PERI MORTEM MUSCLE BIOCHEMISTRY IN AN ANIMAL MODEL OF ACUTE STRESS

Dario G. Pighin[1, 3, 4 *], Sebastian A. Cunzolo[3, 4], Maria Zimerman[2], Ernesto Domingo[2], Adriana A. Pazos[1, 4], Gabriela Grigioni[1, 3, 4]

[1] Instituto Tecnología de Alimentos, Instituto Nacional de Tecnología - INTA, De Los Reseros y Las Cabañas, Morón, Argentina
[2] Estación Experimental INTA Bariloche, Modesta Victoria 4450, San Carlos de Bariloche, Río Negro, Argentina
[3] Consejo Nacional de Investigaciones Científica y Técnicas - CONICET, Av. Rivadavia 1917, Buenos Aires, Argentina
[4] Facultad de Agronomía y Ciencias Agroalimentarias, Universidad de Morón, Cabildo 134, Morón, Argentina.

[*] Corresponding author (phone: +54-11-4621-0446; fax: +54-11-4621-2012; e-mail: dpighin@cnia.inta.gov.ar)

Abstract – Increased levels of stress hormones in the muscle could lead to *post mortem* metabolic/structural modifications which could also be reflected on meat quality. Present study investigated the metabolic effect of either adrenaline or cortisol injected into lambs. Results obtained demonstrated increased glucose metabolism and increased muscle temperature linked to adrenaline or cortisol injection. Muscle pH immediately *post mortem* was affected by adrenaline, but not by cortisol injection. Final muscle pH and temperature were not affected by hormones injection. WHC of fresh muscle was not altered neither by adrenaline nor by cortisol treatments. The animal model of acute stress used succeeded in emulating a biological acute stress. Ageing studies of meat quality are in progress in order to test the hormone effect on the development of meat quality.

Key Words – stress hormones, muscle metabolism, meat quality.

I. INTRODUCTION

Recent data have reported a possible effect of stress hormones (catecholamines and/or cortisol) on metabolic and/or structural *post mortem* modifications in the muscle. These effects would be independent of the glycogen levels or final pH, and may affect *post mortem* proteolytic processes leading to altered water holding capacity, tenderness and flavor during meat aging [1, 2, 3].

It is known that animals´ response against stress usually displays great variability. Also, different stressors usually elicit very different types of biological responses [4]. Thus, experimental models of animals injected with stress hormones represent an interesting approach to study individual hormone effect on the *post mortem* biochemistry of muscle and its consequence/s on meat quality.

The aim of the present study was to investigate the effect of *pre mortem* adrenaline or cortisol injected animals on the biochemistry and key muscle *post mortem* events.

II. MATERIALS AND METHODS

Twenty-seven (27) Merino Australian lambs of 15 months of age and of 36.8 kg mean liveweight were randomly assigned to one of the following groups:

- control group (C): animals injected with saline solution -NaCl 0.9%.

- adrenaline group (A): animals injected with adrenaline (Sigma-Aldrich) solution (0.2 mg adrenaline/kg body weight).

- cortisol group (Co): animals injected with cortisol (Sigma-Aldrich) solution (2.86 mg cortisol/kg body weight).

-

Hormone injection was performed via jugular vein. Injected doses of adrenaline and cortisol were selected considering published experimental protocols and physiological hormone levels in sheep [5, 6].

Once injected, the C group was electrically stunned and exsanguinated. The A and Co groups were stunned and slaughtered 3-5 and 10-12 minutes after injection, respectively. Delay times between injection and slaughter were stated considering half-life of hormones and heart output of sheep.

Rectal temperature was recorded at slaughter procedure (Ti) using a digital thermometer with resolution of 0.1 °C.

Blood samples were collected at exsanguination to determine hematocrit (microhematocrit method), glucose (GOD/POD enzymatic method, Wiener kit, Rosario, Argentina), lactate (enzymatic method, Randox kit, UK), adrenaline (ELISA method, DiaSource kit, Belgium), cortisol (ELISA method, DiaSource kit, Belgium) and CK activity (kinetic method, Wiener kit, Rosario, Argentina).

Post-mortem Longissimus dorsi (*LD*) temperature was recorded at 45 min (T_{45}) and 24 h (T_u) *post-mortem* using temperature data loggers (Maxim, CA) placed 2 cm near to the place of pH-measurement. The muscle pH was also measured at 45 min (pH_{45}) and 24 h (pH_u) *post-mortem* with a Testo pHmeter (model 230, Testo, BA, Argentina) equipped with a glass pH electrode and a temperature probe.

