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Feeding strategy for improving tenderness has opposite effects in two different muscles

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ABSTRACT

Compensatory growth has been shown to affect rate of muscle protein turnover and may potentially improve tenderness of beef. Thus, a study of tenderness in relation to feeding regime and slaughtering at a time with maximal muscle protein degradation was performed. Friesian bull calves (5-month-old) were fed either *ad libitum* ($n = 6$) (AA) or restrictively for 3 months followed by re-alimentation for 6 weeks ($n = 6$) (RA) before slaughter at 10 months. At slaughter the fractional breakdown rate of muscle protein was 2.4% in RA compared with 1.6% in AA ($P < 0.06$). Sensory profiling revealed superior texture of *M. semimembranosus* from RA compared with AA, whereas *M. longissimus* was superior in texture from AA compared with RA, with no difference in proteolysis and shear force. In conclusion it was clear that different muscles in terms of tenderness responded very differently to the nutritional manipulation.

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1. Introduction

High and uniform tenderness of beef is important for the meat industry in order to satisfy consumer demands and consequently maintain market share. As reviewed by several authors, tenderness is dependent on a range of factors including genetics, sex, production strategies, age at slaughter, slaughter procedure and ageing (Ouali, 1990). The link between production strategy and tenderness within specific muscles seems to be the rate of muscle protein degradation in the live animal because the enzymes involved also affect proteolysis postmortem which is essential for tenderisation (Goll, Thompson, Taylor, & Ouali, 1998; Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002).

Muscle growth rate is the difference between the rate of muscle protein synthesis and degradation (protein turnover) and one way of improving tenderness may be to slaughter beef cattle at a time where maximal protein degradation occurs. A production strategy involving compensatory growth has been shown to affect the rate of muscle protein turnover in steers (Jones, Starkey, Calkins, & Crouse, 1990) and bulls (Therkildsen, 2005). Excretion of 3-methyl-histidine in urine reflects the total muscle protein degradation in the body of cattle (Harris & Milne, 1981). Therkildsen (2005) showed by measuring the excretion of 3-methylhistidine, that bull calves, fed restrictively for 14 weeks from the age of 5 months followed by re-alimentation in 20 weeks, had maximal fractional breakdown rate of muscle protein after 5 to 8 weeks of re-alimen-

tation, which exceeded the level in control bull calves fed *ad libitum* throughout the experimental period. Thus the muscle protein degradation was increased in the calves after re-alimentation compared with control calves, and it was hypothesised that slaughtering the bull calves at the time of maximal protein degradation in the muscle would lead to the potential for superior tenderness development compared with control calves.

Thus, the aim of the present experimental work was to study the effect of feeding regime and optimal time of slaughter on tenderness of meat postmortem in order to verify the theory of the advantage of slaughtering meat-producing animals at a time with maximal protein degradation. This strategy could then be implemented in commercial production at relevant slaughter weights.

2. Materials and methods

2.1. Animals

The experiment was approved by and conducted in accordance with the guidelines outlined by the Danish Inspectorate of Animal Experimentation. The experiment included a total of 12 Holstein Friesian bull calves born after two sires. The calves entered the study at the age of 5 months and were allocated to two feeding strategies either *ad libitum* (AA, $n = 6$) or restricted/*ad libitum* (RA, $n = 6$) with respect to sire and age. The calves were loose-housed from 5 months to 6 months of age on straw bedded floors and from 6 months to slaughter at 10 months of age kept in tie stalls.

The calves on the AA feeding strategy had free access to a concentrate mixture and a total mixed roughage (TMR) ration. The

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calves on the RA feeding strategy were restricted in energy intake from 5 to 8 months by allowing them free access to the TMR ration. From $6\frac{1}{2}$ months of age to the end of the restriction period they were supplied with 1 kg of the concentrate mixture each day. From 8 months they were re-alimentated and given free access to the concentrate mixture and the TMR ration. In the first week of re-alimentation the calves were gradually adjusted to the free access of concentrate. The composition of the rations is given in Table 1. The calves were weighed on two consecutive days at the beginning of the experiment, at the end of the restriction period and on two consecutive days prior to slaughter.

2.2. Urine collection and determination of 3-methylhistidine in urine

Two days before slaughter, urine was collected from the animals over a 24 h period in order to calculate the fractional breakdown rate (FBR) of myofibrillar protein from the concentration of 3-methylhistidine in urine as described in Therkildsen (2005). Because of problems with the urine collection, urine was only collected from 8 bull calves, 4 from each feeding strategy.

2.3. Slaughter procedure, sampling and preparation

Slaughtering was carried out on two consecutive days, with 6 calves (3 from each treatment) each day. The calves were transported 500 m to the experimental slaughterhouse at The Faculty of Agricultural Science, University of Aarhus and stunned by captive bolt pistol, hung and bled and a blood sample was collected at the exsanguination. Within 15 min postmortem, samples from *M. longissimus* (LD) were collected, snap frozen in liquid nitrogen and stored at −80 °C for later analysis. Measurements of pH in LD was carried out at the last rib 45 min post mortem with PHM201 pH meter (Radiometer, Denmark) equipped with Metrohm probe type glass electrode WOC (Metrohm, Switzerland). The electrode was calibrated in pH 4.01 and 7.00 IUPAC buffers equilibrated to 35 °C. At the same time a temperature meat logger was inserted in the LD, collecting temperature data every minute. The carcasses were chilled at 12 °C for 4 h postmortem and then stored at 3 °C.

