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Fractionation of whey proteins from red deer (*Cervus elaphus*) milk and comparison with whey proteins from cow, sheep and goat milks

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ABSTRACT

In the present study, we report the first protein fractionation analysis of whey proteins from red deer (*Cervus elaphus*) milk. The aim of this study was to investigate the protein composition of red deer milk and examine the protein profile compared with that of cow, sheep and goat using 1D-PAGE and protein RP-HPLC of defatted milk. Ammonium sulfate fractionated sweet whey proteins of the four species were further subjected to anion exchange chromatography, and 1D- and large format 2D-PAGE analysis. Deer milk caseins displayed different mobility on 1D-PAGE, and chromatographed differently on RP-HPLC to those of other species. The apparent ratio of alpha-lactalbumin to beta-lactoglobulin in red deer milk was different compared to other species. As cheese wheys from other ruminants have been shown to contain proteins and peptides with health promoting benefits, it is of interest to characterize proteins in red deer whey, a by-product of an emerging novel deer cheese manufacture venture. Red deer sweet whey, as well as other species, could be fractionated to obtain a fraction partially depleted of alpha-lactalbumin and beta-lactoglobulin, which facilitated display of lower abundance proteins by 1D- and 2D-PAGE. Furthermore, a difference in casein coagulation during sweet whey production from milks of red deer and other species was observed.

1. Introduction

Although milk production in the dairy industry in many countries is dominated by cow milk, other farmed animals, such as sheep and goats are increasingly used for milk production with products having interesting properties (Casper et al., 1999), including hypoallergenicity compared to cow (El-Agamy, 2007). Milk production from these small ruminants is well established and most of this milk is processed into specialist cheese or low allergenicity milk products. Red deer and also elk are farmed for meat production in New Zealand and more recently dairy herds of red deer and elk have been established, creating potential for developing a novel dairy industry, and trial productions of cheese from these milk sources have been reported (Ashton, 2013). Cheese production results in the generation of significant

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quantities of whey as a by-product, which is often under-utilized in the dairy industry. Whey from non-bovine milk is usually disposed by land spreading (Casper et al., 1999).

Whey proteins have been recognized to have significant nutritional and health-promoting value and have been utilized in various products (see review by Hernandez-Ledesma et al., 2011). Whey protein hydrolysates are increasingly being recognized as an important source of bioactive peptides (Pihlanto-Leppala, 2000). A recent investigation of the *in vitro* production of peptide hydrolysates from red deer whole milk in comparison with that of cow milk demonstrated that red deer milk was more digestible and produced more peptides (Opatha-Vithana et al., 2012). Subsequently it has been shown that red deer milk hydrolysates generated peptides with significant immunomodulatory bioactive properties (Opatha-Vithana, 2012).

To our knowledge there has been only one previous report on red deer, which was limited by the technology available at the time and focused mainly on the most abundant whey protein beta-lactoglobulin (β-Lg) (McDougall and Stewart, 1976). While milk protein composition is reported to vary for several different species and differences in physical characteristics of the proteins are reported to be due to differences in amino acid sequence (Martin et al., 2003), literature on red deer whey proteins is very limited. Although a substantial literature on fractionation of milk proteins from mainly cow but also other species is available, many of the methods are more suitable for laboratory analytical purposes and are not necessarily readily scalable for industry. The analysis of milk proteins by FPLC using ion exchange chromatography on Mono Q and Mono S columns and using urea in the buffer (Andrews et al., 1985), or the use of RP-HPLC using trifluoroacetic acid/acetonitrile buffers (Bordin et al., 2001) are examples of this.

To compare red deer milk proteins with those of other ruminants we initially performed a fractionation of defatted whole milk proteins from red deer, cow, sheep and goat by RP-HPLC and 1D-SDS PAGE. In order to gain further insight into the characteristics of less abundant milk proteins, proteins with higher abundance such as caseins, β-Lg and alpha-lactalbumin (α-La) were depleted. Red deer, cow, sheep and goat sweet whey was subjected to ammonium sulfate fractionation, followed by anion exchange chromatography, which are industry scalable procedures, and the sub-fractionated whey proteins were analyzed by 1D- and 2D-PAGE. Ammonium sulfate fractionation of whey proteins has been reported to be useful for enrichment of the relatively highly abundant whey protein components (Brodbeck et al., 1967) and was used in this report to achieve depletion of these two proteins from the whey protein fraction to improve comparative visualization of other less abundant whey proteins. Anion exchange chromatography is reported to be generally useful to achieve fractionation of whey proteins and we elected to use a HiTrap-Q FF cartridge, as a relatively cost-effective and scalable ion exchange procedure, to further demonstrate differences of deer whey proteins compared to other species.

