

# Impact of Adrenaline or Cortisol Injection on Meat Quality Development of Merino Hoggets

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## Abstract

Increased levels of stress hormones in the muscle could lead to *post mortem* metabolic/structural modifications that could be reflected on meat quality. The present study investigated the metabolic effect of either adrenaline or cortisol injected into lambs in order to obtain an animal model of acute stress. Results showed that adrenaline or cortisol injection lead to glucose metabolism and muscle temperature increase. Muscle pH immediately *post mortem* was affected by adrenaline treatment. Water holding capacity (WHC) of fresh muscle, final muscle pH and temperature registered at 24 h *post mortem* were not affected by injected hormones. Hardness and adhesiveness of LD muscle evaluated 3 d *post mortem* tended to increase as a result of adrenaline or cortisol injection. Results demonstrated that injected hormones were able to affect the *post mortem* muscle biochemistry and the pH/T curve independently of final muscle pH.

**Key words:** stress hormones, muscle metabolism, meat quality

## INTRODUCTION

The biology of animal stress involves the activation of the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (Moberg 2000). The autonomic response, mediated by catecholamines (adrenaline and noradrenaline), is initiated in reaction to stressors that require a rapid response and include several metabolic effects. On the other hand, the activation of HPA axis, manifested by glucocorticoids release (e.g., cortisol), tends to amplify the metabolic effects of catecholamines, although the response is typically slower (Moberg 2000). Emerg-

ing evidences support that the effects of these stress hormones (catecholamines and/or cortisol) include metabolic and/or structural *post mortem* modifications in the muscle (Ferguson and Warner 2008). These events would be independent from the muscle glycogen levels or the consequent final pH (pH<sub>w</sub>, measured at 24 h post-slaughter), and may affect *post mortem* proteolytic processes leading to altered water holding capacity (WHC), tenderness and flavor during meat ageing (Ferguson *et al* 2001; Warner *et al.* 2007; Ferguson and Warner 2008). Regarding this issue, pH and temperature decline curve would play a major role during the first 24 h *post mortem*, being able to impact on many *post mortem* events in the muscle (Dransfield

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1992; Thornberg 1996).

Several difficulties usually arise when trying to study hormone profile and biochemical response associated to animal stress. In this regard, it is known that the response of animals to stress usually shows a great variability and is conditioned by previous experience. Different stressors also induce diverse biological responses (Moberg 2000). These facts make difficult to study the effect of acute stress on meat quality development and the mechanisms involved. Thus, experimental models of animals injected with stress hormones (adrenaline and/or cortisol) represent an interesting approach to study the individual hormone effect on the *peri mortem* biochemistry in a more controlled way.

Previous results have suggested deleterious effects of injected adrenaline into animals. Bond *et al.* (2004) did not find any effect of single dose of adrenaline on lambs in the *Semimembranosus* shear force values. More recently, Warner and co-workers found a reduction in the myofibrillar fragmentation index (MFI) values in the *Longissimus* and *Semimembranosus* muscles of lambs injected with adrenaline at 3 or 24 h pre slaughter (Ferguson and Warner 2008). Regarding glucocorticoids, Sugden and Fuller (1991) have reviewed published data and postulated that if there were effects on muscle protein degradation, these effects were likely to be transient. More recently, Ferguson and co-workers did not find changes in shear force over 5 d ageing in a study where sheep were given ACTH challenge 6 h prior to slaughter to obtain a sustained elevation of blood cortisol (Ferguson and Warner 2008). In spite of this information, the effect of cortisol on the muscle protein metabolism and related meat quality is still poorly understood (Ferguson and Warner 2008). Therefore, the aim of the present study was to investigate the effect of *pre mortem* adrenaline or cortisol injection on hoggets muscle biochemistry and its consequences on meat quality.

## RESULTS AND DISCUSSION

### Levels of blood constituents

Blood parameters determined at exsanguination are

shown in Table 1. Animals injected with adrenaline displayed a significant increase (12 fold) of circulating adrenaline levels in relation to control ones. On the other hand, animals injected with cortisol solution displayed a significant increase (22 fold) of circulating cortisol levels in relation to control ones. Mean cortisol level recorded in control animals was greater than those reported by Zimmerman (2012) (22.4 vs. 10.1  $\mu\text{g dL}^{-1}$ , respectively). Control animals were injected with saline solutions. Then, it could be assumed that this procedure was the reason for cortisol rise. Taking into account the significant differences found in the hormones levels of both adrenaline and cortisol-injected animals, it is possible to believe that the animal models of acute stress initially proposed were successfully obtained.