Forty-eight hours postmortem the carcasses were weighed and pH_{48} was measured as described above, however, at this time the electrode was calibrated in buffers equilibrated at 3 °C. *M. supraspinatus* (SS) and *M. semimembranosus* (SM) were cut from both sides and weighed and SM muscles were vacuum-packed in oxygen impermeable bags and aged until either 7 or 14 days postmortem at 3 °C before storage at −20 °C. The ageing time was randomly assigned to different carcass sides. At the time of preparation of SM for sensory profiling a standardized sample was removed, vacuum-packed and stored at −20 °C, until analysed for IMF. LD from the 5th to 2nd lumbar vetebra from both sides were removed, vac-

uum-packed and aged as described for SM. These cuts were used for sensory profiling. An overview of the total sample variation is presented in Fig. 1. In addition, from each side the LD from the 10th thoracic vertebra to the 13th thoracic vertebra was used as follows (from cranial direction): left side: 7 cm for Warner-Bratzler shear force (WBSF) aged 2 days, 7 cm for WBSF aged 7 days and the rest used for colour and drip loss determination, right side: 7 cm used for determination of intramuscular fat (IMF) and myofibrillar fragmentation index (MFI) and 7 cm for WBSF aged 14 days. The samples were vacuum-packed and aged as described at 3 °C before storage at −20 °C. The sample used for MFI determination was divided in three parts, one part was immediately snap frozen in liquid nitrogen and stored at −80 °C for later analysis of MFI and calpain activity, the other two parts were vacuum-packed and aged for either 7 or 14 days post mortem and then handled as above.

2.4. Chemical and instrumental analysis

2.4.1. Drip loss and colour determination

Drip loss was measured on approximately 100 g of LD muscle using the plastic bag method described by Honikel (1998). Colour was measured on LD samples using a Minolta Chroma Meter CR-300 (Osaka, Japan) calibrated against a white tile ($L^* = 92.30$, $a^* = 0.32$ and $b^* = 0.33$). Samples were allowed to bloom for 1 h at 3 °C prior to the measurements. The three parameters L^*, a^* and b^*, representing lightness, redness and yellowness, were measured on five sites randomly chosen, covering the whole surface.

2.4.2. Determination of intramuscular fat

The amount of intramuscular fat in LD and SM was determined by ether extraction (method NMKL 131:1989, Q481/Q7003), performed by Steins Laboratorium A/S, Holstebro, Denmark as described in Hansen, Therkildsen, and Byrne (2006).

2.4.3. Calpain and calpastatin activity determination

Calpain activity was measured in LD samples collected immediately after slaughter and 2 days postmortem, whereas the calpastatin inhibitory activity was measured only in samples collected immediately after slaughter. The activity of μ-calpain was determined by casein zymography, as described in Therkildsen (2005). The activity of μ-calpain is expressed as density, which is directly correlated to the activity of the enzyme. Calpastatin inhibitory activity against μ-calpain was determined as described by Thompson, Saldaña, Cong, and Goll (2000) with modifications described in Therkildsen (2005) using the commercially available bodipy-labelled casein (ENZCHEK-protease assay kit, E6638, Molecular
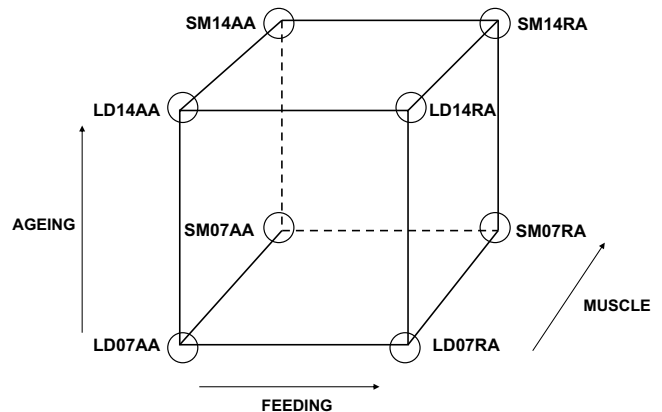
Table 1
Composition of diets

Ingredients (% of feed)	Concentrate	Total mixed ration TMR
Barley straw		59.2
Barley	74.1	5.4
Rapeseed cake	7.0	21.5
Rapeseed meal	6.0	
Soybean meal	6.4	
Sugarcane molasses	3.0	
Sugar beet molasses		13.5
Vegetable fat	1.2	
Minerals and vitamins	2.3	0.3
Net energy content, SFU/kg[a]	1.00	0.53
Metabolic energy, MJ/kg DM	15.3	10.98
Digestible crude protein, g/kg DM	124	96

[a] SFU – Scandinavian feed units.

Fig. 1. Schematic of experimental design. The first two letters designate muscle (LD = *M. longissimus*; SM = *M. semimembranosus*). The numbers designate ageing periods (07 = 7 days; 14 = 14 days), and the latter two letters designate feeding strategy (AA = *ad libitum*; RA = restricted/*ad libitum*).