

Diets were analysed for dry matter, ether extract as described by the AOAC (1990), neutral detergent fibre using the Tecator Fibretec System, and starch using a kit from Megazyme Int. Ltd., Bray, Ireland, after grinding through a 0.5-mm-mesh screen. The titanium dioxide content was obtained after sulfuric acid digestion followed by colorimetric determination (Short, Gorton, Wiseman, & Boorman, 1996). Stomach chyme and ileal digesta were analysed for DM, and titanium dioxide as described above. The total nitrogen content was also determined in diets and digesta samples pooled across rats within each treatment using the Dumas method (AOAC, 1990) on a LECO analyser (LECO Corporation, St. Joseph, MI), in order to determine true ileal nitrogen digestibility.

2.4. Tricine–sodium dodecyl sulfate–polyacrylamide gel electrophoresis and protein degradability

Tricine–SDS–PAGE was used to estimate the degradability of the dietary proteins and was carried out using a Criterion cell (Bio-Rad Laboratories Pty. Ltd., Auckland, New Zealand). Soluble proteins and peptides were extracted from an accurately weighed aliquot (approximately 5 mg) of freeze-dried diet, chyme or digesta samples by mixing with 500 μL Tricine sample buffer (Bio-Rad) containing β -mercaptoethanol (1:100 w:v) for 30 min. The samples were then heated at 100°C for 10 min and centrifuged at 6600 rpm for 3 min. A known amount (15–30 μL) sample was loaded onto a 10–20% gradient Tricine–SDS–PAGE gel (Bio-Rad) and electrophoresed at a constant voltage of 125 V. The gels were then stained using Coomassie Brilliant Blue R. MW standards (10–250 kDa 161-0363/0373/0374 and 1.4–26.6 kDa 161-0326, Bio-Rad) were also loaded in separate wells in each gel.

The measurement of intact proteins and peptides was carried out using gel scanning (Molecular Imager Gel Doc XR, Bio-Rad) followed by densitometry, using the software Quantity One 1-D Analysis (Bio-Rad). The staining density was measured (in arbitrary density units, ADU) horizontally for each protein band.

2.5. Data and statistical analysis

Gastric and ileal protein degradability was calculated for individual proteins in each protein source as follows:

Protein degradability(%)

$$= 100 \times [\text{band intensity}_{\text{diet}} - (\text{band intensity}_{\text{digesta}} \times \text{titanium}_{\text{diet}} / \text{titanium}_{\text{digesta}})] / \text{band intensity}_{\text{diet}}$$

True ileal nitrogen digestibility was calculated as follow (units are $\text{mg g}^{-1} \text{DM}$):

$$\begin{aligned} &\text{True ileal nitrogen (N) digestibility(%) (units are } \text{mg g}^{-1} \text{DM):} \\ &= 100 \times [N_{\text{diet}} - (N_{\text{digesta}} \times \text{titanium}_{\text{diet}} / \text{titanium}_{\text{digesta}} \\ &\quad - \text{ileal endogenous N flow})] / N_{\text{diet}} \end{aligned}$$

Ileal endogenous nitrogen flows were those reported by Hodgkinson, Souffrant, and Moughan (2003), determined using the enzyme-hydrolysed casein technique.

Homogeneity of variance between the Hort16A- and Haywards-based diets was tested using a Bartlett test for each protein source. An ANOVA was conducted using the General Linear Model procedure of Statistical Analysis Systems statistical software package version 9.1 (SAS Institute Inc., Cary, NC). For each protein source, the effect of actinidin supplementation in the diet was tested for the gastric and ileal degradability. When the *F*-value of the ANOVA was significant ($p < 0.05$), the means were compared using Tukey's test.

3. Results

Generally the rats appeared healthy and gained weight over the course of the study. The mean body weight gain across all dietary treatments over the experimental period was $17 \pm 1.8 \text{ g}$. However, one rat died of unknown causes. The macronutrient composition was similar across the experimental diets with perhaps the exception of the NDF which was higher for the actinidin positive (Hayward kiwifruit) diets compared to the actinidin negative (Hort 16A) diets (Table 1).

3.1. Protein degradability

The aim of this study was to determine the gastric and ileal degradability of a range of food proteins in the presence of Hayward kiwifruit (containing actinidin) or Hort16A (actinidin devoid). To determine gastric degradability, duodenal digesta would normally be collected, to represent material leaving the stomach. In this study, while ileal digesta collection was straightforward, duodenal digesta could not be collected in sufficient quantities for SDS–PAGE analysis. Consequently, stomach chyme was collected in place of the duodenal digesta. Furthermore, the amount of jejunal digesta was sufficient for SDS–PAGE analysis but insufficient for titanium analysis. Consequently protein degradability for the jejunal samples was not determined. Degradability was defined as the disappearance of the intact protein by comparing SDS–PAGE analysis of diets with that of chyme or digesta. In addition, true ileal nitrogen digestibility was also determined, based on the nitrogen content of the terminal ileal digesta estimated using an indigestible marker and corrected for endogenous nitrogen flow.

