

# Proteolytic enzyme and inhibitor levels in reindeer (*Rangifer tarandus tarandus* L.) vs. bovine *longissimus* muscle, as they relate to ageing rate and response

V.M.H. Barnier<sup>1\*</sup>, E. Wiklund<sup>2</sup>, A. van Dijk<sup>1</sup>, F.J.M. Smulders<sup>3</sup> & G. Malmfors<sup>2</sup>

<sup>1</sup> Universiteit Utrecht, Department of the Science of Food of Animal Origin, P.O. Box 80 175, 3508 TD Utrecht, The Netherlands (Barnier@vvd.vet.uu.nl).

<sup>2</sup> Swedish University of Agricultural Sciences, Department of Food Science, P.O. Box 7051, 750 07 Uppsala, Sweden.

<sup>3</sup> Institute for Meat Hygiene, Meat Technology and Food Science, Veterinary Medical University of Vienna, Veterinärplatz 1, A-1210 Vienna, Austria.

\* corresponding author.

**Abstract:** Eight reindeer bulls (age 1.5 years) and six Friesian bulls (age 1.5 years) were included in the study for comparison of tenderness. The reindeer were slaughtered at a commercial reindeer slaughter plant in northern Sweden and the Friesian bulls at a commercial slaughter plant in The Netherlands. Samples for determination of calpain/calpastatin activity were taken from the *M. longissimus* (LO) within 1 h *post mortem* (p.m.), and at various times p.m. pH and temperature were registered in LO; ultimate pH values were measured at 24 h p.m. for beef and at 35 h p.m. for reindeer. At day 1 p.m., samples of LO from both carcass sides were excised, divided in two parts, vacuum packaged and stored at 0-2 °C. One part of each muscle was randomly sampled at 1, 3, 7 and 14 days p.m. for determination of shear force, proteolytic enzyme activity, myofibrillar protein degradation, collagen content and heat solubility. pH and temperature fall was faster in reindeer than in beef. Collagen content in reindeer muscle was found to be low but collagen was 4 times less soluble as compared with beef. Reindeer LO was found to be extremely tender, at 3 days p.m. shear force values were only 2-3 kg/cm<sup>2</sup> (8-12 kg/cm<sup>2</sup> for beef LO). In reindeer meat, the  $\mu$ -calpain levels dropped to about 55% within 3 days. Troponin T and 30 kDa values were not related to changes in tenderness in reindeer meat. Cathepsin activities in reindeer were up to ten times higher than in beef. As in beef, cathepsin B+L levels in reindeer increased during storage, which is probably associated with a decrease in cystatin-like inhibitor levels.

**Key words:** collagen, meat quality, proteases, shear force.

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## Introduction

As reviewed by Ouali (1990) and Smulders *et al.* (1991), meat tenderisation is a variable process depending on a number of biological factors (e.g. species, age, sex and muscle type) and environmental factors (nutrition, ante mortem stress, slaughter- and chilling conditions and ageing).

Modern reindeer management often includes various stressful pre-slaughter conditions, such as gathering, herding and long distance animal transport. These conditions can promote the occurrence of DFD (Dark, Firm, Dry) meat (Wiklund *et al.*, 1995; 1996a; 1996b; 1997a). The incidence of DFD in reindeer meat has been measured in Sweden (DFD

limit pH 6.2 in *M. longissimus*), where 6% of 3400 reindeer carcasses were classified as DFD (Wiklund *et al.*, 1995). Usually, reindeer meat is consumed after no more than 2-3 days of ageing, and allegedly is appreciated particularly for its tenderness; this suggests that reindeer muscle might have a higher ageing rate than beef (Wiklund *et al.*, 1997b). The comparison of their proteolytic capacity and tenderness could be helpful in challenging this hypothesis and identifying the underlying muscle biological mechanisms.

The objective of this study was to compare tenderness development and proteolytic enzyme levels and their inhibitors in bovine and reindeer *longissimus* muscle.