**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±3.6	43.4±3.1	43.0±3.5
Adrenaline ( $\text{mg L}^{-1}$ )	0.10±0.08	1.20±0.41**	0.04±0.03
Cortisol ( $\mu\text{g dL}^{-1}$ )	24.4±2.6	44.8±16.5**	550.0±142.3**
Glucose ( $\text{mmol L}^{-1}$ )	0.59±0.08	0.84±0.19*	0.59±0.05
Lactate ( $\text{mmol L}^{-1}$ )	4.80±0.74	6.10±0.72*	6.90±0.50*
CK activity ( $\text{U L}^{-1}$ )	80.1±24.6	116.1±33.6*	105.4±58.8

Values expressed as mean±SD. \*, significant effect between A and C or Co and C,  $P<0.05$ ; \*\*, significant effect between A and C or Co and C,  $P<0.001$ . The same as below.

As can be seen in Table 1, adrenaline injection increased blood glucose ( $P<0.05$ ) and lactate ( $P<0.05$ ) levels and creatin-kinase (CK) activity ( $P<0.05$ ) as well. These findings support the idea that catecholamines induced an increase in the energy metabolism; which also agrees with published data (Kuchel 1991; Moberg 2000). Increased blood CK activity also suggests an increased *peri mortem* muscle activity and, possibly, increased muscle cell damage as a direct or indirect result of this stimulated activity. In this regard, available data diverge. Results published by Warner *et al.* (2000) and Kannan *et al.* (2003) showed significant rises of CK activity as a result of physical stress. On the other hand, Zimmerman *et al.* (2011) did not find significant changes in CK activity in kids submitted to fasting, exercise or fear as pre slaughter stressors. The disagreement observed in these results could be associated to differences in the intensity and

duration of induced stress and the levels of related hormones.

Cortisol injection significantly increased blood lactate level ( $P<0.05$ ). Nevertheless, a non-significant increase of hematocrit and CK activity was found. These findings would suggest changes in the energy metabolism and modifications of the muscle activity. It is worth noting that the effect of cortisol on the biochemical parameters assayed appeared to be less extended when compared to adrenaline effect.

Hematocrit was not affected as a result of the stress hormones injected. It has been established that an increment of circulating adrenaline and/or cortisol levels usually rise red blood cell circulation by means of spleen contraction (Warriss *et al.* 1995; Moberg 2000) and/or increased glomerular filtration and diuresis (Parker *et al.* 2003; Broom and Fraser 2007), respectively. Thus, in the present study increased hematocrit levels were expected. The high deviation found in the measurement of this parameter could be responsible for the absent of significant differences.

### Meat quality traits

Instrumental color and WHC of LD muscle are shown in Table 2. As can be seen, non-significant changes were 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 (Table 3). Nevertheless, since adrenaline has been proposed to affect the *post mortem* muscle protein metabolism, it could also affect WHC by means of non-dependant pH mechanisms (Ferguson *et al.* 2001; Warner *et al.* 2007; Ferguson and Warner 2008). Further effects of injected hormones on color and WHC of aged meat can not be discarded and are being studied.

Rectal temperature ( $T_i$ ) and LD temperature and pH values recorded are shown in Table 3. Hormones injection significantly increased both  $T_i$  and  $T_{45}$  (recorded 45 min *post mortem*) values. These findings agree 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 ( $P<0.001$ ) decrease of LD  $pH_{45}$  (measured 45 min *post mortem*), suggesting an in-

**Table 2** Instrumental color 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*	38.00±1.97	38.40±1.30	37.40±1.60
a*	20.10±1.20	20.80±0.60	19.90±0.93
b*	6.74±0.47	7.20±0.62	7.10±0.93
WHC	30.4±5.8	29.9±3.9	30.1±3.5

**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
$pH_{45}$	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

creased rate of anaerobic glycolysis in the muscle immediately *post mortem*.

Previous research has demonstrated that pH/T decline curve usually plays a major role in the conversion of muscle into meat (Ferguson *et al.* 2001; Devine *et al.* 2002). *Post mortem* pH and temperature kinetics can influence the rate and extent of protein denaturation, oxidation and proteolysis, lipid oxidation, color characteristics, water holding capacity and sensory aspects of meat (Lawrie 1992; Ferguson *et al.* 2001). Results obtained in the present study demonstrated that hormone injection led to changes both in T and pH, which would reinforce the importance of acute stress on meat quality development.

