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TIMING OF NITRIC OXIDE INHIBITION PRE-SLAUGHTER INFLUENCES LAMB MEAT TENDERNESS AND PROTEOLYSIS

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Abstract

Nitric oxide (NO) is a free radical that is constantly produced or released throughout the body by diverse tissues and is known to influence proteolytic activity in human and rodent skeletal muscle as well as being involved in regulation of calcium homeostasis in the muscle cell. The influence of nitric oxide (NO) on development of meat tenderness has been demonstrated through postmortem manipulation and this experiment was designed to manipulate NO synthase activity *in vivo*. The aim was to investigate the effect of timing of NOS inhibition, on meat quality attributes of two different lamb muscles. Sixty-four second cross lambs were used. Endogenous NOS activity was manipulated using infusion of L-NAME (0, vs 30 mg/kg) at either 24 hrs or 3 hrs post-slaughter. Lambs were slaughtered and meat quality attributes measured in the *semimembranosus* (SM) and *longissimus thoracis* (LT). Muscle pH at 1 hr and 24 hrs postmortem was not influenced by L-NAME infusion or timing of infusion in either muscle ($P>0.05$). Inhibition of endogenous NOS using L-NAME resulted in lower WBSF values in the LT ($P<0.05$) and tended to result in lower WBSF values for the SM ($P<0.10$) which was more pronounced in the LT in lambs infused at 24 hrs pre-slaughter ($P<0.05$). Similarly, MFI values in the LT and in the SM were higher in L-NAME infused lambs compared to those infused with saline (P0.05 for both). Sarcomere length tended to be reduced in the LT of L-NAME infused lambs ($P<0.10$), which was more pronounced in the lambs infused at 24 hrs pre-slaughter. Sarcomere length in the SM was not influenced by treatments ($P>0.05$). In conclusion, inhibition of endogenous NOS activity caused an increase in tenderness, as measured by shear force and an increase in proteolysis, as measured by Myofibrillar Fragmentation Index values, in both the LT and SM muscles. The effect was more pronounced in lambs that were infused at 24 hrs pre-slaughter compared to 3 hrs pre-slaughter. The role of skeletal muscle NOS activity pre-slaughter in determining meat tenderness deserves further investigation.

Introduction

Nitric oxide (NO) is a free radical that is constantly produced or released throughout the body by diverse tissues and is known to influence proteolytic activity in human and rodent skeletal muscle (Michetti et al., 1995) as well as being involved in regulation of

calcium homeostasis in the muscle cell (Hare, 2003). Injection of nitric oxide (NO) donors and inhibitors into hot-boned beef *longissimus thoracis* (LT) at 2 hrs post-slaughter has been found to reduce and increase shear force respectively (Cook et al., 1998). Nitric oxide synthase (NOS) activity post-slaughter has been found at 0 hrs post-slaughter in chicken, turkey, pork diaphragm and trout muscle but only the pork diaphragm retained NOS activity until 24 hrs post-slaughter and even then, it was in an *in vitro* system where ample substrate and co-factors were provided (Brannan and Decker, 2002). Cottrell et al. (2002) were the first to show an effect of manipulation of NO levels *in vivo* pre-slaughter on meat tenderness postmortem. They showed that infusion of a NOS inhibitor, L-NAME, into lambs at 3 hrs pre-slaughter resulted in more tender meat in the LT muscle at 3 days post-slaughter but tougher meat in the *semimembranosus* (SM) at 1 and 3 days post-slaughter. The effect of changing the time of infusion of the NOS inhibitor on meat tenderness is unknown and thus was proposed for investigation.

Objectives

To investigate the effect of timing of NOS inhibition on meat quality attributes of two different lamb muscles.