## Material and methods

### Reindeer

In Sweden, eight reindeer bulls (age 1.5 years, average carcass weight 33 kg with standard deviation  $s = 13$  kg), were slaughtered at a commercial slaughter plant. The animals had been exposed to gathering and herding to a corral, a selection procedure, road transport over a distance of about 400 km (i.e. 5-6 hours driving) and overnight rest before slaughter with free access to hay and water. The animals were stunned with a captive bolt, bled and skinned. The pH and temperature in the centre of *M. longissimus* of the righthand carcass side, were measured at 1, 3, 5 and 35 h *post mortem* (p.m.). Temperature was measured with a digital thermometer (Ama-digit ad 40 th, Amarell Electronic, Germany) and pH values were measured with a portable pH meter (Portamess 651-2, Knick Elektronische Messgeräte GmbH & Co, Germany) equipped with a polymere electrode (Xerolyt, lot 406 M-6, Ingold Messtechnik AG, Switzerland). Samples for determination of calpain/calpastatin activity were taken within 1 h p.m. At 1 day p.m., samples of *M. longissimus* from both carcass sides were excised, divided in two parts, vacuum packaged and stored at 0-2 °C. One part of each muscle was randomly sampled at 1, 3, 7 and 14 days p.m. for determination of shear force, proteolytic enzyme activity, myofibrillar protein degradation, collagen content and heat solubility. All samples were frozen in liquid nitrogen (-196 °C) and transported at -80 °C to laboratory facilities in the Netherlands.

### Pilot experiment

From the same commercial slaughter plant as mentioned above, muscle samples (*M. longissimus*) from

six reindeer bulls (age 1.5 years) were collected. The animals had been exposed to the same type of pre-slaughter handling and treatment as described above. Samples for determination of calpain/calpastatin and cathepsin B+L/cystatin activity were taken as soon as possible after slaughter (i. e. 20 min post mortem).

### Beef

Six Friesian bulls (age 1.5 years, average carcass weight 360,  $s = 33$ , kg) were slaughtered at a commercial slaughter plant in the Netherlands. The pH and temperature in the centre of *M. longissimus* of the righthand carcass side, were measured at 45 min, 1.5, 3 and 24 h p.m. Temperature was measured with a digital thermometer (Tastotherm D700, Germany) and pH values were measured with a portable pH meter (type CG 818, Schott Geräte, Hofheim, Germany) equipped with a polymere electrode (Xerolyt, lot 406-M6-DXK S7/25, Ingold, Germany). Samples for determination of calpain/calpastatin activity were taken within 1 h p.m. At 1 day p.m., samples of *M. longissimus* from the lefthand carcass side were excised, divided in four parts, vacuum packaged and stored at 0-2 °C. At random, one part of each muscle was sampled at 1, 3, 7 and 14 days p.m. for determination of shear force, proteolytic enzyme activity, myofibrillar protein degradation, collagen content and heat-solubility. Samples for determination of calpain/calpastatin and cystatin-like inhibitor activities were frozen in liquid nitrogen (-196 °C) and stored at -80 °C until determination.

### Shear force

Warner Bratzler shear force was determined on samples heated in polyethylene bags in a waterbath at 75 °C until a core temperature of 70 °C was reached, whereafter they were chilled in running tap water for 40 min (Boccard *et al.*, 1981). Ten rectangular samples of 1 cm<sup>2</sup> cross section were cut out from each cooked sample, parallel to the muscle fibre direction. Shear force was measured using a draw bench (Adamel Lhomargy, Division d'Instruments S.A. Paris, France) equipped with a Warner Bratzler shearing device. A triangular blade (1.2 mm thick) was used at a crosshead speed of 298 mm/min.

### Determination of calpain and calpastatin activity

Separation of calpains and calpastatin was performed according to Etherington *et al.* (1987) with

minor modifications as described by Geesink (1993).

#### SDS gel electrophoresis and densitometry

Myofibrils were prepared according to the method of Ouali *et al.* (1983). Degradation of myofibrillar proteins was assessed by means of SDS-PAGE according to Greaser *et al.* (1983) on a 12.5% acrylamide separating gel. The intensity of the protein bands was measured with a LKB Ultrascan XL Enhanced Laser Densitometer.

#### Determination of collagen content and heat solubility

The extraction of heat soluble collagen was performed according to Hill (1966) and acid hydrolysis according to Bauer (1991) with a microwave oven. Hydroxyproline was measured according to the method of Stegemann & Stalder (1967) with minor modifications described by Barnier (1995).

