INFUSION OF NITRIC OXIDE DONORS AND INHIBITORS INTO LAMBS INFLUENCES PLASMA METABOLITES, POSTMORTEM MUSCLE METABOLITES AND MEAT QUALITY

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Introduction

Nitric oxide (NO) is a signalling molecule that regulates processes such as force production, blood flow, glucose metabolism, calcium homeostasis, and proteolysis in skeletal muscle (Balon and Nadler, 1997; Zhang et al., 2004). The generation of NO by nitric oxide synthase (NOS) activity in animal skeletal muscle is directly related to the degree of relaxation (stretching), leading to an increased provision of blood/nutrient supply (Rubinstein et al., 1998) and swelling of muscle cells. Due to the multi-faceted effects of NO in skeletal muscle, it is postulated that altered levels of NO pre-slaughter may affect muscle contraction and glycogen depletion in skeletal muscle pre-slaughter which may impact on meat quality. Infusion of the NOS inhibitor, L-NAME 2 h prior to slaughter has been reported to increase post-slaughter glycolysis and improve tenderness of muscle *longissimus thoracis* (LT) but not the *semimembranosus* (SM) muscle in lambs (Cottrell et al., 2002).

Objectives

To investigate the effect of infusion of a NOs inhibitor (L-NAME) or a NO donor (L-Arginine) or both on plasma and muscle metabolites and subsequent effects on meat quality.

Methodology

Forty second cross ((Merino x Border Leicester) x Poll Dorset) wether lambs (12 m old) weighing 43-45 kg were blocked into four groups on body weight. The groups were brought into a shed, acclimatised for 2 weeks and then randomly divided into one of four treatments in a 2 x 2 factorial design. At 24 h prior to slaughter, lambs were catheterised via the jugular vein and on the day of slaughter, at 190 min pre-slaughter, the lambs were treated with either (1) Control (30 mL 0.9% Nacl), (2) L-Arginine.HCl = Arginine (ARG, 500 mg/kg body weight), (3) L-NAME = L-NAME (30 mg/kg body weight) or (4) L-Arginine plus L-NAME = BOTH. Blood samples were collected from all lambs at regular intervals before and after the infusion time. Plasma was centrifuged and separated, then stored frozen until analysis for glucose, lactate and NOx (nitrate abd nitrite) as described in Cottrell et al. (2004). At 190 min post infusion, lambs were slaughtered, samples of LT

and SM were collected at 15 min, 1h, 3h and 24h post-slaughter for muscle glycogen and lactate determination and muscle pH recorded at 1 (initial) and 24 h (ultimate) post-mortem. Samples of LT and SM were removed at 24 h post-slaughter and vacuum packaged for the measurement of Warner-Bratzler shear force (WBSF), Myofibrillar Fragmentation Index (MFI), sarcomere length and surface colour (L*a*b*) after a 30 min bloom, after 0 and 3 days of ageing at 2^{o}C. Muscle metabolites and meat quality characteristics were determined as described by Cottrell et al. (2002;2004). Data were subjected to ANOVA with a 2x2 factorial design using day of infusion and lamb as blocks. Main treatment effects of ARG, L-NAME and their interaction on plasma metabolites, muscle glycogen and muscle lactate was compared with sampling time relative to time of infusion (ARG x L-NAME x Time). Meat quality aspects were reported for arginine, L-NAME main effects and their interaction (ARG x L-NAME) as there was no treatment x time effect.

Results & Discussion

Lambs receiving either arginine alone or both arginine and L-NAME had higher plasma glucose ($P < 0.001$) compared with control or L-NAME treatment (Figure 1a). There was an ARG x Time interaction ($P < 0.001$) such that ARG caused an increase in plasma glucose up to 120 min post-infusion compared to those lambs receiving control or ARG. Plasma lactate concentration was reduced ($P < 0.001$) by ARG compared to L-NAME (Figure 1b). However, there was an interaction (Arg x L-NAME; $P < 0.03$) such that ARG and L-NAME together caused a prolonged reduction in lactate. There was no effect of arginine or L-NAME or interactions on plasma NOx concentration measured at 120 min post-infusion ($P > 0.05$; Table 1). Over the 15 min to 24 h post-mortem, L-NAME treatment tended to have higher ($P = 0.09$; 0.98 vs 1.04 g/100g muscle) LT glycogen while ARG ($P = 0.08$; 1.27 vs 1.42 g/100g muscle) treatment tended to have higher SM glycogen compared to control lambs. Arginine increased (0.39 vs 0.41 g/100g muscle; $P < 0.041$) and L-NAME decreased (0.42 vs 0.38 g/100g muscle; $P < 0.039$) SM lactate content over the 15 min to 24 h period, respectively.

L-NAME treated lambs had a higher 1 h pH post-slaughter in the SM ($P < 0.01$) while ARG treated lambs had a lower 1 h pH in the LT ($P < 0.08$) and SM ($P < 0.05$) muscles but there were no differences in 24 h pH (Table 1). Neither surface colour of the LT and SM muscle measured as lightness (L*), redness (a*) and yellowness (b*) after 0 and 3 days of ageing nor meat quality characteristics evaluated as sarcomere length, purge, MFI or cook loss was affected by any treatment ($P > 0.05$, data not presented). L-NAME decreased the WBSF of the SM for 0 day aged muscle (4.38 vs 4.73, $P < 0.03$) particularly when arginine was simultaneously infused (L-NAME x Arg; $P< 0.05$).

