International Food Research Journal 19(4): 1367-1374 (2012)
Journal homepage: *http://www.ifrj.upm.edu.my*

In vitro digestion of Red Deer (*Cervus elaphus*) and Cow (*Bos taurus*) milk

[1,*]Opatha Vithana, N. L., [1]Mason, S. L., [2]Bekhit, A. E. A. and [1]Morton, J. D.

[1]*Department of Wine, Food and Molecular Bioscience, Lincoln University, PO Box 84, Lincoln 7647, New Zealand*
[2]*Department of Food Science, University of Otago, PO Box 56, Dunedin, New Zealand*

Article history

Received: 8 March 2012
Received in revised form: 12 April 2012
Accepted: 13 April 2012

Keywords
Deer milk
milk protein
digestion
peptides

Abstract

The aim of this study was to compare the digestion of milk proteins from deer and cow milk using *in vitro* digestion and to quantify the production of peptides using the O-phthaldialdehyde assay. Deer milk contained on average 8.8 ± 0.13% protein which is twice the levels found in cow milk (4.1 ± 0.02%). Deer and cow milk were digested in two steps; imitating both the human stomach (Pepsin, pH 2.5, 30 min) and the duodenum (Corolase PP, pH 7.5, 30 min). The degradation patterns of the milk proteins were visualized by SDS-PAGE and quantified using ImageJ software. Peptide production was significantly higher in deer milk than cow milk ($P< 0.05$). The commercial proteolytic enzymes degraded milk protein from deer more rapidly than those from cow. Deer casein was completely digested at 40 min of digestion (10 min into duodenum digestion) where as 14% of the casein was still present in cow milk. The digestibility of α-lactalbumin and immunoglobulin were also higher in deer milk than cow milk. However, β-lactoglobulin from both species appeared to be resistant to both gastric and duodenal digestion. This study shows that deer milk proteins were more digestible and produced more peptides than protein from cow milk. The bioactive functions of deer peptides are currently under investigation.

Introduction

Milk proteins are considered the most important commercial source of bioactive peptides but information on these peptides from species other than bovine is very limited. Digestion of milk proteins produces peptides which exhibit significant physiological roles in addition to their nutritional importance. These peptides can contribute to the high biological value attributed to milk. A large number of medium and low molecular weight peptides resulting from cow milk proteolysis by digestive enzymes have been identified (Korhonen and Pilanto, 2006; Korhonen, 2009). Moreover, hydrolysis of purified casein and whey proteins from cow milk with gastric and pancreatic enzymes results in the release of bioactive peptides. Bioactive peptides are inactive within the sequence of their parent protein and can be released by enzymatic hydrolysis during gastrointestinal digestion. Following digestion, bioactive peptides can either be absorbed through the intestine to enter the blood circulation intact and exert systemic effects, or produce local effects in the gastrointestinal tract. There is now a substantial body of evidence to indicate that cow milk contains a number of very potent immunomodulatory peptides (Kayser and Meisel, 1996; Gill, 2000; Prioult *et al*., 2004; Phelan *et al*., 2009). Pepsin, trypsin and chymotrypsin have been shown to release a number of immunomodulatory peptides from both different casein (α-, β-, and κ- casein) and whey proteins, e.g. α-lactalbumin, β-lactoglobulin and glycomacropeptide (Gauthier *et al*., 2003; Gobbetti *et al*., 2006; Phelan *et al*., 2009).

The proteins in milk from different species vary in concentration and amino acid composition. The protein content in milk from bovine species is approximately 3.3 %, whereas reindeer milk contains more protein, approximately 7.8% (Park and Haenlein, 2006). Casein is the main protein component of bovine milk constituting about 80% of the total milk proteins (Shah, 2000). The casein fraction consists of αs1-, αs2-, β- and κ-casein. The main proteins in the whey fraction are β-lactoglobulin (β-lg), α-lactalbumin (α-la), serum albumin (SA), immunoglobulins (IGs), lactoferrin (LF) and lysozyme (LZ) (Inglingstad *et*

*Corresponding author.
Email: *nelum.opathavithana@lincolnuni.ac.nz*

al., 2010). The amino acid composition of casein and whey fractions varies in different species and will affect their digestibility (Almaas *et al*., 2006).

Protein digestion starts in the stomach by the action of acid and pepsin and is followed by intestinal digestion by trypsin, chymotrypsin and various carboxypeptidases and aminopeptidases. Milk protein represents an important dietary source for human, providing that they are digested suitably.The high digestibility of milk protein (~ 95%), combined with a superior amino acid composition for human requirements, makes milk a "high quality protein" source (Bos *et al*., 1999). *In vivo* results of Dangin *et al*. (2002, 2003) led to the concept of "slow" digested caseins and "fast" digested whey protein. *In vivo* studies with either milk or purified milk proteins in healthy human showed that whey proteins were taken up more rapidly in the upper jejunum than the casein (Mahe *et al*., 1995, 1996). However, studies by Almaas *et al*. (2006) using human gastric and duodenal juices for the *in vitro* digestion of cow and caprine milk proteins revealed that the whey proteins, α-lactalbumin and β-lactoglobulin were very resistant to hydrolysis and that the caseins were degraded faster.