#### Cathepsin B+L and their inhibitors

In reindeer meat, determination of total cathepsin B+L activity was performed on frozen meat according to Etherington *et al.* (1987). In beef the determination of free cathepsin B+L activity was performed on fresh meat samples according to Wu *et al.* (1985), subsequently aliquots were frozen in liquid nitrogen (-196 °C) and stored at -80 °C. Muscle samples were prepared according to Bige *et al.* (1985) to extract the cysteine proteinase inhibitors, omitting the ultimate purification step. The amount of cystatin-like inhibitors was determined using a papain active-site titration based on the method of Anastasi *et al.* (1983), described by Barnier (1995).

#### Statistical analyses

Significance of difference between ageing times was tested with a one-way analysis of variance (SPSS/PC+, Chicago, IL).

## Results and discussion

#### pH, temperature, collagen content and heat solubility

Due to their low dressed carcass weights (Table 1), the temperature fall was relatively fast in reindeer (Fig. 1b). The pH fall was also rather fast in reindeer in comparison with beef (Fig. 1a). The present results are in good agreement with earlier studies, where consistently a rapid pH fall in reindeer meat was found (Wiklund *et al.*, 1995; Gundersen & Nummedal, 1996).

Table 1. Carcass weight (kg), collagen content ( $\mu\text{g}$  hydroxyproline/g muscle) and heat-solubility (%) of reindeer ( $n = 8$ ) and beef ( $n = 6$ ) *M. longissimus* (means and standard deviations).

	Beef	Reindeer
Carcass weight (kg)	360 ( 33)	33 ( 13)
Collagen content ( $\mu\text{g}/\text{g}$ )	1158 (210)	500 ( 78)
Heat-solubility (%)	11.1 ( 2.2)	2.8 (0.4)

Table 2. Shear force at 70 °C ( $\text{kg}/\text{cm}^2$ ), intensity of troponin T (% of troponin relative to actin) and 30 kDa peptide (% of 30 kDa peptide relative to actin) in beef ( $n = 6$ ) and reindeer ( $n = 8$ ) *M. longissimus* at 1, 3, 7 and 14 days *post mortem* (means and standard deviations).

Trait	Beef	Reindeer
<b>Shear force, <math>\text{kg}/\text{cm}^2</math></b>		
1 day	13.4 <sup>a</sup> (2.6)	n.d.
3 days	10.0 <sup>b</sup> (1.9)	2.9 <sup>a</sup> (1.0)
7 days	9.8 <sup>b</sup> (2.8)	2.6 <sup>a,b</sup> (0.5)
14 days	6.5 <sup>c</sup> (1.4)	1.9 <sup>b</sup> (0.6)
<b>Troponin T</b>		
1 day	8.6 <sup>a</sup> (2.7)	9.4 <sup>a</sup> (4.6)
3 days	n.d.	5.0 <sup>b</sup> (4.4)
7 days	2.7 <sup>b</sup> (1.1)	1.8 <sup>b</sup> (2.1)
14 days	1.4 <sup>b</sup> (1.7)	n.m.
<b>30 kDa</b>		
1 day	1.4 <sup>a</sup> (1.6)	n.m.
3 days	n.d.	1.9 <sup>a</sup> (1.7)
7 days	6.0 <sup>b</sup> (1.2)	4.2 <sup>a</sup> (2.8)
14 days	7.3 <sup>b</sup> (1.8)	8.9 <sup>b</sup> (3.3)

n.d.: not determined.

n.m.: not measurable.

Within-trait and columns, means having a superscript containing a common letter are not significantly different ( $P > 0.05$ ).

Collagen content in reindeer muscle was rather low but the collagen was quite insoluble (4 times less soluble as compared with beef, Table 1).

#### Tenderness, proteolysis and myofibrillar protein degradation

In the present study, reindeer *longissimus* muscle was found to be extremely tender. As early as 3 days post mortem, shear force values were only 2 to 3  $\text{kg}/\text{cm}^2$ , which was very low compared with measured shear force values in beef (values around 10  $\text{kg}/\text{cm}^2$ ) after the same ageing time (Table 2). The speed of p.m.