LD and *Psoas Major (PM)* instrumental color (L*, a* and b*) was measured using a Minolta CR-400 colorimeter (Konica Minolta Sensing Inc., Bergen, NJ) using D65 illuminant and an 8-mm aperture. Two scans were collected from the surface of each muscle, avoiding areas of connective tissue or intramuscular fat. Each muscle was allowed to bloom for 30 min at 2 ± 1 °C before color measurement.

Water holding capacity (WHC) of LD muscle 24 h *post-mortem* was determined according to the compression method described by Pla Torres [7].

Results are expressed as mean ± standard deviation. Data collected was analyzed according to the PROC MIXED procedure of SAS v.9.1 statistical package (SAS Inst. Inc. Cary, USA). Differences between the mean values of each treatment vs. control were analyzed by Kruskal-Wallis´ test.

III. RESULTS AND DISCUSSION

Blood parameters determined at exsanguination are shown in Table 1. As can be seen, adrenaline injection increased blood glucose and lactate levels and CK activity. These findings agree with previously published results demonstrating that catecholamines changed energy metabolism [8].

In the present study, increased blood activity of CK suggests increased *peri-mortem* muscle activity and, possibly, increased muscle damage as a result of this activity. This finding agrees with previous published results but diverges with others [9, 10]. The intensity of stress and its related hormone level could be the reason for this variability.

Hematocrit was not affected as a result of the treatments. It was expected that adrenaline treatment increased red blood cell circulation by means of spleen contraction. The reduced period of time between adrenaline injection and slaughter (2-5 min) was not probably enough to exhibit increased hematocrit levels.

Instrumental color of LD and PM muscles and WHC of LD muscle are shown in Table 2. As showed, non-significant changes could be recorded as a result of adrenaline or cortisol injection. These results could be associated to the fact that non-significant differences in pH_u were observed between treatments and control group.

Rectal temperature (T_i) and LD muscle temperature and pH values recorded are shown in Table 3. Hormones injection significantly increased T_i and T_{45} values. This finding agrees with previous research that showed an increased body temperature as a result of stressing events (Pighin *et al.*, 2010). Interestingly, only adrenaline injection led to a significant decreased of LD pH_i, suggesting an increased rate of anaerobic glycolysis in the muscle immediately *post-mortem*.

Table 1 Blood parameters recorded in lambs injected with saline solution (C), adrenaline (A) or cortisol (Co)

	Control group (C)	Adrenaline group (A)	Cortisol Group (Co)
Hematocrit (%)	41.1 ± 3.57	43.4 ± 3.1	43.0 ± 3.46
Adrenaline (mg/L)	0.10 ± 0.08	1.20 ± 0.41 **	0.04 ± 0.03
Cortisol (µg/dL)	24.4 ± 2.6	44.8 ± 16.5 **	550.0 ± 142.3 **
Glucose (mM)	0.59 ± 0.08	0.84 ± 0.19 **	0.59 ± 0.05
Lactate (mM)	4.80 ± 0.74	6.10 ± 0.72 *	6.90 ± 0.50 *
CK activity (U/L)	80.1 ± 24.6	116.1 ± 33.6 *	105.4 ± 58.8

* Significant effect between A and C or Co and C, $p<0.05$; ** significant effect between A and C or Co and C, $p<0.001$

Table 2 Instrumental color of *LD* muscle and *PM* muscle and WHC of *LD* muscle from lambs injected with saline solution (C), adrenaline (A) or cortisol (Co)

	Control Group (C)	Adrenaline Group (A)	Cortisol Group (Co)
L* LD	38.00 ± 1.97	38.40 ± 1.30	37.40 ± 1.60
a* LD	20.10 ± 1.20	20.80 ± 0.60	19.90 ± 0.93
b* LD	6.74 ± 0.47	7.20 ± 0.62	7.10 ± 0.93
WHC LD	30.4 ± 5.8	29.9 ± 3.9	30.1 ± 3.5
L* PM	41.80 ± 2.13	43.10 ± 1.70	42.50 ± 1.80
a* PM	23.75 ± 1.30	24.10 ± 1.40	24.30 ± 1.30
b* PM	9.00 ± 0.59	9.40 ± 0.80	9.50 ± 0.75

* Significant effect between A and C or Co and C, $p<0.05$; ** significant effect between A and C or Co and C, $p<0.001$