This study investigates the *in vitro* digestion of deer and cow milk by commercial gastric and duodenal enzymes with the major focus being on comparison of the degradation of protein to evaluate the differences in digestibility and peptide production. No information on the digestion of deer milk, which is unique from other species in its composition, is available and only basic composition of red deer milk is reported. This information could be potentially useful in understanding the functional roles in animal nutrition and evaluating its potential for human use.

Materials and Methods

Materials

Oxytocin was purchased from Pharm Distributors (Auckland, New Zealand). Pepsin from porcine stomach mucosa, OPA (O-phthaldialdehyde), Leucine and TCA (trichloroacetic acid) were purchased from Sigma (St. Louis, MO, USA) and Corolase PP (CPP) from pig pancreas was from AB Enzymes (Darmstadt, Germany). Colrolase PP is a mixture of trypsin, chymotrypsin and several amino and carboxypeptidases. NuPAGE 4-12% Bis-Tris precast gels were purchased from Invitrogen USA. Bio-Rad Precision Plus Protein TM standard was used as molecular weight marker.

Preparation of milk samples

Pooled deer milk was obtained from Lincoln University deer farm at Lincoln (New Zealand). Calves were weaned from twenty hinds which were then milked twice-daily. Immediately prior to milking, each hind received an injection of 1 ml oxytocin (10 IU/ml) to enable the 'let down' of milk. The hinds were milked, one at a time, in a side-loading crush, using a commercial machine designed for milking sheep and goats. Approval for this work was given by Lincoln University Animal Ethics Committee (# 377). Pooled cow milk was obtained from Lincoln University Dairy Farm. Fat was removed by centrifugation at 4000 ×g for 30 min at 4°C. Defatted milk was freeze dried and stored at room temperature in airtight containers until analysis.

Protein content

Total nitrogen (TN), non-protein N (NPN) and non-casein nitrogen (NCN) were measured using the Kjeldahl method (Barbano *et al*., 1991). NPN was estimated after precipitation of protein with 24% trichloracetic acid followed by centrifugation at 27,000 xg for 60 min. The supernatant was filtered using Whatman No 1 filter paper and the filtrate was used for Kjeldahl analysis. NCN was measured after precipitation of casein by adjusting the milk to pH 4.6 with slow addition of 1M HCL while stirring and centrifugation at 10,000 ×g for 15 min at 200C. The supernatant was filtered and protein content was determined using the Kjeldahl method. Total protein [(TN-NPN) x 6.38], Casein protein [(TN-NCN) x 6.38] and whey protein [(NCN-NPN) x 6.38] concentrations were calculated.

Dry matter content

Weights of deer and cow milk were taken before and after freeze drying of milk. Dried weight was calculated as percentage of total milk weight.

In vitro digestion

Defatted deer and cow milk samples (20 ml) were prepared in triplicate by rehydrating freeze dried defatted milk in 26% and 12% rehydration ratio respectively. *In vitro* protein digestion was performed using pepsin and CPP according to Eriksen *et al*. (2008). The procedure mimics "normal digestion" in the human gastro-intestinal tract. The first incubation which mimics digestion in the stomach was, adjusted to pH 2.5 with 1M HCL, and used pepsin (~4mg/g milk protein) at 37°C for 30 min. The second incubation used CPP (~4 mg/g milk protein) at 37°C for 60 min after the pH was adjusted to 7.5 with 1M NaOH. Continuous shaking (150 rpm) was maintained during digestion. The digestion of milk protein was monitored by measuring pH, the production of N terminals by O-phthaldialdehyde (OPA) assay and Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Small samples were drawn for OPA assay

(250 µl) and SDS- polyacrylamide gel electrophoresis (1 ml) every 10 min of digestion.

Proteolysis assesment using OPA assay

The OPA assay followed the method of Church *et al*. (1983). The OPA reagent contained 2.5 ml of 20% (w/v) SDS, 25 ml of 100 mM sodium tetraborate, 40 mg OPA (previously dissolved in 1 ml methanol), 100 µl of 2-mercaptoethanol and distilled water up to 50 ml. Samples were incubated with 0.75 M TCA at a sample: TCA ratio of 1:3 (250 µl: 750 µl) at 4°C for 30 min and then centrifuged (4000 ×g for 10 min) to eliminate any interference of the undigested protein fractions as suggested by Church *et al*. (1983). A 10 µl aliquot of the supernatant was diluted by adding 140 µl H_2O, 1 ml of OPA reagent was added and then the tube was incubated at room temperature for 2 min. Absorbance at 340 nm was measured using UNICAM 8625 UV/VIS spectrophotometer. Leucine (Leu) was used for the construction of a standard curve and the proteolytic activity was expressed as mM Leu equivalent.