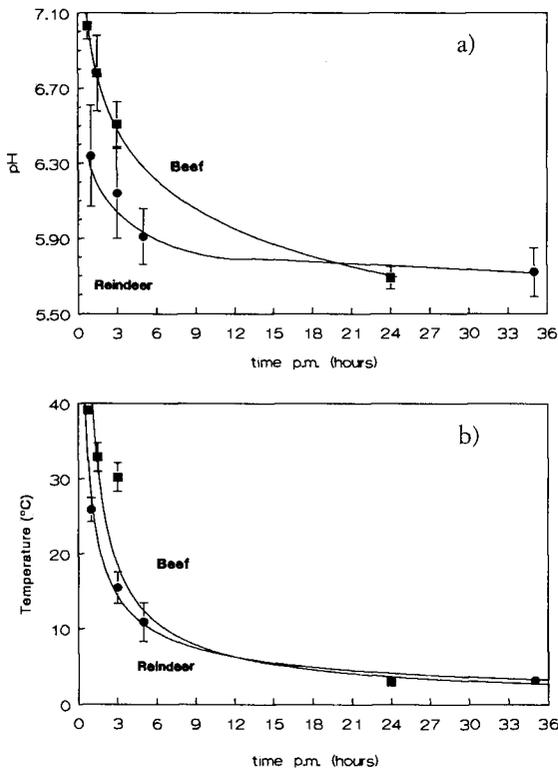


Fig. 1. a) pH and b) temperature (°C) decline in beef ( $n = 6$ ) and reindeer ( $n = 8$ ) *M. longissimus* (means and standard deviations).

proteolysis in reindeer muscle appears to be very fast, therefore. Whereas initial values of  $\mu$ -calpain assessed in reindeer meat in a pilot experiment on very early p.m. samples (20 min p.m.) were in the 42 - 79 unit range ( $n = 6$ ), these levels dropped to about 55% within 3 days (Fig. 2a). As  $\mu$ -calpain, once activated, gradually loses its activity through an autolytic process, a decrease in  $\mu$ -calpain activity would mean that the enzyme has been active and possibly caused tenderisation. During p.m. storage, muscle calpastatin levels were lower in reindeer than beef (Fig. 2b). Assuming a similar enzyme autolysis/activation mechanism for both beef and reindeer, this finding suggests that most of the proteolysis in reindeer meat has already taken place within the first few days after slaughter.

Degradation of troponin T during p.m. storage and consequent appearance of a 30 kDa breakdown product (Ho *et al.*, 1994) have been reported as an indicator of beef tenderness (Uytterhaegen *et al.*, 1992). Surprisingly, troponin T and 30 kDa values (Table 2), following a similar pattern as in beef, are

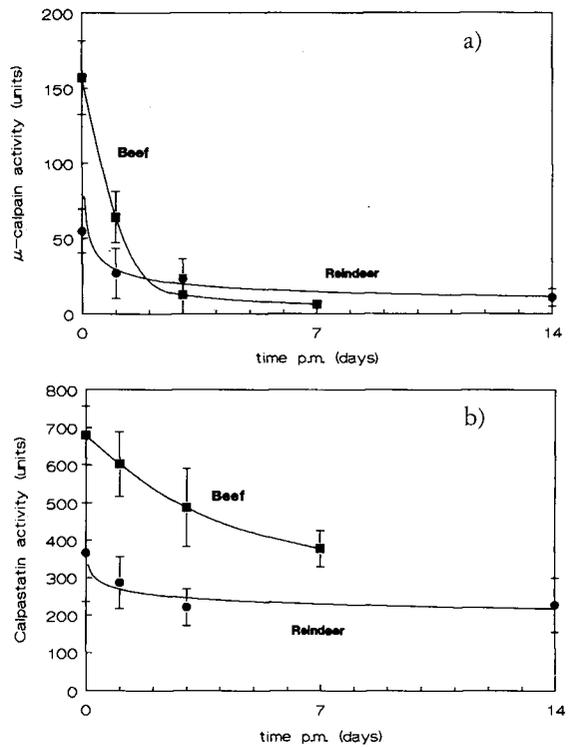


Fig. 2. a)  $\mu$ -calpain and b) calpastatin activities of beef ( $n = 6$ ) and reindeer ( $n = 8$ ) *M. longissimus* (means and standard deviations).

difficult to relate to tenderness changes in reindeer meat in the present study.