The electrophoretic profiles (SDS–PAGE gel) of six food protein-based diets formulated either with Hayward kiwifruit (containing actinidin) or with Hort16A kiwifruit (devoid of actinidin) and stomach chyme, jejunal and ileal digesta collected from rats fed these diets are shown in Fig. 1. Gastric and ileal degradability of dietary proteins with MWs similar to those of the endogenous proteins (as determined using the rats fed the protein-free diet) were not determined, since it is possible that the latter dietary proteins would co-migrate with the endogenous proteins.

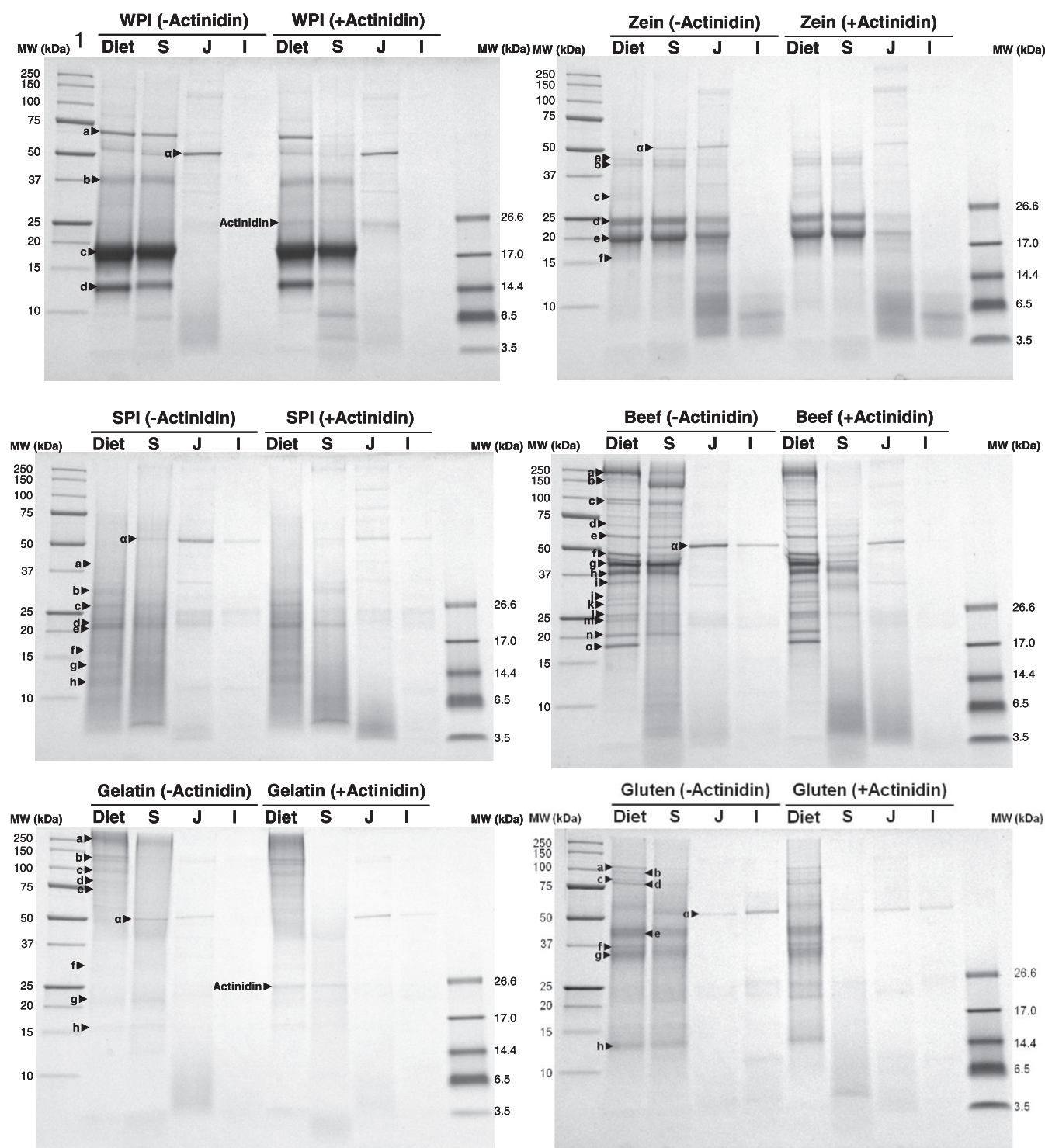


Fig. 1. SDS-PAGE analysis of six food protein-based diets and stomach chyme (S), jejunal digesta (J) and ileal digesta (I) of selected rats fed the six food protein-based diets. The amount of sample loaded in each lane was normalised to the concentration of titanium dioxide (indigestible marker). For WPI, protein bands were identified as follows: (a) BSA, (b) β -Lg dimer, (c) β -Lg, (d) α -Lac. For zein, protein bands were identified as: (a) Z22 dimer, (b) Z19 dimer, (c) Z15 dimer, (d) Z22, (e) Z19, (f) Z15. For SPI, protein bands were identified as: (a–b) acidic subunits A–B of 11S globulin, (c–f) basic (A–D) subunits of 11S globulin, (g) 14 kDa protein, (h) 12 kDa protein. For beef muscle protein, protein bands were identified as: (a) myosin heavy chain, (b) β -actinin, (c) α -actinin, (d) 71 kDa protein, (e) 64 kDa protein, (f) 45 kDa protein, (g) actin, (h) tropomyosin β chain, (i) tropomyosin α chain, (j) 32 kDa protein, (k) 29 kDa protein, (l) 25 kDa protein, (m) myosin light chain1, (n) troponin I, (o) myosin light chain2. For gelatin, protein bands were identified as: (a) high MW aggregate, (b) α 1 type I collagen, (c) α 2 type I collagen, (d) 88 kDa protein, (e) 74 kDa protein, (f) 30 kDa protein, (g) 21 kDa protein, (h) 15 kDa protein. For gluten, protein bands were identified as: (a–c) (HMW) subunits of glutenin, (d) ω -gliadin, (e) LMW subunit B of glutenin, (f–g) LMW subunit C of glutenin, (h) 13 kDa protein. α represents α -amylase in all gels.

3.2. Gastric and ileal digestion of whey protein isolate

WPI is composed mainly of β -lactoglobulin (β -Lg; 18.4 kDa) and α -lactalbumin (α -Lac; 14.2 kDa) proteins but also contains bovine

