

Original article

Post mortem proteolysis and tenderization of beef muscle through infusion of calcium chloride

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Abstract — A study involving 48 beef carcasses was conducted in order to evaluate the effects of 0.3 M calcium chloride (CaCl_2) injection on final tenderness in muscle *Longissimus thoracis et lumborum*. Injection of beef carcasses with CaCl_2 accelerated post mortem tenderization process. Ca^{2+} -dependent proteases (μ -calpain and m-calpain) and their inhibitor (calpastatin) activities were all significantly ($P < 0.01$) decreased in CaCl_2 injected animals ($n = 24$) compared with control animals ($n = 24$). Tenderness, assessed by measuring shear force, was significantly improved ($P < 0.05$) by CaCl_2 injection both at two and eight days post mortem.

beef cattle / meat / tenderness

Résumé — Protéolyse post mortem et tendreté de la viande de taurillons après injection de chlorure de calcium. Quarante-huit carcasses de taurillons ont été utilisées afin d'évaluer les effets d'une injection de chlorure de calcium (CaCl_2 , 0,3 M) sur la tendreté finale du muscle *Longissimus thoracis et lumborum*. L'injection de CaCl_2 pratiquée dans les carcasses de taurillons a permis d'accélérer le processus d'attendrissage post mortem. L'activité des protéases dépendantes du calcium (μ -calpaïne et m-calpaïne) et de leur inhibiteur (calpastatine) a diminué de façon significative dans le groupe des carcasses traitées ($n = 24$) comparativement au groupe témoin ($n = 24$). L'injection de CaCl_2 a augmenté ($P < 0,05$) la tendreté, déterminée via les valeurs de résistance à la coupe, le deuxième et le huitième jours post mortem.

taurillons / viande / tendreté

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1. INTRODUCTION

Factors affecting muscle tenderness have been extensively researched over the past 50 years [11, 15, 19]. Meat tenderness is primarily determined by two muscle components such as connective tissue and the contractile apparatus [2]. Over the last two decades, most investigations have been focused on the nature of the changes that occur at the level of the endogenous proteolytic systems [1, 4, 5, 10]. The infusion of CaCl_2 or NaCl into carcasses has been used for acceleration of post mortem proteolysis and tenderization process [6, 7, 12]. The present study was conducted to determine whether the activation of the calcium-dependent proteases I (μ -calpain) and II (m-calpain) is the mechanism through which infusion of beef carcasses with CaCl_2 immediately after death accelerates the tenderization process.

2. MATERIALS AND METHODS

Forty-eight crossbred beef cattle reared on the same farm were used in this trial. All animals were slaughtered at a mean body weight of about 550 kg, and carcasses were divided into two groups. Twenty-four carcasses were used as control group (slaughtered according to normal procedures), and the remaining twenty-four carcasses were infused (500 ml) with 0.3 M CaCl_2 . The above solutions were infused using a pumping device (five injection sites, the distance between each injection site was 7.5 cm) in a section of the *Longissimus Thoracis et Lumborum* (LTL) muscle, 40 cm in length, from first to the sixth lumbar vertebra [8].

After completion of the infusion process (within 45 min after slaughter), infused and control carcasses were put in a cold room at a controlled temperature of 2 °C. Twenty-four h after slaughter, the entire LTL was removed from each carcass. Samples designated for shear force determination, weighing approximately 100 g, were removed

from LTL between the 12th and 13th rib interface. Chops were taken from the mid-region of each sample and roasted on a metal tray, according to the procedures of Riley et al. [13].

Samples for sarcomere length determinations were prepared at 48 h after slaughter, following the procedures of Koolmes et al. [9]. The Ca^{2+} dependent proteases-I (μ -calpain), -II (m-calpain) and their inhibitor were prepared from 100 g of LTL at 24 h post mortem, according to Koohmaraie et al. [7]. The total calcium contents of the LTL was measured using standard techniques, with an atomic absorption spectrophotometer [6].

Data were analysed by the method of least squares using the general linear model procedure of the SAS [14] and results were expressed as least square means. The statistical model used in this study was a simple one way analysis of variance.

3. RESULTS AND DISCUSSION

The calcium content ($\mu\text{g}\cdot\text{g}^{-1}$ wet tissue) of the carcasses infused with CaCl_2 was significantly ($P < 0.01$) increased (approximately $\times 100$) compared to the control groups (Tab. I), in similarity with the results obtained by Koohmaraie et al. [7]. In another study by Koohmaraie et al. [8] calcium content in beef loins injected with 0.3 M CaCl_2 was increased about 120-fold compared to the control group.

Infusion of carcasses with CaCl_2 significantly ($P < 0.05$) lowered LTL shear force values both at two and at eight days post mortem compared with the control groups (Tab. I), according to a previous study [18] in which shear force values of lamb carcasses infused with 0.3 M CaCl_2 were found to be significantly decreased both at one and seven days post mortem compared to the control carcasses. In another study conducted on beef carcasses [8], CaCl_2 injection of LTL muscle resulted in a

Table I. Effects of treatment on calcium content, shear force values, enzyme activity and sarcomere length in *Longissimus* muscle.

	Treatment group		SEM	P value
	Control (n = 24)	Infusion with CaCl ₂ (n = 24)		
Calcium (µg·g ⁻¹ tissue)	12.9	1265.4	50.13	< 0.01
Shear force 2 days (kg·cm ⁻²)	9.60	8.33	0.357	< 0.05
Shear force 8 days (kg·cm ⁻²)	6.99	5.44	0.396	< 0.05
µ-calpain ^a	64.1 (sem 5.72)	nd	na	na
m-calpain ^a	134.2	39.1	7.22	< 0.01
Calpastatin ^b	178.2	27.3	3.64	< 0.01
Sarcomere length (µm)	1.77	1.82	0.024	ns

^aTotal activity·100 g⁻¹ muscle (caseinolytic assay).

^bTotal activity·100 g⁻¹ muscle (inhibition of casein hydrolysis by m-calpain).

nd: not detected; ns: not significant; na: not applicable.

significant acceleration of post mortem tenderization as determined by shear force value at 1 day after slaughter. As previously reported by Koochmarai et al. [6, 7], of several concentrations of calcium chloride solution examined, a 0.3 M solution was most effective in reducing shear force value in injected carcasses.

The m-calpain activity and inhibitor activity at 24 h post mortem were lowered ($P < 0.01$) in carcasses infused with 0.3 M CaCl₂, while in the treated carcasses there was no µ-calpain activity remaining (Tab. I). Based on the results of the present experiment, CaCl₂ injection results in activation of µ-calpain and m-calpain, which eventually results in loss of their activities because of autolysis [16, 20].

Calcium infusion did not have a significant effect on sarcomere lengths (Tab. I). Therefore, differences in shear force values between treatment groups cannot be attributed to differences in sarcomere length, according to the results obtained also in previous experiments [3, 17].

The results of this study indicated that infusion of beef carcasses with calcium chloride accelerated post mortem tenderization. The loss of µ-calpain activity and the

significant decrease in m-calpain activity are due to autolysis of these proteases in the presence of calcium [7, 8]. Based on the results obtained in the present study, we suggest that activation of Ca²⁺-dependent proteases results in tenderization in meat tenderness. More research is required to investigate properties controlling the Ca²⁺-dependent proteases system to consistently produce tender meat.

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