SDS -polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis was used to visualise the protein profile of the samples taken at different hydrolysis times during digestion. SDS-PAGE was performed according to a standard protocol (Laemmli, 1970) using NuPAGE 4-12% Bis-Tris gels. Bio-Rad Precision Plus Protein TM standard was used as a molecular weight marker. Samples were diluted to 2 mg /ml protein with 6x sample buffer (30 µl) containing 0.35 M Tris-HCL, 10.28% SDS, 30% glycerol 0.012% Bromophenol blue and 5% Mercaptoethanol. The volume was adjusted to 180 µl with water and the sample was heated at 95°C for 4min. Samples (15 µl) and 10 µl of molecular marker were loaded in to the wells. Electrophoresis was performed at a constant voltage of 200v for 40 min. Gels were fixed in 50% Methanol and 7% acetic acid for 15 min with rocking and then washed 3 times for 15 min with water. Gels were stained with Coomassie Brilliant Blue (Gel Code Blue) and de stained with continuous shaking in water overnight.

Quantification of protein bands

The protein bands in the gels were quantified (Inglingstad *et al*., 2010) by ImageJ software (version 1.42). Gels were scanned using Coral Photo Paint 12. Image rectangle was applied for background subtraction, and rolling ball radius was fixed to 30 pixels. Bands of interest were marked in each lane to be able to investigate the degradation pattern of the different proteins. In order to measure degradation, the amount of intact protein remaining in the digested samples was calculated as a percentage of its undigested counterpart. Three gels from each sample were quantified, and the results are given as a mean value.

Statistical analysis

All experiments were carried out in triplicate and values are mean ± standard deviation. The data were subjected to one way analysis of variance (ANOVA), followed by Tukey's test to determine the significant differences between samples at $p< 0.05$ level using Minitab statistical software(16 version, Minitab Inc., USA).

Results and Discussion

Milk composition

The dry matter content and protein composition of deer and cow milk are shown in Table 1. Dry matter content of deer milk is 25.7% while cow milk has 12.1% dry matter. Hence deer milk has more than twice the dry matter of cow milk. Similarly total protein content in deer milk is 8.8% which is more than twice that of cow milk (4.1%). Total protein is the combination of casein and whey proteins. Deer milk has 8.7% casein while cow milk has only 4.0% casein. Both deer and cow milk have very low amounts of whey proteins which are 0.64% and 0.57% respectively. Therefore both milks have a high casein to whey protein ratio.

The milk from ruminants is characterized by high casein:whey protein ratio when compared with other groups of mammals (McDougall and Stewart, 1975; Vincenzetti *et al*, 2008). Among ruminants, most domesticated species are bovids and their milk is readly available and economicaly important. Hence our knowledge of the milk proteins of ruminants is mainly confined to bovids, but it would be useful to investigate the milk of other ruminant families. The cervids are the largest of these families and appear to have evolved earlier than the bovids (Young, 1962). Consistent with data of Arman *et al*. (1974) our results showed that deer milk casein and whey contents (Table 1) had a similar ratio to that found in other ruminants.

In vitro digestion

In human beings pepsin digestion and acid hydrolysis at pH of 1.5 – 2.5 are the first steps in the gastrointestinal degradation of protein, followed by stomach emptying and further digestion in the duodenum by pancreatic enzymes at pH = 7. To

Table 1. Dry matter and protein content of red deer and cow milk

Milk Type	Dry matter (%)	Total Protein (%)	Casein (%)	Whey protein (%)
Deer Milk	25.7 ± 0.76b	8.8 ± 0.13b	8.7 ± 0.13b	0.64 ± 0.002
Cow Milk	12.1 ± 0.01a	4.1 ± 0.02a	4.0 ± 0.02a	0.57 ± 0.004

Values are mean ± standard deviation (n= 3).
Means within the same column that have different letters (a-b) are statistically different (P < 0.05) as determined by Tukey's test.

mimic *in vivo* digestion the present work has used *in vitro* digestion of defatted deer and cow milk by commercial pepsin and duodenum enzymes trypsin and chymotrypsin was use. Pepsin is an aspartic protease which prefer to cleaves peptides at bonds with Phe, Tyr, Trp and Leu in position P1 or P1' (Fujimoto *et al*., 2004). Trypsin cleaves peptide bonds following a positively charged amino acid and chymotrypsin cleaves peptides bonds following bulky hydrophobic amino acid residues (Antal *et al*., 2001). Therefore digestion pattern with these enzymes will vary depending on the protein stucture of cow and deer milks and this could lead to differences in digestibility and digestive products with different bioactivity.

The pH affects the activity of enzymes and during in vivo digestion. The pH of stomach increases rapidly from 2 (pH of gastric juice) to pH of the diet immediately following the consumption of meal and then decrease progressively toward its initial value (Savalle *et al*., 1989). At the begining of digestion the pH was adjusted to 2.5 with 1 M HCL for the pepsin digestion. The pH increase during digestion was greater (Figure 1) in cow milk than deer milk which could indicate a higher buffering effect of deer milk. Milk acts as a complex buffer because it contains carbon dioxide, protein, phosphate, citrate and a number of minor constituents (Ismail *et al*., 1973). Goat milk has a higher buffering capacity due to its higher content of major buffering components including minerals (Park, 1992). Deer milk had 1.1 ± 0.05 % ash while cow milk had only 0.70 ± 0.04 % (n = 6). This finding was in accordence with mineral composition analysis reported by Arman *et al*. (1974) which showed a 1.11 % ash content in deer milk (average of 6 hinds in mid lactation). Our results showed (data not given) that deer milk had higher buffering effect than cow milk. This could be due to high protein and mineral content and this high buffering quality of deer milk could enhance its value for sufferers of peptic ulcers and other such gastric ailment. At 40 min (10 min into the second stage of digestion) deer milk shows only 0.8 unit pH drop where as the pH of cow milk decreased by 1.3 (Figure 1). Again the buffering effect of deer milk could contribute to the observed differences.