serum albumin (BSA; 66 kDa) (Feng, Konishi, & Bell, 1991) and a range of minor proteins not considered here. The gastric and ileal degradability of WPI proteins in the stomach and terminal ileum of rats fed diets either with Hayward kiwifruit (containing actini-

Table 2

Mean gastric and ileal ($n = 8$) degradability (%) of WPI proteins determined in the presence [Hayward kiwifruit, (+) Act] and absence [Hort16A kiwifruit, (–) Act] of dietary actinidin using the growing rat.

	Mean degradability (%)		Overall SE	Significance
	(–) Act	(+) Act		
<i>Gastric</i>				
BSA	38.8	62.2	8.6	NS
β-Lg dimer	23.7	9.5	11.9	NS
β-Lg	12.6	0.0	12.6	NS
α-Lac	28.5	32.9	11.1	NS
<i>Ileal</i>				
BSA	96.7	97.1	0.5	NS
β-Lg dimer	96.9	97.7	0.6	NS
β-Lg	97.4	98.1	0.4	NS
α-Lac	96.7	97.9	0.4	*
True ileal N digestibility ^a	87.8	87.9		

NS, not significant $p > 0.05$, * $0.05 > p > 0.01$.

^a Determined using pooled digesta from six rats.

din) or Hort16A kiwifruit (devoid of actinidin) are shown in Table 2. The gastric degradability of BSA, β -Lg, the dimer of β -Lg (37 kDa) and α -Lac ranged from 12.6% to 38.8% in the presence of Hort16A. Furthermore, there was no effect when actinidin-containing Hayward kiwifruit was present in the WPI-based diet ($p > 0.05$). The ileal degradability of the four WPI proteins was greater than 97%. The presence of dietary Hayward kiwifruit (containing actinidin) did not significantly ($p > 0.05$) increase the ileal degradability of any of the WPI proteins with the exception of α -Lac which was significantly ($p < 0.05$), but only slightly (1% unit) higher, when Hayward kiwifruit was present in the diet. True ileal nitrogen digestibility for the WPI-based diets containing either Hayward or Hort16A kiwifruit was 87.9% and 87.8%, respectively.

3.3. Gastric and ileal digestion of zein proteins

Zein is a mixture of proteins that constitute 50–60% of the total endosperm proteins in maize (*Zea mays*) with the α -zeins being the most abundant of the four zein classes (α -, β -, γ -, and δ -zein) (Hamaker, Mohamed, Habben, Huang, & Larkins, 1995). Within the α -zeins, Z19 (19 kDa) and Z22 (22 kDa) have been characterised

Table 3

Mean gastric and ileal ($n = 8$) degradability (%) of zein proteins determined in the presence [Hayward kiwifruit, (+) Act] and absence [Hort16A kiwifruit, (–) Act] of dietary actinidin using the growing rat.