2. Materials and methods

2.1. Materials

All chemicals were obtained from Sigma Aldrich New Zealand Ltd., Auckland, New Zealand, unless otherwise stated. 1D-PAGE electrophoresis materials were from Life Technologies, Auckland, New Zealand. 2D-PAGE electrophoresis materials and ion exchange chromatography materials were from GE Healthcare, Auckland, New Zealand. Bovine milk protein standards were purchased from Sigma Aldrich New Zealand Ltd., Auckland, New Zealand.

2.2. Milk samples

Unpasteurized milk samples were collected from red deer (*Cervus elaphus*) ($n = 6$), Jersey cow ($n = 10$), East Friesian sheep ($n = 6$) and Saanen goat ($n = 6$) at 4 weeks into the lactation period. Red deer whole milk was obtained from Lincoln University deer farm, Lincoln, New Zealand, and cow, sheep and goat (raw) whole milk was obtained fresh from local farm supply sources and was aliquoted and stored frozen at −20 °C. Frozen milk samples were thawed on ice and centrifuged (4000 × *g*, 30 min, 4 °C) to remove the cream fraction. Any pelleted (casein) protein was re-suspended and then aliquots of the 'defatted' milk were used for the various experiments.

2.3. Preparation of sweet whey from defatted whole milk samples

Preparation of sweet whey was performed according to the method of Parris et al. (1991). Defatted whole milk from each species was incubated at 37 °C for 1 h after the addition of 1 mL rennet (Renco New Zealand, Eltham, New Zealand) per 1 L of milk. The samples were then centrifuged at 10,000 × *g* for 30 min using an Allegra X-15R centrifuge (Beckman Coulter, USA) at 4 °C. The supernatant (sweet whey) was decanted, aliquoted and stored at −20 °C until required for analysis.

2.4. 1D-SDS PAGE

Whey protein samples were analyzed by 1D-SDS PAGE using a Novex BOLT mini-gel electrophoresis system (Life Technologies, Auckland, New Zealand). Aliquots of protein samples were added to Novex BOLT LDS sample buffer (4×) and Novex BOLT sample reducing agent (10×) according to supplier's recommendations, and heated prior to loading on a Novex BOLT 4-12% bis-Tris electrophoresis gel. Novex pre-stained protein standards (Life Technologies, Auckland, New Zealand) were run in one lane for calibration. After electrophoresis the gels were washed in MQ-water 3× 5 min and then stained with Simply Blue Safestain (Invitrogen, Auckland, New Zealand) according to the supplier's instructions.

2.5. 2D-PAGE

Whey protein samples for 2D-PAGE were desalted using a '2D clean-up kit' (GE Healthcare) and the protein pellet dissolved in an isoelectric focusing (IEF) rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS, 4 mM TCEP, 50 mM DTT), containing 5 μL IPG buffer concentrate per 1 mL of rehydration buffer. The whey protein samples dissolved in rehydration buffer were used to rehydrate 18 cm IPG 3-10 NL IEF strips (GE Healthcare, Auckland, New Zealand) overnight, followed by isoelectric focusing in an IPGphor IEF instrument (Pharmacia Biotech, Sweden). After the first dimension IEF, the proteins focused in the IEF strips were subjected to reduction for 10 min (6 M urea, 130 mM DTT) followed by alkylation for 10 min (6 M urea, 135 mM iodoacetamide) and then applied to large format 12.5% SDS-PAGE for the second dimension electrophoresis in an ETTAN DALTsix electrophoresis unit (GE Healthcare, Auckland, New Zealand). Gels were then fixed in 10% methanol, 7% acetic acid for 2 × 30 min, before staining with colloidal Coomassie blue following the method of Candiano et al. (2004).

2.6. Reversed-phase HPLC of defatted whole milk samples

Reversed-phase HPLC (RP-HPLC) of defatted whole milk was performed according to the method of Bobe et al. (1998). The Gilson HPLC system used consisted of a model 322 pump module, model 234 auto-injector, model FC204 fraction collector and a model 156 UV/VIS