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TIME FOR BEEF CATTLE TO RECOVER FROM A BOUT OF STRESS AND THE UTILITY OF CARCASS ELECTRICAL STIMULATION TO AUGMENT THIS PROCESS

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I. INTRODUCTION

Color is a critical beef quality attribute that significantly influences consumer purchasing decisions and product value. Meat color is predominantly regulated by myoglobin content and the rate and extent of postmortem pH decline, the latter ranging from 7.4 in living muscle to around 5.5 in meat. If glycogen reserves are exhausted prior to harvest, postmortem acidification will be reduced, resulting in a condition known as dark-cutting (DC) or dark, firm, and dry (DFD) beef. Dark-cutting beef can be induced through the administration of epinephrine injections [1]. Although single doses of epinephrine pre-harvest mimic atypical dark beef, and two doses recapitulate typical DFD [2], the exact duration required for an animal to recover from this insult and attain a normal ultimate pH remains unclear. Therefore, the aim of this study is to determine the duration of time required for an animal's full physiological recovery following exposure to stress, aiming to avoid DFD occurrence, and investigate the use of electrical stimulation to reduce the incidence of stress-induced dark beef development.

II. MATERIALS AND METHODS

All procedures used were approved by the Virginia Tech Institutional Animal Care and Use Committee (23-244). Thirty-two market-weight steers were randomly assigned to one of four treatments. Twenty-four animals received two subcutaneous injections of epinephrine (0.06mg/kg.BW) 24 hrs apart and were assigned to three different pre-harvest recovery periods, one (T1D, n=8), three (T3D, n=8), or five (T5D, n=8) days after injection. The remaining eight animals served as negative controls (CON). At harvest, blood was collected for metabolite analyses. One hr post-exsanguination, the liver was collected, carcasses were split, and one side was subjected to electrical stimulation (ES, 300V, 15 Hz), while the other side served as a non-stimulated control (NS). Samples from the *Longissimus lumborum* muscle (LL) were collected at 1 hr (pre-ES) 24 hrs postmortem for metabolite and pH analyses. Moreover, after 24 hrs of chilling, 2.54 cm LD steaks were collected from each side and allowed to bloom for 0.5 hr for color measurements. Data were analyzed as a completely randomized design considering the fixed effects of treatments and harvest day as a random effect. Differences were considered statistically significant when $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Animals T1D showed lower ($P = 0.0139$) plasma lactate concentrations at harvest. Plasma lactate is an indicator of stress and was expected in T1D animals, however, we speculate that because these animals were still under prolonged stress, their energy reserves were getting exhausted, slowing down glucose recycling through lactate in the liver (Table 1). Although no differences in glucose between treatments were observed in the liver, animals T3D and T1D showed lower glycogen levels ($P = 0.0021$). Glycogen concentrations in LL were higher ($P < 0.0001$) in the CON cattle, followed by T5D, while the T3D and T1D animals had the lowest glycogen concentrations. Moreover, the LL muscle G6P was higher ($P < 0.03$) in animals CON and T5D, suggesting a faster rate of glycolysis in these treatments, which was interesting as we expected a higher glycogen phosphorylase activity in more stressed animals (T3D and T1D). We speculate that the lower G6P concentrations at 1 hr postmortem in T1D carcasses could be due to the lower glycogen concentrations at harvest, suggesting that these animals were reaching the limit of glycogen metabolism earlier than CON and T5D, slowing down the formation of G6P. Even though treated animals had lower glycogen concentrations than CON animals, T5D and T3D had enough substrate for proper acidification, as indicated by similar ultimate pH as CON tissue. Muscle from T1D cattle had low glycogen at harvest and the highest ultimate pH (Table 2). Yet, glycogen levels were similar between T1D and T3D cattle muscle, but differences in ultimate pH

were detected (P < 0.0001). These data suggest the type of glycogen deposited in T3D muscle may differ from that of T1D cattle, or regulation of glycolysis may differ with time post-stress. Additional analyses will be necessary to elucidate the exact mechanism.