	Mean degradability (%)		Overall SE	Significance
	(–) Act	(+) Act		
<i>Gastric</i>				
Z22 dimer	0.0	0.0	8.3	NS
Z19 dimer	2.4	7.2	7.1	NS
Z15 dimer	2.8	2.9	7.4	NS
Z22 ^a	0.0	0.0	7.2	NS
Z19	0.0	0.0	7.4	NS
Z15	0.0	0.0	8.9	NS
<i>Ileal</i>				
Z22 dimer	92.7	94.8	1.5	NS
Z19 dimer	93.2	95.7	1.3	NS
Z15 dimer	93.6	93.4	1.7	NS
Z19	94.7	92.0	1.2	*
Z15	74.7	54.0	6.6	*
True ileal N digestibility ^b	17.7	35.7		

NS, not significant $p > 0.05$, * $0.05 > p > 0.01$.

^a Ileal degradability was not determined for Z22 since this protein co-migrated with an endogenous protein as assessed from ileal digesta of rats fed a protein-free diet.

^b Determined using pooled digesta from six rats.

(Hamaker et al., 1995). The Z15 (15 kDa) and Z10 (10 kDa) zein proteins have also been reported in maize (Shewry & Mifflin, 1985). In this study, the Z22 and Z19 proteins were the major zein proteins present (Fig. 1, zein SDS–PAGE gel, bands d and e, respectively). Bands corresponding to the MWs of dimers of the Z22, Z19 and Z15 proteins were also present, albeit in lower amounts (Fig. 1, zein SDS–PAGE gel, bands a, b and c, respectively).

At the gastric level, there was no degradation observed for the Z22 dimer, Z22, Z19 and Z15 protein bands determined in either the presence or absence of dietary actinidin, indicating that no digestion of these proteins occurred (Table 3). In addition, the degradability of the Z19 dimer and Z15 dimer ranged between 2.4% and 7.2%. There was no significant effect of dietary Hayward kiwifruit (containing actinidin) on the gastric degradability of any of the zein proteins in comparison with the Hort16A (devoid of actinidin). The ileal degradability of the Z19 zein protein and its dimer and the Z15 dimer was greater than 90% (Table 3). The inclusion of Hayward kiwifruit in the zein-based diet did not influence the ileal degradability of the Z15, Z19 and Z22 dimers ($p > 0.05$), in comparison with Hort16A. However, the degradability of the Z19 and Z15 proteins was significantly ($p < 0.05$) reduced when Hayward as opposed to Hort16A kiwifruit was included in the diet. The Z15 protein was particularly poorly digested. True ileal nitrogen digestibility for the zein-based diets containing either Hayward or Hort16A kiwifruit was 35.7% and 17.7%, respectively, reflecting the greater presence of peptides in the ileal digesta (4–12 kDa), in comparison with the other protein sources (Fig. 1).

3.4. Gastric and ileal digestion of soya protein isolate

Soya proteins comprise lipoxxygenase (94 kDa), β -amylase (60 kDa), α' , α , β -subunits (80, 76, and 50 kDa) of conglycinin (7S globulin), the acidic subunits (38, 35, and 33 kDa) and the basic subunits (25, 22, and 18 kDa) of glycinin (11S globulin) (Parris & Gillespie, 1988; Qi, Hettiarachchy, & Kalapathy, 1997). Due to the partial hydrolysis that is carried out during manufacture to achieve

Table 4

Mean gastric and ileal ($n = 8$) degradability (%) of SPI proteins determined in the presence [Hayward kiwifruit, (+) Act] and absence [Hort16A kiwifruit, (–) Act] of dietary actinidin in the growing rat.

	Mean degradability (%)		Overall SE	Significance
	(−) Act	(+) Act		
<i>Gastric</i>				
Acidic subunit A of 11S globulin	6.8	45.4	5.8	***
Acidic subunit B of 11S globulin ^a	18.7	48.0	5.4	**
Basic subunit A of 11S globulin ^a	6.9	34.0	6.3	**
Basic subunit B of 11S globulin ^a	7.2	36.2	5.9	**
Basic subunit C of 11S globulin	10.0	36.3	5.6	**
Basic subunit D of 11S globulin	1.7	26.6	5.4	**
14 kDa protein	0.0	8.0	5.2	*
<i>Ileal</i>				
Acidic subunit A of 11S globulin	90.5	89.8	1.2	NS
Basic subunit C of 11S globulin	92.3	91.7	1.0	NS
Basic subunit D of 11S globulin	92.0	91.5	1.0	NS
14 kDa protein	91.8	91.5	1.0	NS
True ileal N digestibility ^b	95.4	97.1		

NS, not significant $p > 0.05$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $p < 0.001$.

^a Ileal degradability was not determined for these proteins since they co-migrated with endogenous proteins as assessed from ileal digesta of rats fed a protein-free diet.