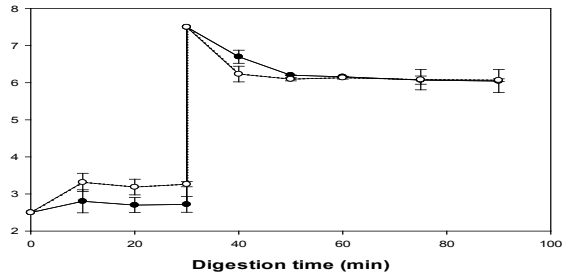

Figure 1. pH during in vitro digestion of deer (●) and cow (○) milk. 0 to 30 min digestion is simulated stomach. At 30 min pH was adjusted to7.5. 30 to 90 min digestion is simulated duodenum. Data are mean ± S.D. (n = 3).

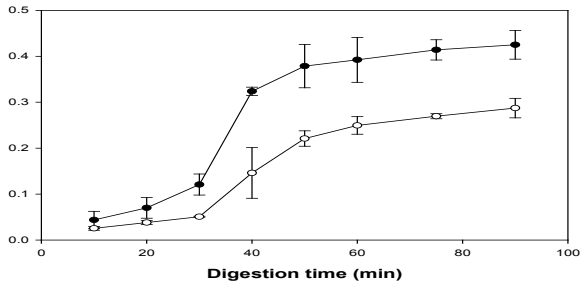

Figure 2. Peptide production during In vitro digestion of deer (●) and cow (○) milk (up to 30 min: simulated stomach (stage 1) and then simulated duodenum (stage 2)). Peptide production was measured using OPA assay after TCA precipitation. Data are mean ± S.D. (n = 3).

In deer milk, pH change during the start of second stage of digestion was higher than that for the first stage of digestion. It could be a result of the higher digestibility of deer protein in simulated duodenum by trypsin and chymotrypsin than pepsin. Cow caseins were more susceptible to hydrolysis by trypsin than camel caseins, whereas camel caseins were more prone to hydrolysis by chymotrypsin than cow casein (Salami *et al*., 2008). To examine the differences in digestability of deer and cow protein, peptide production and protein profile were evaluated using OPA assay and SDS – PAGE using quantification by imageJ software.

Proteolysis assessment using OPA assay

In this study, the hydrolysis of milk protein was measured using a rapid, sensitive and simple OPA-based spectrophotometric assay (Pescuma *et al*., 2008; Salami *et al*., 2008; Salami *et al*., 2009). The peptide production from the *in vitro* digestion of milk proteins is shown in Figure 2. Deer milk produced more hydrolysed products (peptides and amino acids) than cow milk which is consistent with deer milk containing twice the protein available in cow milk. However, peptide production was not two times greater than cow milk. This could be due to differences in protein structures and hence enzyme target sites

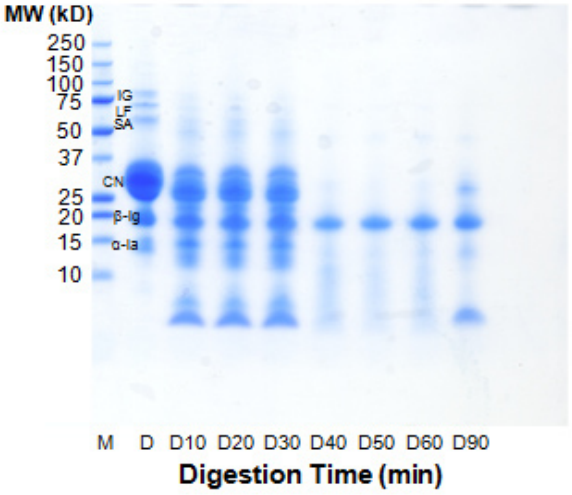

Figure 3. SDS- PAGE (4-12%) of defatted Deer Milk & Deer Milk digested with pepsin and CPP. Major bands; immunoglobulins (IG), lactoferrin (LF), serum albumin (SA), casein (CN), β-lactoglobulin (β-lg) and α-lactalbumin (α-la). Standard molecular weight markers are shown on left-hand side of the gel. The wells contain: Molecular marker(M), Deer milk(D), Digest after 10min (D10), 20min (D20), 30min (D30) in simulated stomach and digest after 40min (D40), 50min(D50), 60min(D60), 90min(D90) (simulated duodenum).