Muscle pH plays a major role in color development. Our data showed that T1D animals had higher ultimate pH values (P < 0.0001) and lower (P < 0.0001) overall color measurements than other treated and CON cattle. Interestingly, although T3D animals had similar ultimate pH values to those of CON cattle, their luminosity (L*) values were lower (P < 0.0001). In contrast, a* values appeared to correlate well with the ultimate pH of muscle. Use of electrical stimulation at 1 hr postmortem in these carcasses had some effect on the ultimate pH in the muscle of CON and T1D cattle, but not enough to impact color characteristics.

Table 1. Mean, SEM, and probability of metabolite concentrations in plasma and *Longissimus lumborum*.

Metabolites	Control	T5D	T3D	T1D	P-value
		Plasma			
Lactate, mM	5.38 ± 0.73^{a}	5.36 ± 0.67^{a}	6.52 ± 0.67^{a}	3.03 ± 0.67^{b}	0.0139
Glucose, mM	4.87 ± 0.43^{ab}	4.71 ± 0.46^{ab}	5.95 ± 0.43^{a}	4.05 ± 0.43^{b}	0.0408
		Liver			
Glucose, µM/g	221.91±2.39	215.18±2.39	220.77±2.56	222.22±2.56	0.1737
Glycogen, µM/g	204.18±16.48^{a}	189.98±16.48^{ab}	126.90±17.26^{c}	163.03±17.26^{bc}	0.0021
		Longissimus lumborum			
Lactate, µM/g	16.49 ± 0.17	14.52 ± 1.37	16.70 ± 1.47	15.40 ± 1.59	0.6829
Glucose-6-phosphate, µM/g	4.13 ± 0.49^{ab}	4.49 ± 0.52^{a}	3.04 ± 0.49^{bc}	2.83 ± 0.52^{c}	0.0280
Glucose, µM/g	1.76 ± 0.29	1.63 ± 0.34	1.34 ± 0.31	1.31 ± 0.29	0.6485
Glycogen, µM/g	78.73 ± 4.38^{a}	67.85 ± 4.38^{b}	52.31 ± 4.38^{c}	42.08 ± 4.38^{c}	<.0001

Table 2. Mean, SEM, and probability of pH and color measurements (L*, a*, and b*) of *Longissimus lumborum* at 24 hours post-harvest.

Traits	Control		T5D		T3D		T1D		*P-value*
	NS	ES	NS	ES	NS	ES	NS	ES	
pH	5.62±0.03^{cd}	5.49±0.03^{e}	5.58±0.03^{cde}	5.54±0.03^{de}	5.65±0.03^{c}	5.63±0.03^{cd}	5.96±0.04^{a}	5.83±0.04^{b}	<.0001
L*	39.26±0.72^{ab}	39.72±0.72^{a}	38.72±0.77^{abc}	39.48±0.77^{ab}	37.26±0.72^{cd}	37.67±0.72^{bcd}	34.82±0.72^{e}	35.84±0.72^{de}	0.0001
a*	22.57±0.49^{a}	22.51±0.49^{a}	21.67±0.53^{a}	22.78±0.53^{a}	22.03±0.49^{a}	22.14±0.49^{a}	18.54±0.49^{b}	18.77±0.49^{b}	<.0001
b*	12.85±0.39^{a}	12.43±0.39^{ab}	11.75±0.42^{b}	12.58±0.42^{ab}	11.71±0.39^{b}	11.83±0.39^{ab}	9.14±0.39^{c}	9.46±0.39^{c}	<.0001

Means bearing different superscripts within row differ.
L*- luminosity
a*- redness
b*- yellowness.

IV. CONCLUSION

In conclusion, these data suggest that a three-day recovery period was sufficient for animals to achieve normal ultimate pH values and redness. However, luminosity appeared to require a longer recovery. A five-day period was necessary for animals to match the color and pH values observed in non-treated control cattle. Ongoing analyses aim to further explore the relationship between pH and color, and to identify additional factors contributing to the atypically dark beef observed in muscle of T3D animals.

REFERENCES

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