Data of texture profile analysis (TPA) compiled in Table 4 shows that hormone treatments tended to increased hardness and adhesiveness values measured in LD muscle 3 d *post mortem*. This tendency was especially evident in cortisol treatment, where high deviation could be responsible for the absent of significant differences. This finding indicates that a higher force is necessary to attempt a given deformation of the muscle samples. On this regard, Caine *et al.* (2003) had shown that TPA hardness and adhesiveness can be useful in explaining a significant proportion of variation in tenderness of rib steaks, where these parameters showed an inverse relationship with overall tenderness. It has been proposed that meat tenderness varies not only with the rate of glycolysis and rigor onset post-slaughter, but also with the extent of glycolysis, classically identified through the ultimate pH ( $pH_u$ ) achieved in a muscle (Ferguson *et al.* 2001). No

significant difference in  $pH_u$  was observed in the present study. Thus, it could be possible to associate the changes observed in the hardness and adhesiveness to differences in pH/T decline curve.

On the other hand, springiness and resilience evaluated in LD muscle (Table 4) showed similar values in all treatments. It has been shown that these parameters were included in prediction models of juiciness, flavor desirability and flavor intensity through stepwise regression analysis (Caine *et al.* 2003). Hardness, chewiness and springiness are usually very useful parameters for the assessment of meat texture (Ruiz de Huidobro *et al.* 2005).

Present results suggest that injected hormones can affect meat texture independently from the muscle final pH. However, subsequent studies are necessary to look further into the relationship between TPA profile – of fresh and aged meat – with stress parameters and sensory attributes.

**Table 4** Descriptive statistics for texture profile analysis parameters of LD muscle (3 d *post mortem*) from lambs injected with saline solution (C), adrenaline (A) or cortisol (Co)

Texture profile analysis parameters	Control group (C)	Adrenaline group (A)	Cortisol group (Co)
Hardness (N)	78.5±5.2	80.3±7.4	84.6±8.9
Cohesiveness	0.47±0.02	0.47±0.02	0.49±0.02
Springiness	0.43±0.02	0.44±0.02	0.45±0.02
Resilience	0.15±0.01	0.15±0.01	0.16±0.02
Adhesiveness (g s)	-22.08±3.96	-25.73±3.50	-27.88±3.84
Chewiness	16.14±1.34	16.48±2.10	18.11±1.74

## CONCLUSION

The single *pre mortem* dose of adrenaline or cortisol used in the present study changed the *peri mortem* animal metabolism, imitating the physiological response to acute stress. Adrenaline induced an increase in 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. Even though no effect of injected hormones was observed on instrumental color or WHC, stress hormones tended to affect texture profile (hardness and adhesiveness) of LD muscle. Ageing studies are being carried out in order to observe further alterations of meat quality development

as a result of mentioned *peri mortem* changes.

## MATERIALS AND METHODS

The study was carried out in the Experimental Station of INTA (National Institute of Agricultural Technology) in the Río Negro Province of Argentina.

### Animals and experimental treatments

Merino hoggets (n=27) with a mean age of 15 mon and a mean live weight of 36.8 kg were used. The study was carried out using hoggets in order to work with animals that are being promoted as meat source in Argentina. Furthermore, the selection of hoggets has the advantage of avoiding the stress associated to weaning procedure. Animals were reared under an extensive rangeland production system in the Experimental Farm Pilcaniyeu of INTA (70°35'21''W and 41°01'42''S, 970 m a.s.l.) in the Río Negro Province of Argentina. The location is characterized by shrubby-grassland steppe dominated by *Mulinum spinosum*, *Senecio filaginoides*, *Poa ligularis*, and *Stipa speciosa* (Bran *et al.* 2000). Animal handling and experimental procedures were conducted in accordance with regulation procedures for animal welfare of the National Service of Animal Health (SENASA) of Argentina.

Animals were randomly assigned to one of the following groups: control group (C), adrenaline group (A) and cortisol group (Co). Groups C, A and Co were injected with saline solution-NaCl 0.9% (p/v), adrenaline (Sigma-Aldrich, USA) solution (0.2 mg adrenaline  $kg^{-1}$  body weight) and cortisol (Sigma-Aldrich) solution (2.86 mg cortisol  $kg^{-1}$  body weight), respectively. 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 (Watanabe *et al.* 1996; Bond *et al.* 2004).