Table 3 Temperature and pH decrease in LD muscle from lambs injected with saline solution (C), adrenaline (A) or cortisol (Co)

	Control Group (C)	Adrenaline Group (A)	Cortisol Group (Co)
T_i	39.4 ± 0.2	40.0 ± 0.2 **	39.8 ± 0.1 *
T_{45}	35.0 ± 0.3	35.9 ± 1.0 *	36.2 ± 0.5 **
T_u	13.3 ± 1.4	13.2 ± 1.6	13.3 ± 1.4
pH_i	6.50 ± 0.10	6.20 ± 0.10 **	6.40 ± 0.20
pH_u	5.40 ± 0.03	5.40 ± 0.03	5.45 ± 0.47

* Significant effect between A and C or Co and C, $p<0.05$; ** significant effect between A and C or Co and C, $p<0.001$

IV. CONCLUSION

The single *ante-mortem* dose of adrenaline or cortisol used in the present study changed the *peri-mortem* animal metabolism, mimicking the physiological response to acute stress. Adrenaline induced increased glucose metabolism and affected initial pH and temperature of LD muscle, suggesting a faster anaerobic glycolytic rate. Cortisol also affected muscle *pre-rigor* temperatures (T_i and T_{45}), but did not affect the initial muscle pH. No effect of injected hormones was observed on fresh meat quality measured by means of instrumental color or WHC. Ageing studies are being carried out in order to observe possible alterations of meat quality development as a result of mentioned *peri-mortem* changes.

ACKNOWLEDGEMENTS

Authors thank the financial support from INTA and University of Moron. Authors also express their gratitude to Lic. Ana Sancho for performing the statistical analysis.

REFERENCES

1. Lensink, B. J., Fernandez, X., Gozzi, G., Florand, L. & Veissier, I. (2001). The influence of farmer behaviour on calves reactions to transport and quality of veal meat. Journal of Animal Science, 79: 642–652.
2. Warner, R. D., Ferguson, D. M., Cottrell, J. J. & Knee, B. (2007). Acute stress induced by the use of electric prodders pre-slaughter causes tougher beef meat. Australian Journal of Experimental Agriculture, 47: 782–788.
3. Ferguson, D.M. & Warner, R.D. (2008). Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? Meat Science, 80: 12–19.
4. Moberg, G. P. (2001). Biological response to stress implications to animal welfare. In G. P. Moberg & J. A. Mench (Eds.), The biology of animal stress – Basic principles and implications for animal welfare (pp. 1–22). Oxon, UK: CABI Publishing.
5. Watanabe, A., Dalyb, C.C. & Devine, C.E. (1996) The Effects of the Ultimate pH of Meat on Tenderness Changes During Ageing. Meat Science, Vol. 42, No. 1: 67-78.
6. Bond, J.J., Can, L.A. & Warner, R.D. (2004) The effect of exercise stress, adrenaline injection and electrical stimulation on changes in quality attributes and proteins in Semimembranosus muscle of lamb. Meat Science, 68: 469–477.
7. Pla Torres, M. (2005). Capacidad de retención de agua. In: Caneque, V., Sañudo, C. (Eds.), Estandarización de las metodologías para evaluar la calidad del producto (animal vivo, canal, carne y grasa) en los rumiantes. Monografías INIA, Madrid, España, Serie Ganadera No. 3, pp. 243–250.
8. Kuchel, O. (1991). Stress and catecholamines. In G. jasmin & M. Cantin (Eds.), Stress revisited 1. Neuroendocrinolgy of stress (pp. 80–103). Basel, Switz: Karger.
9. Warner, R.D., Bond, J.J. & Kerr, M.G. (2000). Meat quality traits in lamb *M. Longissimus thoracis et lumborum*: The effect of pre-slaughter stress and electrical stimulation. In Proceedings 46th International Congress of Meat Science and Technology (pp. 154–155), 27 August-1 September 2000, Buenos Aires, Argentina.
10. Zimerman, M. Grigioni, G., Taddeo, H. & Domingo, E. (2011) Physiological stress responses and meat quality traits of kids subjected to different pre-slaughter stressors. Small Ruminant Research, Vol. 100, No.2-3: 137-142.
11. Pighin, D.G, Warner, R.D., Brown, W., Ferguson, D.M. & Fisher, A.D. (2010) Predicting post-slaughter muscle glycogen content and dark-cutting through changes in pre-slaughter body temperature of lambs. In Proceedings 56th International Congress of Meat Science and Technology (pp. 100), 15-20 August 2010, Jeju, South Korea.