NITRIC OXIDE PRODUCTION IN PRE-RIGOR *SEMIMEMBRANOSUS* PORK MUSCLE

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2019. Amino acid alternative curing system. Patent Attorney Docket No. TAMU-19489 December 2019, issued 2019.,

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I. OBJECTIVES

The objective of this study was to determine whether the L-arginine activated nitric oxide synthase (NOS) system found in living skeletal muscle is still functional in pre-rigor porcine muscle via the addition of L-arginine to produce nitric oxide (NO) and residual nitrite.

II. MATERIALS AND METHODS

Pre-rigor pork *semimembranosus* muscle samples (60–75 g) were collected from 4 pre-rigor pork carcasses harvested at 6 separate times over a 4-d period ($n = 24$). Subsamples (~5 g) were placed in separate test tubes and treated with one of 5 concentrations of 2 mL L-arginine solution (2, 4, 8, 16, or 32 mM) with control water (0 mM). Additionally, a solution containing 72 mg NaCl, 576 ppm sodium erythorbate, and 8 mL of water was added to each sample ($n = 288$). Samples were either heated (water bath cooking samples to 62°C within 60 min) or left raw (uncooked). All treated samples were analyzed for residual nitrite by analyzing supernatant and pellet samples using modified ultraviolet/visible spectrophotometric method. Cooked sample supernatant and pellets were analyzed for nitrosylation (determination of nitrosylhemochromagen) after storage (−30°C; freezer for 3 wk, then thawed in 0°C cooler).

III. RESULTS

For raw supernatants, the 32 mM L-arginine treatment had higher residual nitrite levels (14.3 ppm) compared to the control (0.08 ppm; $P < 0.05$). The 4 mM (8.00 ppm), 8 mM (9.60 ppm), and 16 mM (9.23 ppm) treatments were different ($P < 0.05$) than the control and 32 mM treatment. Residual nitrite levels of 4 mM cooked supernatants were higher (8.79 ppm; $P < 0.05$) compared to other treatments and control (0.07 ppm). The observed bimodal effect is similar to endogenous activation of the NOS system by L-arginine and NO synthesis. The NO produces stable nitrite when extracellular L-arginine concentrations increase from 0.5 to 5 mM. The L-arginine addition to pre-rigor samples activated the NOS system (4 mM) and at higher L-arginine concentrations (32 mM) resulted in more efficient NO generation. This occurred by reducing the interference of citrulline slowing NOS system to regenerate L-arginine as a substrate for the NOS system for residual nitrite and nitrosylhemachromagen (NO-heme) formation. For cooked muscle pellets, the 32 mM L-arginine treatment exhibited the highest residual nitrite values (15.7 ppm; $P < 0.05$) compared to other treatments and the control (0.04 ppm). The 2 mM (10.5 ppm), 4 mM (9.35 ppm), 8 mM (11.2) and 16 mM (14.1 ppm) L-arginine treatments were different than the control but not significantly different between each other. In cooked supernatants, no differences were observed for any L-arginine concentration for NO-heme or nitrosylation. However, all L-arginine concentrations exhibited nitrosylation values over 100%. This suggests that the NOS system did generate NO and residual nitrite. In cooked pellets, there was a difference ($P < 0.05$) in NO-heme levels for all L-arginine treatments compared to the

control. For total heme levels, all treatment concentrations were higher than the control, while no differences existed between the treatments. Percent nitrosylation was greatest for the 32 mM L-arginine treatment (156.72%) and was different ($P < 0.05$) than the other concentrations.

IV. CONCLUSION

In conclusion, raw supernatant and pellet samples had higher residual nitrite levels compared to cooked supernatants and pellets. Cooked pellets' nitrosylation was significantly higher compared to the control. Based upon these study results, there was evidence that the NOS system can generate NO and residual nitrite by L-arginine addition in pre-rigor pork muscle.

Keywords: L-arginine, nitric oxide synthase, nitrite