^b Determined using pooled digesta from six rats.

the desired functionality (Personal communication, The Solae Company, St. Louis, MO), a number of the proteins reported to be present in soya beans were not observed in the SPI sample used in this study (Fig. 1). The protein bands that were identified based on MW were some of the acidic (35 and 38 kDa) and basic (25, 22 and 18 kDa) subunits of 11S globulin.

The gastric degradability of all the identified proteins in the SPI-based diet in the presence of dietary Hort16A (devoid of actinidin) kiwifruit ranged from 0% to 18.7% (mean degradability across proteins = 7.3%) (Table 4). However, in the presence of dietary Hayward kiwifruit (containing actinidin), the gastric degradability of all the 11S globulin subunits (acid and basic) and the unidentified 14 kDa protein increased significantly ($p < 0.05$) by between 15% and 38% units. Ileal degradability of the soy proteins was greater than 89% and there was no significant effect of Hayward kiwifruit compared to Hort16A kiwifruit. Ileal degradability of the acidic subunit B (35 kDa) and basic subunit A and B (25 and 22 kDa) of 11S globulin was not determined, due to co-migration with endogenous proteins, as determined based on alimentation with a protein-free diet. True ileal nitrogen digestibility for the soya protein-based diets containing either Hayward or Hort16A kiwifruit was 97.1% and 95.4%, respectively.

3.5. Gastric and ileal digestion of meat proteins

Muscle tissue contains a large number of proteins, the main components being myosin, actin, troponin and tropomyosin (Xiong, 1997). A number of major protein bands were observed

Table 5

Mean gastric and ileal degradability (%) of beef muscle proteins determined in the presence [Hayward kiwifruit, (+) Act; $n = 8$] and absence [Hort16A kiwifruit, (–) Act; $n = 7$] of dietary actinidin in the growing rat.

	Mean degradability (%)		Overall SE	Significance
	(–) Act	(+) Act		
<i>Gastric</i>				
Myosin heavy chain	37.5	73.9	4.9	***
β-Actinin	14.3	65.6	11.3	**
α-Actinin	5.1	62.9	7.5	***
71 kDa protein	0.0	37.1	8.3	***
64 kDa protein	0.0	39.2	9.9	*
45 kDa protein ^a	14.2	49.2	7.4	**
Actin	10.1	55.0	6.9	***
Tropomyosin α chain	0.0	19.8	8.8	NS
Tropomyosin β chain	14.9	30.5	7.7	NS
32 kDa protein	0.5	14.6	8.4	NS
29 kDa protein ^a	3.0	28.1	7.5	*
25 kDa protein ^a	4.1	31.1	8.7	*
Myosin light chain (MLC1)	8.0	34.0	8.2	*
Troponin I	0.0	42.7	7.2	***
Myosin light chain (MLC2)	30.9	49.2	7.0	NS
<i>Ileal</i>				
Myosin heavy chain	99.2	98.1	0.4	NS
β-Actinin	98.2	96.1	1.1	NS
α-Actinin	96.0	95.4	0.9	NS
71 kDa protein	93.7	95.5	1.4	NS
64 kDa protein	93.5	96.0	1.2	NS
Actin	94.3	97.2	1.0	*
Tropomyosin α chain	96.6	98.0	0.7	NS
Tropomyosin β chain	97.2	98.3	0.6	NS
32 kDa protein	97.3	97.4	0.7	NS
Myosin light chain (MLC1)	91.0	92.4	0.9	NS
Troponin I	96.7	97.9	0.9	NS
Myosin light chain (MLC2)	99.0	98.2	0.3	NS
True ileal N digestibility ^b	93.3	95.3		

NS, not significant $p > 0.05$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $p < 0.001$.

^a Ileal degradability was not determined for these proteins since they co-migrated with endogenous proteins as assessed from ileal digesta of rats fed a protein-free diet.

^b Determined using pooled digesta from six rats.

in the SDS–PAGE gel of beef muscle and, of these, eight protein bands were identified based on their determined MW (Fig. 1). These proteins consisted of myosin heavy chain (MHC, 220 kDa), β-actinin (130 kDa), α-actinin (95 kDa), actin (42 kDa), tropomyosin β chain (36 kDa), tropomyosin α chain (34 kDa), myosin light chain (MLC1, 23 kDa), troponin I (21 kDa), and myosin light chain (MLC2, 17 kDa). Nebulin and titin (600–800 kDa and 2500 kDa, respectively) were not observed on the SDS–PAGE gel.