Methodology

Sixty-four second cross (Border Leicester /Merino dam x Poll Dorset sire) lambs approximately six months old, ranging between 33.5 and 51kg live weight, were selected from a flock, blocked on liveweight and were allocated to one of four slaughters (n=16 per slaughter). On the day prior to slaughter, lambs were brought into a shed and an indwelling jugular catheter was inserted intravenously into the jugular vein. Animals were deprived of food approximately 12 hrs pre-slaughter, but retained *ad libitum* access to water. Lambs were allocated to an L-NAME infusion treatment (30 mg/kg of L-NAME in 0.9% saline vs saline; infused via the indwelling catheter) and two infusion times (3 hrs vs 24 hrs pre-slaughter) in a 2 x 2 factorial with a balanced randomised block design. Lambs were quietly moved from pens in the shed to the abattoir and then stunned using 200 volts and 1 amp for 4 seconds applied to the head. Animals were exsanguinated and carcasses eviscerated before entering the chiller (2 ^{o}C $\pm$1^{o}C). The postmortem pH was measured at 1 and 24 hours post-slaughter in the SM and LTL. The LT and SM were removed at 24 hrs postmortem. Tenderness was measured by Warner-Bratzler shear force (WBSF) after 1, 3 or 9 days of ageing by methods described in Channon et al. (2000). Samples were removed from the LT at 24 hrs postmortem for snap freezing in liquid nitrogen and freezer storage prior to measurement of sarcomere length and from the LT and SM for measurement of myofibrillar fragmentation index (MFI). Muscle surface colour (L*a*b*) was measured after 30 min. bloom at 1, 3 and 9 days postmortem. All data were tested for significance with an analysis of variance (ANOVA) blocked for the slaughter day. For MFI and WBSF, days of ageing was analysed in the AOV. As there were no interactions between time of infusion pre-slaughter, L-NAME treatment and days of ageing, means are presented as least squares means across all ageing periods.

Results & Discussion

The effects of L-NAME infusion and timing of infusion on major meat quality traits are shown in Table 1. Muscle pH at 1 hr and 24 hrs postmortem was not influenced by L-NAME infusion or timing of infusion in either muscle (P>0.05). Inhibition of endogenous NOS using L-NAME resulted in lower WBSF values in the LT (P<0.05) and tended to result in lower WBSF values for the SM (P<0.10) which was more pronounced in the LT in lambs infused at 24 hrs pre-slaughter (P<0.05). Similarly, MFI values in the LT and in the SM were higher in L-NAME infused lambs compared to those infused with saline (P0.05 for both). Sarcomere length tended to be reduced in the LT of L-NAME infused lambs (P<0.10), which was more pronounced in the lambs infused at 24 hrs pre-slaughter. Sarcomere length in the SM was not influenced by treatments (P>0.05). There were no major effects of treatments on any surface colour measurements (P>0.05; data not presented).

Conclusions

Inhibition of endogenous NOS activity caused an increase in tenderness, as measured by shear force and an increase in proteolysis, as indicated by Myofibrillar Fragmentation Index values, in both the LT and SM muscles. The effect was more pronounced in lambs that were infused at 24 hrs pre-slaughter compared to 3 hrs pre-slaughter. The role of skeletal muscle NOS activity pre-slaughter in determining meat tenderness deserves further investigation.

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Tables and Figures

Table 1: Effect of injection time (IT, 3 hrs vs 24 hrs pre-slaughter) and L-NAME infusion (L, 0 vs 30 mg/kg) on Warner-Bratzler shear force (WBSF), Myofibrillar Fragmentation Index (MFI), sarcomere length (SL) and the pH and 1 hr or 24 hrs (ultimate) post-slaughter for the muscles *longissimus thoracis* and *semimembranosus*.

Injection time	3 hrs		24 hrs			F-value		
L-NAME	0	30	0	30	SED	L	IT	L x IT
Longissimus thoracis								
WBSF (kg)	3.88	3.81	4.39	3.61	0.195	0.002	0.262	0.010
MFI	74.2	80.8	70.5	77.2	3.31	0.005	0.121	0.981
SL (um)	2.08	2.09	2.22	2.01	0.077	0.072	0.688	0.048
pH 1 hr	6.58	6.67	6.58	6.57	0.084	0.495	0.374	0.403
pH ultimate	5.53	5.52	5.54	5.52	0.013	0.221	0.565	0.755
Semimembranosus								
WBSF (kg)	3.78	3.67	3.99	3.84	0.147	0.088	0.057	0.897
MFI	70.2	73.7	66.8	73.2	3.57	0.049	0.440	0.570
SL (um)	2.10	2.07	2.01	2.06	0.064	0.876	0.316	0.384
pH 1 hr	6.35	6.43	6.43	6.47	0.055	0.144	0.137	0.580
pH ultimate	5.51	5.50	5.50	5.50	0.015	0.349	0.513	0.711