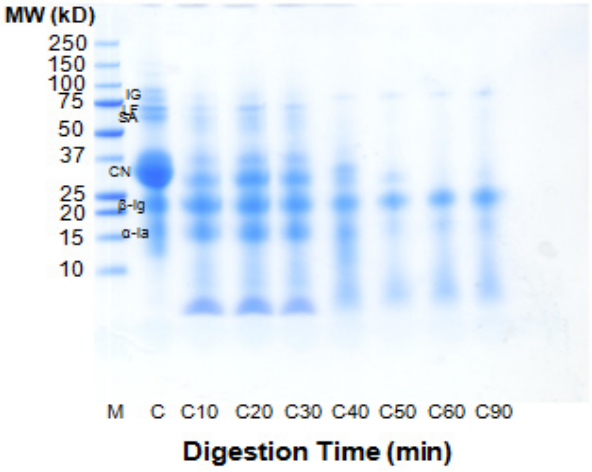

Figure 4. SDS- PAGE (4-12%) of skimmed CowMilk & CowMilk digested with pepsin and CPP. The wells contain; immunoglobulins (IG), lactoferrin (LF), serum albumin (SA), casein (CN), β-lactoglobulin (β-lg) and α-lactalbumin (α-la). Standard molecular weight markers are shown on left-hand side of the gel. The wells contain: Molecular marker(M), Cow milk(C), Digest after10min (C10), 20min (C20), 30min (C30) in simulated stomach and digest after 40min (C40), 50min(C50), 60min(C60), 90min(C90) (simulated duodenum)

in deer and cow milk that may leads to formation of different peptides with different lengths. Kopf-Bolanz *et al*. (2012) demonstrated the formation of different number of peptides with different length during in vitro digestion of cow milk. Therefore the number of N terminals produced by deer milk digestion might not be twice that of cow milk digestion.

There was a gradual increase of peptide production during digestion with pepsin in the simulated stomach for both milks and then a rapid increase in the simulated duodenum. This was faster

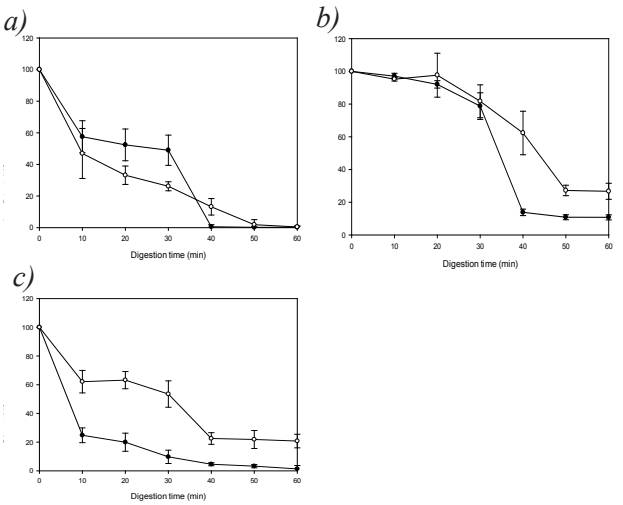

Figure 5. *In vitro* digestion of casein (a), α-lactalbumin (b), immunoglobulin (c) in raw milk from deer (●) and cow (○) by pepsin at pH 2.5 / 37 °C (1 step of digestion up to 30 min) and CPP at pH 7.5 / 37 °C (step 2 until 60 min). Values were obtained using ImageJ of SDS-PAGE. Data are mean ± S.D. (n = 3)

in deer milk than cow milk. Peptide production of simulated duodenum was significantly ($P<0.05$) higher than simulated stomach for both milk. The analysis of hydrolysis of deer and cow milk (Figure 2) revealed greater production ($P<0.05$) of peptides from deer milk than cow milk in simulated duodenum. Therefore hydrolysis activity was higher in simulated duodenum in deer milk compare to cow milk. After 60 min of digestion, peptide production had plateaued. No new peptides were produced by extending the reaction time. Therefore, for gel band quantification only 30 min was used for simulated duodenum digestion (stage 2).

SDS – polyacrylamide gel electrophoresis (SDS – PAGE)

The protein patterns on SDS – PAGE (Figures 3 and 4), illustrated the protein profile of red deer (lane D of Figure 3) and cow (lane C of Figure 4) milk and these milks during in vitro digestion at 10 min intervals. The intensity of casein bands reduced from 0 min to 30 min in digests of both milks. Deer casein was totally digested after 40 min of digestion (10 min after second stage of digestion) whereas traces of casein (14%) were still present in cow milk (Figures 3, 4 and 5 a). The casein band in cow milk was completely digested after 50 min (Figures 4 and 5a). This result confirms that deer casein was digested more rapidly than cow casein. The gel results supported the suggested higher hydrolysis activity of deer milk than cow milk in simulated duodenum which resulted from OPA results. About half (49%) of intact major milk protein (casein) started to degrade at the beginning of simulated duodenum digestion in deer milk while only about quarter (27%) of intact casein

Table 2. Protein content (%) remaining in raw milk before digestion (start), after stage 1 digested with pepsin at pH 2.5 for 30 min / 37 °C and after stage 2 digested with CPP at pH 7.5 for 30 min / 37 °C. Values are obtained from SDS-PAGE using Image J (n=3)