The gastric degradability of the identified proteins ranged from 0% to 37.5% with an average degradability of 9.5% in the Hort16A kiwifruit diet (Table 5). In the presence of dietary Hayward kiwifruit the degradability of myosin heavy chain, β-actinin, α-actinin, actin, MLC1 and troponin I was significantly ($p < 0.05$) higher, in comparison with the Hort16A kiwifruit-containing diet, with a mean increase of 32% units. For tropomyosin α chain, tropomyosin β chain and MLC2 and 32 kDa protein there was no significant ($p > 0.05$) difference in degradability determined in the presence of either Hayward or Hort16A kiwifruit. There were several unidentified proteins present in the beef muscle (71, 64, 45, 29 and 25 kDa) for which the degradability was significantly ($p < 0.05$) higher in the presence of dietary Hayward kiwifruit compared to Hort16A kiwifruit (mean increase = 33%). The ileal degradability across all identified proteins was greater than 91%. For most of the proteins, including myosin heavy chain, β-actinin, α-actinin, tropomyosin α chain, tropomyosin β chain, MLC1, MLC2 and several unidentified proteins of MWs 71, 64 and 32 kDa, there was no significant difference between the ileal degradability determined in the presence of either Hayward or Hort16A kiwifruit. In contrast, the ileal degradability of actin increased significantly ($p < 0.05$) (approximately 3% units) in the presence of Hayward kiwifruit in the diet. True ileal nitrogen digestibility for the meat protein-based diets containing either Hayward or Hort16A kiwifruit was 95.3% and 93.3%, respectively.

3.6. Gastric and ileal digestion of gelatin

Gelatin is a commercial product derived from the alkaline or acidic extraction of collagen-containing animal byproducts

Table 6

Mean gastric and ileal ($n = 8$) degradability (%) of gelatin proteins determined in the presence [Hayward kiwifruit, (+) Act] and absence [Hort16A kiwifruit, (–) Act] of dietary actinidin in the growing rat.

	Mean degradability (%)		Overall SE	Significance
	(–) Act	(+) Act		
<i>Gastric</i>				
High MW aggregate	26.4	88.0	7.9	***
α1 type I collagen	14.4	84.4	9.1	***
α2 type I collagen	17.0	83.2	8.9	***
88 kDa protein ^a	15.3	78.7	9.2	***
74 kDa protein	8.7	65.7	10.2	**
30 kDa protein	19.8	68.3	6.2	***
21 kDa protein	0.0	21.5	5.7	NS
15 kDa protein ^a	13.1	6.7	8.2	NS
<i>Ileal</i>				
High MW aggregate	98.5	97.2	0.4	NS
α1 type I collagen	94.2	92.3	0.7	NS
α2 type I collagen	98.0	97.6	0.3	NS
74 kDa protein	94.1	94.5	1.0	NS
30 kDa protein	92.7	95.7	1.4	NS
21 kDa protein	89.2	85.8	1.1	*
True ileal N digestibility ^b	87.8	87.9		

NS, not significant $p > 0.05$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $p < 0.001$.

^a Ileal degradability was not determined for these proteins since they co-migrated with endogenous proteins as assessed from ileal digesta of rats fed a protein-free diet.

^b Determined using pooled digesta from six rats.

(Aewsiri, Benjakul, & Visessanguan, 2009; Gilsenan & Ross-Murphy, 2000). It mainly consists of the γ -component and α 1- and α 2-collagen chains but also contains smaller proteins probably present as a result of the gelatin manufacturing process (Aewsiri et al., 2009; Muyonga, Coleb, & Duodub, 2004). Bands corresponding to the high MW aggregate, α 1 type I collagen (125 kDa), α 2 type I collagen (106 kDa) were identified in this study (Fig. 1). In addition, bands corresponding to proteins of MW 88, 74, 30, 21 and 15 kDa were also observed in the diet samples.

The gastric degradability of all the observed proteins ranged from 0% to 26.4% (mean degradability = 14%) (Table 6). The degradability of the high MW aggregate, α 1 type I collagen, α 2 type I collagen, as well as the 88, 74, 30 kDa proteins increased significantly ($p < 0.01$) by 48–70% units in the presence of dietary Hayward kiwifruit (containing actinidin), in comparison with Hort16A kiwifruit. The presence of Hayward kiwifruit did not significantly increase the gastric degradability of the 21 and 15 kDa proteins. The ileal degradability of all the gelatin proteins was greater than 89%. However, their degradability was not significantly influenced by the presence of dietary Hayward kiwifruit, with the exception of 21 kDa protein for which ileal degradability was slightly lower (3% units; $p < 0.05$). True ileal nitrogen digestibility for the gelatin-based diets containing either Hayward or Hort16A kiwifruit was 87.9% and 87.8%, respectively.

3.7. Gastric and ileal digestion of wheat gluten

Wheat gluten comprises several proteins including the α -, β -, γ -, and ω -gliadins, as well as the low MW (LMW) subunits of glutenin and high MW (HMW) subunits of glutenin (Shewry, Tatham, Forde, Kreis, & Mifflin, 1986). Most of these proteins were observed in the gluten-based diet as shown using SDS-PAGE (Fig. 1).