Remarkably, cathepsin activities in reindeer meat in the present study were up to ten times higher than in beef (Fig. 3). As in beef (Barnier *et al.*, 1993; Barnier, 1995), reindeer cathepsin B+L levels increased during storage of the meat, which is probably associated with a decrease in cystatin levels

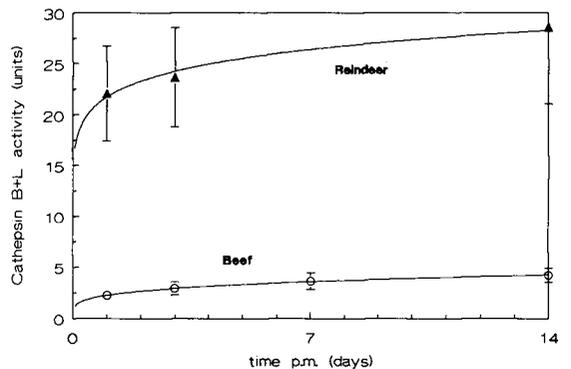


Fig. 3. Cathepsin B+L activity (nmol/min/g muscle) of beef ( $n = 6$ ) and reindeer ( $n = 8$ ) *M. longissimus* (means and standard deviations).

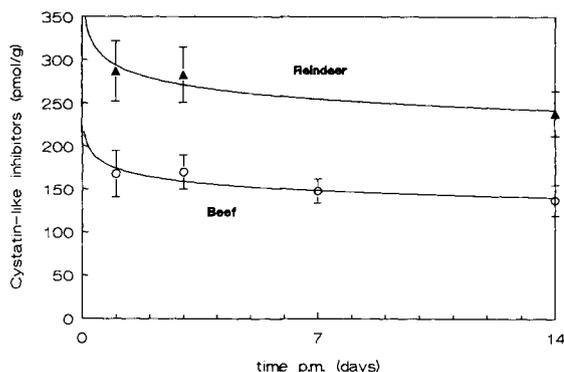


Fig. 4. Cystatin-like inhibitor levels (pmol/g muscle) of beef ( $n = 6$ ) and reindeer ( $n = 8$ ) *M. longissimus* (means and standard deviations).

(Fig. 4). The results of the present study support this hypothesis and are consistent with the latter author's data on beef.

## Conclusions

In reindeer longissimus muscle ultimate tenderness was almost reached at 3 days p.m. Reindeer meat samples had much higher proteolytic enzyme activity and lower inhibitor levels as compared with beef. One should be cautious, however, to extrapolate muscle physiological observations in beef to other ruminant species. As no relationship between the degradation of troponin T and tenderness (shear force) was found in reindeer meat in the present study, further studies on the degradation pattern of high molecular weight proteins and the changes occurring at an ultrastructural level during the post mortem tenderisation process are required. The observations made in the present experiment justify further muscle proteolytic enzyme- and tenderness studies in reindeer as this species might prove to be of interest for model studies of a comparative nature.

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## References

- Anastasi, A., Brown, M. A., Kembhavi, A. A., Nicklin, M. J. H., Sayers, C. A., Sunter, D. C. & Barrett, A. J. 1983. Cystatin, a protein inhibitor of cysteine proteinases. - *Journal of Biochemistry* 211: 129-138.
- Barnier, V. M. H. 1995. *Determinants and predictors of beef tenderness. Involvement of muscle proteinases and their inhibitors in post mortem tenderisation*. Dissertation, Department of the Science of Food of Animal Origin, Utrecht University, The Netherlands.
- Barnier, V. M. H., van Laack, H. L. J. M. & Smulders, F. J. M. 1993. Cathepsin B+L activity in beef as affected by ageing. - *In: 39th International Congress of Meat Science and Technology*. Calgary, Canada, p.148.
- Bauer, F. 1991. Rapid determination of collagen in meat products. - *In: 37th International Congress of Meat Science and Technology*. Kulmbach, Germany, pp. 1127-1130.
- Bige, L., Ouali, A. & Valin, C. 1985. Purification and characterization of a low molecular weight cysteine proteinase inhibitor from bovine muscle. - *Biochimica et Biophysica Acta* 843: 269-275.
- Boccard, J. L., Buchter, L., Casteels, M., Cosentino, E., Dransfield, E., Hood, D. E., Joseph, T. L., MacDougall, D. B., Rhodes, D. N., Schön, I., Tinbergen, B. J. & Touraille, C. 1981. Procedures for measuring meat and meat quality characteristics in beef production experiments. Report of a working group in the commission of the european communities (C.E.C) beef production research programme. - *Livestock Production Science* 8: 385-397.
- Etherington, D. J., Taylor, M. A. J. & Dransfield, E. 1987. Conditioning of meat from different species. Relationship between tenderising and the levels of cathepsin B, cathepsin L, calpain I, calpain II and  $\mu$ -glucuronidase. - *Meat Science* 20: 1-18.
- Geesink, G. H. 1993. *Post mortem proteolysis and beef tenderness with special reference to the action of the calpain/calpastatin system* Dissertation, Department of the Science of Food of Animal Origin, Utrecht University, The Netherlands.
- Greaser, M. L., Yates, L. D., Krzywicki, K. & Roelke, D. L. 1983. Electrophoretic methods for the separation and identification of muscle proteins. - *In: 36th Rec. Meat Conf. Proc.*, pp. 87-91.
- Gundersen, L.-A. & Nummedal, K. 1996. *Kvalitet av norsk reinsdyrkjøtt (Quality of Norwegian reindeer meat)*. - *InforMAT* 9: 11-14 (in Norwegian).
- Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. - *Journal of Food Science* 31: 161-166.
- Ho, C. Y., Stromer, M. H. & Robson, R. M. 1994. Identification of the 30 kDa polypeptide in post mortem skeletal muscle as a degradation product of troponin-T. - *Biochimie* 76: 369-375.