	Deer			Cow		
	Start	Stage 1	Stage 2	Start	Stage 1	Stage 2
IG	100	10 ± 5*	1 ± 2*	100	53 ± 9*	21 ± 5*
LF	100	25 ± 2	0 ± 1	100	32 ± 4	1 ± 1
SA	100	11 ± 6*	0 ± 0	100	25 ± 3*	1 ± 1
CN	100	49 ± 9*	0 ± 0	100	27 ± 3*	1 ± 1
β-lg	100	94 ± 3	54 ± 5	100	91 ± 4	55 ± 8
α-la	100	79 ± 8	11 ± 2*	100	82 ± 10	27 ± 5*

S1 (stage 1) = simulated stomach
S2 (stage 2) = simulated duodenum
IG: immunoglobulins
LF: lactoferrin
SA: serum albumin
CN: casein
β-lg: β-lactoglobulin
α-la: α-lactalbumin
* statistically different between deer & cow ($P < 0.05$) as determined by Tukey's test

was digested during simulated duodenum in cow milk under the same conditions of time, temperature and pH (Figures 3 and 4, Figure 5 a). The major protein components of milk, the αs1- and β- caseins, contain covalently attached phosphate groups bound to residues of serine and threonine (Medina *et al*., 1992). The bound phosphate groups influence many functional properties of these proteins, including their digestibility, bioavailability of divalent cations and immunogenicity (Tezcucano Molina *et al*., 2007).

The specific protein pattern and in particular the physico-chemical and biological properties of milk proteins may influence their digestibility (Salami *et al*., 2008). There is limited information on red deer milk proteins. McDougall (1976) reported amino acid analysis of deer milk β-lactoglobulin, adjusted to lysine = 15 residues (the number of residues is given relative to 15 residues of lysine) showed that it contains one more residue of aspartic acid, alanine and methionine and one less glutamic acid residue and two less leucine residue than cow β-lactoglobulin. From this McDougall (1976) concluded there was only a small difference in amino acid composition of β-lactoglobulin in deer milk compared to cow milk and demonstrated the similarity by gel chromatography and electrophoretic methods. This similarity of β-lactoglobulin in the two species could lead to similarities in digestibility of this protein in milk. β-lactoglobulin is considered the dominant cow milk allergen and its rigid spatial conformation exhibits high resistance to gastric digestion, which in part explain its allergenicity (Prioult *et al*., 2005).

Our results confirmed that the digestibility of β-lactoglobulin is low in milk from both species. After *in vitro* digestion 54% and 55% of intact β-lactoglobulin was still intact in deer and cow milk respectively. With respect to digestion of whey proteins, Inglingstad *et al*. (2010) reported that β-lactoglobulin and α-lactalbumin of cow and goat were very resistant to human gastric and duodenal enzyme digestion, while horse milk showed rapid duodenal degradation of β-lactoglobulin. Cow milk α-lactalbumin possesses 26 potential chymotrypsin-specific target sites and 13 trypsin-specific target sites in its primary structure (Salami *et al*., 2008). The digestibility of α-lactalbumin was significantly ($P<0.05$) higher in deer milk than cow milk after total digestion (Table 2). At 40 min of digestion there was only 14% α-lactalbumin remaining undigested in deer milk, where as 62% remained in cow milk (Figure 5b). Present results are in accordance with those of Pintado and Malcata (2000), who also found that cow milk α-lactalbumin was resistant to hydrolysis by trypsin. β-lactoglobulin and α-lactalbumin are the major milk allergen whey proteins (Wal, 1998; El-Ghaish *et al*., 2011). The slightly less β-lactoglobulin and significantly ($P<0.05$) less α-lactalbumin in deer digest than cow digest (Table 2) may suggest less allergenic effect of deer milk than cow milk.

The other whey proteins such as lactoferrin and serum albumin were highly degraded by the gastrointestinal enzymes. In this study, lactoferrin and serum albumin bands disapeared after digestion of both milk samples (Figures 3 and 4). But for serum albumin this was significanly faster in deer milk than cow milk, with only 11% intact serum albumin remaining after simulated stomach in deer while cow milk had 25% (Table 2). Our results showed that deer immunoglobulin is more susceptible to digestion by commercial enzymes than cow milk. Deer immunoglobulin was almost completely digested (1% intact) after simulated stomach and duodenal digestion while 21% remained in cow milk immunoglobulins (Figure 5c). Many *in vitro* studies have shown that cow immunoglobulins is resistant to proteolysis by digestive enzymes and are not inactivated by gastric acid (Korhonen *et al*., 2000; Hurley and Theil, 2011).

The results reveal that less intact protein is present after simulated stomach and duodenal digestion of deer milk than cow milk (Table 2). The rate of hydrolysis was different between proteins and between the two species as shown in Figures 3-5. Commercial proteolytic enzymes degraded milk protein from deer more rapidly than those from cow. This suggests deer milk may also be more digestible *in vivo* than cow milk.