The mean gastric degradability across all the gluten proteins in the presence of dietary Hort16A kiwifruit was 37% (Table 7). The degradability of three of the HMW subunits (110, 99 and 90 kDa) of glutenin and an unidentified protein of MW 13 kDa was not significantly influenced by the presence of dietary actinidin. In contrast, the degradability of ω -gliadin and three LMW subunits (41,

37 and 35 kDa) of glutenin was significantly ($p < 0.05$) higher in the presence of dietary Hayward kiwifruit, in comparison with dietary Hort16A kiwifruit. The latter increase ranged from 17% to 35% units with an average increase of 29% units. The ileal degradability of all the observed proteins was high (>95%) and with the exception of the LMW subunit B of glutenin, a small (<2.3% units) but significant ($p < 0.05$) decrease in ileal degradability was observed in the presence of dietary Hayward kiwifruit. True ileal nitrogen digestibility for the gelatin-based diets containing either Hayward or Hort16A kiwifruit was 98.5% and 96.8%, respectively.

4. Discussion

Actinidin is a proteolytic enzyme present in green Hayward, but not gold Hort16A, kiwifruit (Bublin et al., 2004). There is some anecdotal evidence and a popularly held belief that dietary actinidin enhances digestion. In addition, the presence of dietary actinidin may result in the formation of different bioactive peptides from those generated from gut enzymes, particularly pepsin alone. In the present study, the effect of dietary actinidin on the gastric and ileal degradability of selected food proteins (WPI, SPI, zein, beef muscle protein, gelatin and gluten) was tested *in vivo* in the growing rat, based on the dietary inclusion of either Hayward kiwifruit which contains actinidin or Hort16A kiwifruit which does not. Initially, gastric degradability was to be determined based on digesta collected from the upper duodenum of the rats. However, the presence of only very small amounts of duodenal digesta meant that gastric chyme had to be used instead.

In this study, protein degradability was determined based on the disappearance of intact proteins rather than the reduction of the protein to amino acids. Furthermore, quantitation of the intact protein is based on the SDS-PAGE analysis of diet, chyme and digesta samples and corrected for an indigestible marker (titanium dioxide). This technique permits the semi-quantitative determination of degradability for any given protein within a complex protein mixture (e.g., food protein sources) but is limited to dietary proteins that do not co-migrate with endogenous proteins since the band intensity for such proteins will be overestimated, due to the presence of co-migrating endogenous proteins. While no correction of apparent degradability values to true values is made, it is likely that degradability data determined in this study represent true degradability values. Overall, the method offers a novel and useful approach for assessing the degradability of individual food proteins *in vivo*.

For all six food protein sources examined in this study, a number of peptides less than 12 kDa were observed in either gastric chyme or jejunal digesta that were not present in the diets and it is possible that these represent undigested peptides. One limitation of SDS-PAGE is that small peptides (3.5 kDa) are difficult to detect and it is possible that such peptides are present but not observed.

4.1. The effect of actinidin on the gastric degradability of food proteins

The predominant digestive activity in the stomach is the proteolytic activity of pepsin, which has a well-defined specificity (Fruton, Bergmann, & Anslow, 1939) and can only hydrolyse a limited range of peptide bonds. Actinidin, in contrast, has a very broad specificity (Boland and Hardman, 1972) and can hydrolyse a wide range of peptide bonds unavailable to pepsin. In so doing, it can open up protein structures and expose new sites to pepsin activity. Dietary actinidin did not increase the gastric degradability of the major components of WPI and zein but appeared to markedly increase the gastric digestion of the main components of SPI, beef muscle protein, gelatin and gluten (Table 8).

Table 7

Mean apparent gastric and ileal ($n = 8$) degradability (%) of wheat gluten proteins determined in the presence [Hayward kiwifruit, (+) Act] and absence [Hort16A kiwifruit, (–) Act] of dietary actinidin using the growing rat.

	Mean degradability (%)		Overall SE	Significance
	(–) Act	(+) Act		
<i>Gastric</i>				
HMW subunits of glutenin	46.1	59.4	4.9	NS
HMW subunits of glutenin	42.7	57.2	4.8	NS
HMW subunits of glutenin	42.1	55.7	4.6	NS
ω-Gliadin	44.3	61.0	4.3	*
LMW subunit B of glutenin	39.7	72.7	4.6	***
LMW subunit C of glutenin	34.6	64.9	4.8	***
LMW subunit C of glutenin	36.0	70.6	4.6	***
13 kDa protein	10.6	18.8	6.8	NS
<i>Ileal</i>				
HMW subunits of glutenin	98.5	96.2	0.3	**
HMW subunits of glutenin	98.4	96.4	0.3	**
HMW subunits of glutenin	98.7	96.3	0.3	**
ω-Gliadin	98.7	97.2	0.3	*
LMW subunit B of glutenin	98.3	98.0	0.2	NS
LMW subunit C of glutenin	99.2	98.1	0.2	**
LMW subunit C of glutenin	99.3	98.4	0.2	**
13 kDa protein	97.1	95.1	0.5	*
True ileal N digestibility ^a	96.8	98.5		

NS, not significant $p > 0.05$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $p < 0.001$.