- Malmfors, G. & Wiklund, E. 1996. Pre-slaughter handling of reindeer effects on meat quality. – *Meat Science* 43S: S257–S264.
- Ouali, A. 1990. Meat tenderisation: possible causes and mechanisms. A review. – *Journal of Muscle Foods* 1: 129–165.
- Ouali, A., Obled, A., Cottin, P., Mercadi, N., Ducastaing, A. & Valin, C. 1983. Comparative effects of postmortem storage and low calcium requiring CANP on bovine and rabbit myofibrillar proteins. – *Journal of the Science of Food and Agriculture* 34: 466–476.
- Smulders, F. J. M., van Laack, R. & Eikelenboom, G. 1991. Muscle and meat quality: biological basis, processing, preparation. – In: Smulders, F. J. M. (ed.). *The European meat industry in the 1990's*. Audet, Nijmegen, pp. 121–159.
- Stegemann, H. & Stalder, K. 1967. Determination of hydroxyproline. – *Clinica Chimica Acta* 18: 267–273.
- Uytterhaegen, L., Claeys, E. & Demeyer, D. 1992. The effect of electrical stimulation on beef tenderness, protease activity and myofibrillar protein degradation. – *Biochimie* 74: 275–281.
- Wiklund, E., Andersson, A., Malmfors, G. & Lundström, K. 1996a. Muscle glycogen levels and blood metabolites in reindeer (*Rangifer tarandus tarandus* L.) after transport and lairage. – *Meat Science* 42: 133–144.
- Wiklund, E., Andersson, A., Malmfors, G., Lundström, K. & Danell, Ö. 1995. Ultimate pH values in reindeer meat with particular regard to animal sex and age, muscle and transport distance. – *Rangifer* 15: 47–54.
- Wiklund, E., Barnier, V. M. H., Smulders, F. J. M., Lundström, K. & Malmfors, G. 1997b. Proteolysis and tenderisation in reindeer (*Rangifer tarandus tarandus* L.) bull longissimus thoracis muscle of varying ultimate pH. – *Meat Science* 46: 33–43.
- Wiklund, E., Malmfors, G. & Lundström, K. 1997a. The effects of pre-slaughter selection of reindeer bulls (*Rangifer tarandus tarandus* L.) on technological and sensory meat quality, blood metabolites and abomasal lesions. – *Rangifer* 17: 65–72.
- Wiklund, E., Malmfors, G., Lundström, K. & Rehbinder, C. 1996b. Pre-slaughter handling of reindeer bulls (*Rangifer tarandus tarandus* L.) – effects on technological and sensory meat quality, blood metabolites and muscular and abomasal lesions. – *Rangifer* 16: 109–117.
- Wu, F. Y., Dutson, T. R., Valin, C., Cross, H. R. & Smith, S. B. 1985. Ageing index, lysosomal enzyme activities, and meat tenderness in muscles from electrically stimulated bull and steer carcasses. – *Journal of Food Science* 50: 1025–1028.

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