Animals from the C and A groups were electrically stunned and slaughtered 3-5 min after injection. Animals from Co group were stunned and slaughtered 10-12 min after injection. The delay between injection and slaughter procedure was established according to hormones half life and heart output of sheep.

Rectal temperature was recorded in all animals at slaughter procedure ( $T_r$ ) using a digital thermometer with a 0.1°C resolution.

### Levels of blood constituents

Blood samples were collected at exsanguination in tubes with/without anticoagulant solution (EDTA), and were immediately placed in ice. Blood tubes were then centrifuged at 1 000×g for 20 min and the resulting plasma was placed



into safe-lock microtubes and stored at  $(-20\pm 1)^{\circ}\text{C}$  until analysis.

Hematocrit (expressed as a percentage) was determined using microhematocrit capillary tubes (Tecnon, BA, Argentina) and a micro-capillary reader (catalogue number 2201, International Equipment Co., Norfolk, MA, USA). The plasma glucose level was evaluated by means of the GOD/POD Trinder color test without deproteinization (Wiener, Rosario, Argentina). Lactic acid concentration was measured using a commercial enzymatic kit (Randox Labs Ltd., UK). Creatin-kinase activity was evaluated using a commercial enzymatic kit (Wiener, Argentina). Serum adrenaline and plasma cortisol levels were measured using commercial kits provided by DiaSource (INS-EASIA, DiaSource S.A., Belgium). Intra- and inter-assay coefficients of variation were 6 and 13% for adrenaline, 3 and 5% for cortisol, respectively.

## Meat quality traits

*Post mortem Longissimus dorsi (LD)* temperature was recorded at 45 min ( $T_{45}$ ) and 24 h ( $T_u$ ) using temperature data loggers (Maxim, CA, USA) placed 2 cm from the pH-measurement site. The muscle pH was also measured at 45 min ( $\text{pH}_{45}$ ) and 24 h ( $\text{pH}_u$ ) *post mortem* with a Testo pH meter (model 230, Testo, BA, Argentina) equipped with a glass pH electrode and a temperature probe.

The instrumental color parameters expressed in CIELab system ( $L^*$ ,  $a^*$  and  $b^*$ ) of *LD* muscle was measured using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Bergen, NJ, USA) using D65 illuminant and an 8-mm aperture. Two scans were collected from the surface of the muscle, avoiding areas of connective tissue or intramuscular fat. Each sample was allowed to bloom for 30 min at  $(2\pm 1)^{\circ}\text{C}$  before color measurement.

Water holding capacity of *LD* 24 h *post mortem* was determined according to the compression method described by Pla Torres (2005). Briefly,  $(2.5\pm 0.2)$  g sample was placed on a sheet of filter paper (type 585, Schleicher & Schulle, Dassel, Germany), and compressed during 2.5 min. The area of the moisture ring was measured and expressed as a percentage of released juice.

The texture profile analysis (TPA) was performed in cooked *LD* samples. Samples were placed on aluminum-folded strips and cooked to an endpoint temperature of  $(71.5\pm 0.5)^{\circ}\text{C}$  on an electric grill (Philips, Ciudad Autónoma de Buenos Aires, Argentina). Internal temperature was monitored with a T-type thermocouple inserted in the geometric centre of each sample. After cooking, steaks were cooled at room temperature for 30 min and then chilled in a refrigerator at  $4^{\circ}\text{C}$  for 24 h. Six 1.3 cm wide and 1.3 cm high cores were obtained from *LD* section, parallel to muscle fibers. Instrumental texture assessment was done by a Texturometer TA-XT2 of Stable Micro Systems (UK) with muscular fibers parallel to the force direction. Measure-

ments were conducted using a cylindrical probe SMS P/35 (35 mm diameter) and the following settings: probe speed during test  $1\text{ mm s}^{-1}$ , final strain 70% and 1 s between the first and second stroke. Each sample was assessed 6 times in average.

In the force-by-time curve, the following TPA parameters were calculated (Caine *et al.* 2003): hardness, cohesiveness, springiness, resilience, adhesiveness, and chewiness were determined.

## Statistical analysis

Results are expressed as mean $\pm$ standard deviation. Data collected were 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 ( $P=0.05$ ).

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