^a Determined using pooled digesta from six rats.

Table 8Summary of the effects^a of actinidin on the gastric degradability of food proteins determined *in vivo* in the growing rat and using an *in vitro* model.

Protein	Effect of actinidin (<i>in vivo</i>) ^b	Effect of actinidin (<i>in vitro</i>) ^c	Comments for the <i>in vivo</i> effect of actinidin
<i>Gastric degradability</i>			
WPI	–	–	No significant effect on digestion
Zein	–	Not determined	No significant effect on digestion
SPI	+	+	Significant ($p < 0.05$) and sizable increase in the digestion of many proteins
Beef muscle protein	+	+	Significant ($p < 0.05$) and sizable increase in the digestion of many proteins
Gelatin	+	–	Significant ($p < 0.05$) and sizable increase in the digestion of many proteins
Gluten	+	+	Significant ($p < 0.05$) and sizable increase in the digestion of many proteins
<i>Ileal degradability</i>			
WPI	+/-	+	Significant ($p < 0.05$) but small increase in α -Lac degradability
Zein	–	+	No positive effect
SPI	–	–	No positive effect
Beef muscle protein	+/-	–	Significant ($p < 0.05$) but small increase in the degradability of several proteins
Gelatin	–	–	No positive effect of actinidin was observed
Gluten	–	+	Negligible negative effect

^a Positive effect (+), no positive effect (–).^b Based on the results of the presently reported study.^c Based on the results of Kaur et al. (2010a).

Kaur et al. (2010a, 2010b) conducted an *in vitro* study investigating the effect of an actinidin-containing Hayward kiwifruit extract on the digestibility of the same proteins as those examined in the present study. A comparison of the *in vitro* (Kaur et al., 2010a, 2010b) and the *in vivo* (present study) data are shown in Table 8. Consistent with the findings in this study, Kaur et al. (2010a) showed no effect of the actinidin-containing Hayward kiwifruit extract on the gastric digestion of WPI, but a significant effect on the gastric digestion of SPI, beef muscle protein and gluten. In contrast, Kaur et al. (2010a) reported no effect of Hayward kiwifruit extract on the simulated gastric digestion of gelatin, while in this *in vivo* study dietary Hayward kiwifruit significantly enhanced the gastric degradability of gelatin proteins, in comparison with Hort16A kiwifruit. The other main difference between the two studies was the protein profile of SPI. In the present study, no major proteins larger than 39 kDa were detected. In contrast, Kaur et al. (2010a) found proteins ranging from 12 to approximately 75 kDa. The reason for this discrepancy is not clear but may be due to the fact that SUPRO 670 was used in this study while Kaur et al. (2010a) used SUPRO[®] XT 34 N IP; these two products undergo different hydrolyses during their processing (personal communications, The Solae Company).

The variability around the mean gastric degradability values (overall SEM = 7.9%) was much higher than for the ileal degradability values (overall SEM = 0.9%). This is likely to be a consequence of the presence of food proteins in the stomach at various stages of digestion, since stomach chyme rather than duodenal digesta was collected and since the rats were not killed at set times after feeding. In addition, SDS–PAGE gel is only a semi-quantitative technique for determining the amount of a protein present in a sample and consequently, protein degradability determined using SDS–PAGE does have its limitations. Despite the obvious limitations, statistically significant increases in gastric protein degradability in the presence of dietary actinidin were still observed for several of the food proteins.

4.2. The effect of actinidin on ileal degradability of food proteins

The mean ileal degradability of individual proteins in the six food protein sources tested in this study was very high and, with the exception of zein, reflected the true ileal nitrogen digestibility, which was also high for all the proteins sources except zein. The presence of dietary actinidin (in the form of Hayward kiwifruit) did not increase the ileal degradability of zein, SPI or gelatin proteins but did alter the ileal degradability of some proteins in WPI, beef muscle and gluten (Table 8). However, for all the latter

proteins the effect in degradability was minor. Clearly, the impact of dietary actinidin (in the form of Hayward kiwifruit) on ileal protein degradability was much less than that observed for gastric protein degradability.

5. Conclusion

Overall, dietary actinidin (in the form of Hayward kiwifruit) significantly increased the gastric digestion of the main proteins of beef muscle, SPI, gelatin and gluten but not of WPI or zein. This result generally correlates with earlier *in vitro* studies (Kaur et al., 2010a) that showed, using simulated gastric digestion, that actinidin enhanced the gastric digestion of proteins in beef muscle, SPI, gluten but not WPI. All the protein sources examined in this study (with the exception of zein) were almost completely digested at the terminal ileum and dietary actinidin had no or little effect on the extent of ileal protein degradability.

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