In summary, infusion of a NO donor (L-Arginine.HCl) at 500 mg/kg body weight significantly altered blood glucose and lactate concentration in lambs over the 120 min post-infusion relative to control and L-NAME. Infusion of a NOS inhibitor (L-NAME) at 30 mg/kg body weight did not change blood glucose or lactate levels compared with control lambs. There was a tendency for increased glycogen in the LT and SM with L-NAME treatment over the post-slaughter period. Post-slaughter muscle lactic acid production was significantly increased by ARG and decreased by L-NAME only in the SM but not in the LT. These were attributed to a decrease and increase in muscle pH in

the SM 1h post-mortem with ARG and L-NAME treatment, respectively. Tenderness, as measured by WBSF, was reduced in the SM at 1 day post-slaughter by infusion of the NOS inhibitor, particularly when combined with infusion of the NO donor. It is not clear whether the lower lactate levels and higher pH in muscle SM with NOS inhibition pre-slaughter was causative in the significant increase in toughness of the SM at one day post-slaughter.

Conclusions

Manipulation of endogenous nitric oxide levels *in vivo* through the infusion of nitric oxide donors and nitric oxide synthase inhibitors pre-slaughter influenced plasma metabolites and post-slaughter muscle metabolites and the tenderness of a leg muscle.

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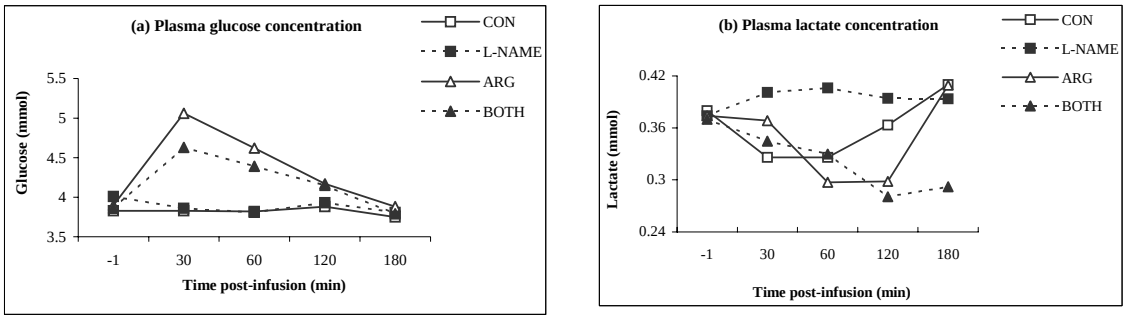
Tables and Figures

Table 1: Plasma NOx (nitrate and nitrite) concentration, initial (1 h) and ultimate pH (24 h) and Warner-Bratzler shear force (WBSF) values of muscle *longissimus lumborum* (LT) and *semimembranosus* (SM) measured after 0 (0d) and 3 days (3d) of ageing from lambs treated with L-arginine methyl ester hydrochloride (L-NAME, 0 or 30 mg/kg body weight) or arginine (0 or 500 mg/kg body weight)

L-NAME (L)	0		30		S.E.D.	P-Value		
Arginine (A)	0	500	0	500		A	L	L x A
Plasma NOx (μmol)	11.10	13.38	12.38	11.02	2.0	0.75	0.71	0.21
Initial pH-LT	6.89	6.84	6.93	6.83	0.06	0.08	0.68	0.65
Ultimate pH-LT	5.70	5.71	5.71	5.72	0.02	0.31	0.61	0.88
Initial pH-SM	6.42	6.36	6.64	6.48	0.08	0.05	0.01	0.36
Ultimate pH-SM	5.62	5.62	5.63	5.64	0.02	0.31	0.20	0.69
0d WBSF LT (kg)	6.40	6.28	6.23	5.75	0.44	0.35	0.28	0.58
3d WBSF LT (kg)	5.71	5.29	5.49	5.41	0.43	0.42	0.86	0.59
0d WBSF SM (kg)	4.56	4.20	4.57	4.89	0.22	0.88	0.03	0.04
3d WBSF SM (kg)	4.22	3.87	3.88	4.16	0.31	0.86	0.92	0.16

Means of plasma NOx, muscle pH, and WBSF are average of 10 lambs, 10 lambs x 2 and 10 lambs x 6 measurements, respectively.

Figure 1: Plasma (a) glucose and (b) lactate concentration of lambs infused with saline (control, CON), L-NAME, arginine (ARG) or arginine and L-NAME (BOTH) in relation to time of infusion. P



= 0.001, 0.340, 0.116, 0.800 for arginine, L-NAME, ARG x L-NAME and ARG x L-NAME x Time, respectively for glucose and P = 0.001, 0.737, 0.030, 0.220 for arginine, L-NAME, ARG x L-NAME and ARG x L-NAME x Time, respectively for lactate concentration.