Conclusion

In vitro digestion using commercial proteolytic enzymes has provided new knowledge of deer milk protein digestion. Commercial proteolytic enzymes degraded milk protein from deer milk more rapidly than those from cow. Most noticeable was the

difference observed in casein, α-lactalbumin and immunoglobulin. Deer and cow milk β-lactoglobulin were resistant to both gastric digestion (simulated stomach) and simulated duodenal digestion. This study provides better knowledge about the digestion of deer milk, compared with cow milk and may reveal important issues with regard to the proteins in nutrition. The results obtained may be relevant for development of easily digestable products for consumer groups with special needs, such as infants, athletes and the elderly. However, as this is an *in vitro* model system, clinical studies will be needed in order to confirm results. Deer milk produced more peptides than cow milk after *in vitro* digestion using commercial enzymes. This may mean that deer milk will have more bioactive peptides. The bioactivity of the peptides produced is currently under investigation.

Acknowledgments

We acknowledge the support and cooperation of Martin Ridgway and Martin Wellby, Lincoln University deer farm and financial support from Lincoln University Research Funding.

References

Almaas, H., Cases, A.-L., Devold, T. G., Holm, H., Langsrud, T., Aabakken, L., Aadnoey, T. and Vegarud, G. E. 2006. *In vitro* digestion of bovine and caprine milk by human gastric and duodenal enzymes. International Dairy Journal 16(9): 961-968.

Antal, J., Pál, G., Asbóth, B., Buzás, Z., Patthy, A. and Gráf, L. 2001. Specificity assay of serine proteinases by Reverse-Phase High-Performance Liquid Chromatography analysis of competing oligopeptide substrate library. Analytical Biochemistry 288(2): 156-167.

Arman, P., Kay, R. N. B., Goodall, E. D. and Sharman, G. A. M. 1974. The composition and yield of milk from captive red deer (*Cervus elaphus* l.). Journal of Reproduction and Fertility 37(1): 67-84.

Barbano, D. M., Lynch, J. M. and Fleming, J. R. 1991. Direct and indirect determination of true protein content of milk by Kjeldahl analysis. Journal of the Association of Official Analytical Chemists (AOAC) 74: 281–288

Bos, C., Mahé, S., Gaudichon, C., Benamouzig, R., Gausserès, N. and Luengo, C. 1999. Assessment of net postprandial protein utilization of N-labelled milk nitrogen in human subjects. British Journal of Nutrition 81(03): 221-226.

Church, F. C., Swaisgood, H. E., Porter, D. H., and Catignani, G. L. 1983. Spectrophotometric assay using o-Phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins. Journal of Dairy Science, 66(6), 1219-1227.

Dangin, M., Boirie, Y., Guillet, C. and Beaufrere, B. 2002. Influence of the protein digestion rate on protein turnover in young and elderly subjects. Journal of Nutrition 132(10): 3228-3233.

Dangin, M., Guillet, C., Garcia-Rodenas, C., Gachon, P., Bouteloup-Demange, C., Reiffers-Magnani, K., Fauquant, J., Ballevre, O. and Beaufrere, B. 2003. The rate of protein digestion affects protein gain differently during aging in humans. The Journal of Physiology 549(2): 635-644.

El-Ghaish, S., Ahmadova, A., Hadji-Sfaxi, I., El Mecherfi, K. E., Bazukyan, I. and Choiset, Y. 2011. Potential use of lactic acid bacteria for reduction of allergenicity and for longer conservation of fermented foods. Trends in Food Science andTechnology 22(9): 509-516.

Eriksen, E. K., Vegarud, G. E., Langsrud, T., Almaas, H. and Lea, T. 2008. Effect of milk proteins and their hydrolysates on *in vitro* immune responses. Small Ruminant Research 79(1): 29-37.

Fujimoto, Z., Fujii, Y., Kaneko, S., Kobayashi, H. and Mizuno, H. 2004. Crystal structure of aspartic proteinase from irpex lacteus in complex with inhibitor pepstatin. Journal of Molecular Biology 341(5): 1227-1235.

Gauthier, S. F. and Pouliot, Y. 2003. Functional and biological properties of peptides obtained by enzymatic hydrolysis of whey proteins. Journal of Dairy Science 86(13): E78-87.

Gill, H. S., Doull, F., Rutherfurd, K. J. and Cross, M. L. 2000. Immunoregulatory peptides in bovine milk. British Journal of Nutrition 84(Supplement S1): 111-117

Gobbetti, M., Minervini, F. and Rizzello, C. G. 2006. Bioactive peptides in dairy products. In Hui Y. H. (Eds). Handbook of Food Products Manufacturing, p. 489-517. Hoboken, NJ, John Wiley and Sons, Inc.

Hurley, W. L. and Theil, P. K. 2011. Perspectives on immunoglobulins in colostrum and milk. Nutrients 3(4): 442-474.

Inglingstad, R. A. E., Devold, T. G., Eriksen, E. K., Holm, H., Jacobsen, M., Liland, K. H., Rukke, E. O. and Vegarud, G. E. 2010. Comparison of the digestion of caseins and whey proteins in equine, bovine, caprine and human milks by human gastrointestinal enzymes. Dairy Science and Technology 90(5): 549-563.

Ismail, A. A., El Deeb, S. A. and El Difrawi, E. A. 1973. The buffering properties of cow and buffalo milks. Zeitschrift für Lebensmitteluntersuchung und -Forschung A 152(1): 25-31.

Kopf-Bolanz, K. A., Schwander, F., Gijs, M., Vergeres, G. and Portmann, R. 2012. Validation of an *in vitro* digestive system for studying macronutrient decomposition in humans. The Journal of Nutrition 142 (2): 245-250.

Kayser, H. and Meisel, H. 1996. Stimulation of human peripheral blood lymphocytes by bioactive peptides derived from bovine milk proteins. FEBS Letters 383(1-2): 18-20.

Korhonen, H. 2009. Milk-derived bioactive peptides:

From science to applications. Journal of Functional Foods 1(2): 177-187.

Korhonen, H., Marnila, P. and Gill, H. S. 2000. Milk immunoglobulins and complement factors. British Journal of Nutrition 84(S1): 75-80.

Korhonen, H. and Pihlanto, A. 2006. Bioactive peptides: Production and functionality. International Dairy Journal 16(9): 945-960.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature 227(5259): 680-685.

Mahé, S., Benamouzig, R., Gaudichon, C., Huneau, J. F., De Cruz, I., Rautureau, J. and Tome, D. 1995. Nitrogen movements in the upper jejunum lumen in human fed low amounts of casein or lactoglobulin. Gastroentérologie clinique et biologique 19(1): 20-26.

Mahe, S., Roos, N., Benamouzig, R., Davin, L., Luengo, C. and Gagnon, L. 1996. Gastrojejunal kinetics and the digestion of [15N] beta-lactoglobulin and casein in humans: the influence of the nature and quantity of the protein. The American Journal of Clinical Nutrition 63(4): 546-552.

McDougall, E. I. and Stewart, J. C. 1975. The whey proteins of the milk of red deer. Biochemistry Journal 153(1): 647-655.

Medina, A. L., Colas, B., Le Meste, M., Renaudet, I. and Lorient, D. 1992. Physicochemical and dynamic properties of caseins modified by chemical phosphorylation. Journal of Food Science 57(3): 617-621.

Park, Y. W. 1992. Comparison of buffering components in goat and cow milk. Small Ruminant Research 8(1-2): 75-81.

Park, Y. W. and Haenlein, G. F. W. (Eds.). 2006. Handbook of milk of non-bovine mammals. Iowa: Blackwell Publishing.

Pescuma, M., Hébert, E. M., Mozzi, F. and Font de Valdez, G. 2008. Whey fermentation by thermophilic lactic acid bacteria: evolution of carbohydrates and protein content. Food Microbiology 25(3): 442-451.

Phelan, M., Aherne-Bruce, S. A., O'Sullivan, D., FitzGerald, R. J. and O'Brien, N. M. 2009. Potential bioactive effects of casein hydrolysates on human cultured cells. International Dairy Journal 19(5): 279-285.

Pintado, M. E. and Malcata, F. X. 2000. Hydrolysis of ovine, caprine and bovine whey proteins by trypsin and pepsin. Bioprocess and Biosystems Engineering 23(3): 275-282.

Prioult, G., Pecquet, S. and Fliss, I. 2004. Stimulation of interleukin-10 production by acidic {beta}-lactoglobulin-derived peptides hydrolyzed with *Lactobacillus paracasei* NCC2461 peptidases. Clinical and Diagnostic Laboratory Immunology 11(2): 266-271.

Prioult, G., Pecquet, S. and Fliss, I. 2005. Allergenicity of acidic peptides from bovine [beta]-lactoglobulin is reduced by hydrolysis with *Bifidobacterium lactis* NCC362 enzymes. International Dairy Journal 15(5): 439-448.

Salami, M., Yousefi, R., Ehsani, M. R., Dalgalarrondo, M., Chobert, J.-M., Haertlé, T., Seyed Hadi, R. S., Ali Akbar, S., Amir, N. and Ali Akbar, M. 2008. Kinetic characterization of hydrolysis of camel and bovine milk proteins by pancreatic enzymes. International Dairy Journal 18(12): 1097-1102.

Salami, M., Yousefi, R., Ehsani, M. R., Razavi, S. H., Chobert, J.-M., Haertlé, T., Ali Akbar, S., Sadat, A. M., Amir, N., Faizan, A. and Ali Akbar, M. 2009. Enzymatic digestion and antioxidant activity of the native and molten globule states of camel [alpha]-lactalbumin: Possible significance for use in infant formula. International Dairy Journal 19(9): 518-523.

Savalle, B., Miranda, G. and Pelissier, J. P. 1989. In vitro simulation of gastric digestion of milk proteins. Journal of Agricultural and Food Chemistry 37(5): 1336-1340.

Shah, N. P. 2000. Effects of milk-derived bioactives: an overview. British Journal of Nutrition 84(Supplement S1): 3-10.

Tezcucano Molina, A. C., Alli, I., Konishi, Y. and Kermasha, S. 2007. Effect of dephosphorylation on bovine casein. Food Chemistry 101(3): 1263-1271.

Vincenzetti, S., Polidori, P., Mariani, P., Cammertoni, N., Fantuz, F. and Vita, A. 2008. Donkey's milk protein fractions characterization. Food Chemistry 106: 640-649.

Wal, J. M. 1998. Immunochemical and molecular characterization of milk allergens. Allergy 53: 114-117.