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# Physiological stress responses and meat quality traits of kids subjected to different pre-slaughter stressors

M. Zimerman[a,*], G. Grigioni[b,c], H. Taddeo[a], E. Domingo[a]

[a] Estación Experimental Agropecuaria Bariloche, Instituto Nacional de Tecnología Agropecuaria INTA, San Carlos de Bariloche, Río Negro, Argentina
[b] Instituto de Tecnología de Alimentos, Centro de Investigación de Agroindustria, Instituto Nacional de Tecnología Agropecuaria INTA, Morón, Buenos Aires, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas CONICET, Buenos Aires, Argentina

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ABSTRACT

Crossbred Criollo Neuquino castrated male kids, 6 months of age and 24 kg of live weight, were used to investigate the effects of pre-slaughter stressors on physiological characteristics and meat quality attributes. On four separate days, 16 kids were randomly assigned to one of the four pre-slaughter stressor treatments (4 kids per treatment per day): (A) no stress (control); (B) 24 h of food deprivation (fasting); (C) physical stress of forced exercise by an animal handler for 30 min at approximately 3 km/h (exercise); or (D) psychological stress by placing kids in a pen with barking dogs for 5 min (fear). Fasted goats had greater ($P < 0.05$) hematocrit, urea and total protein concentrations than controls. Exercised kids had greater ($P < 0.05$) cortisol concentration than controls and goats exposed to barking dogs had greater ($P < 0.05$) hematocrit and cortisol concentration compared with controls. Even though the stressors imposed on the kids induced changes in blood constituents typically associated with the stress response, the intensity and/or duration of these stressors had little or no effect on meat quality.

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## 1. Introduction

Pre-slaughter handling has been described as one of the most stressful procedures endured by farm animals (Cockram and Corley, 1991; Wermer and Gallo, 2008). Stress produced by pre-slaughter management causes metabolic changes that can affect meat quality (Fernandez and Tornberg, 1991; Kannan et al., 2003; O'Neill et al., 2006; Muchenje et al., 2009). Meat with high ultimate pH (>5.9), commonly referred to as dark, firm and dry (DFD), is characterized by a dark colour, high water holding capacity and reduced shelf life (Ferguson et al., 2001) and depending on the ultimate pH value it has increased toughness (Purchas and Aungsupakorn, 1993).

Stressors produce a perturbation on the animal's homeostasis, consequently, an adaptive response is triggered to restore balance. Knowles and Warriss (2000) proposed some blood parameters to evaluate different stressors and indicated that the change of a variable over time in an individual animal is an indication of the extent of the response to a stressful or injurious situation. As stated by Ferguson and Warner (2008) more research is required regarding the effect of specific individual pre-slaughter stressors and the interactions between them, the biophysical changes in muscle and the consequential effects on meat quality traits.

Most of the available information on the effects of pre-slaughter handling on meat quality has been conducted using beef, pigs, broilers and sheep (Kannan et al., 1997; Brown et al., 1998; Geesink et al., 2001; Daly et al., 2006; Ferguson et al., 2007; Bond and Warner, 2007). However, there is limited information on the effects of pre-slaughter

* Corresponding author at: Instituto Nacional de Tecnología Agropecuaria INTA, Estación Experimental Agropecuaria Bariloche, Modesta Victoria 4450, CC 277, San Carlos de Bariloche, Río Negro, Argentina. Tel.: +54 2944 422731; fax: +54 2944 424991.
*E-mail address:* mzimerman@bariloche.inta.gov.ar (M. Zimerman).

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stressors on the physiological responses and meat quality of goats (Kannan et al., 2003).

In a study made by Domingo (2005), 69% of the carcasses of 'Chivito de Veranada' (5–7 month-old kids) slaughtered in a commercial abattoir had 24 h pH values greater than 6.0. This category of kids is produced in extensive systems and animals are herded for long distances from the summer rangelands before being loaded in a lorry. Hence, total transportation time may last 24 h or more followed by a variable lairage period imposing not only physical stress but also a long period of fasting. Furthermore, goats are usually herded with dogs imposing additional stress. Therefore, the aim of the present study was to determine the effects of different known short-term pre-slaughter stressors on blood parameters indicative of stress and meat quality traits in crossbred Criollo Neuquino kids.

## 2. Materials and methods

### 2.1. Site description

The study was carried out in the Experimental Farm Pilcaniyeu of INTA in the Río Negro province of Argentina (70°35′21″W and 41°01′42″S) at 970 m above sea level. The site is characterized by shrubby-grassland steppe dominated by *Mulinum spinosum*, *Senecio filaginoides*, *Poa ligularis* and *Stipa speciosa* (Bran et al., 2000). Field work was carried out in April when the maximum and minimum daily temperatures were 15–22 °C and 0.5–9.5 °C, respectively.

### 2.2. Animals and experimental treatments

Castrated, 3/4 Criollo Neuquino – 1/4 Angora kids ($n = 64$), with the mean age and live weight of 189 days and 24 kg, respectively were used. All kids originated from the same flock, were reared under an extensive rangeland production system and were weaned at an average age of 130 days. Kids were of similar phenotypic characteristics, clinically healthy, and had been vaccinated according to the standard health management practice of the experimental farm.

Animal handling and experimental procedures were conducted in accordance with regulation procedures for animal welfare of the National Service of Animal Health (Servicio Nacional de Sanidad Animal, SENASA) of Argentina. A week before the study live weight of animals was recorded. The experiment was carried out in four different days and 16 kids were randomly assigned to each day (blocking effect). Each day, four of the 16 animals were also randomly assigned to one of the four experimental treatments, constituting a total of 16 groups with four animals each. The term "group" is used to define the four animals subjected the same day to the same treatment. Before starting the treatments, all kids were penned in an open paddock and deprived of food for 6 h with free access to water.

(A) Non-stressed control: kids remained in an open paddock with *ad libitum* access to water.
(B) Fasting: kids were deprived of food, but not water, for a total of 24 h before slaughter.
(C) Exercise: kids were constantly moved for 30 min before slaughter in an open and flat paddock by a livestock handler at an estimated rate of 3 km/h.
(D) Fear: kids were penned with two barking dogs for 5 min before slaughter in an open paddock. Kids and dogs were not allowed direct tactile contact to avoid injury.

It should be noted that food (not water) was withheld for 6 h before the control, exercise and fear stressors were applied. Whereas, the 24 h fasting included the 6 h food withdrawal period that all goats received before treatment application.

### 2.3. Blood sampling

Blood samples were collected 72 h before (basal value) and immediately after stressor treatment (post-treatment), via jugular venipuncture into vacuum tubes containing 0.117 ml of 15% $K_3$ EDTA (Becton, Dickinson & Co., Bergen, NJ, USA) by trained personnel. Basal value samples were collected at 09:30 h, whereas post-treatment samples were collected at 10:00, 15:00, 16:30 and 17:30 h for kids in the fasting, control, exercise and fear treatment groups, respectively. Blood tubes were then centrifuged at $1006 \times g$ for 20 min and the resulting plasma was placed into safe-lock microtubes and stored at $-20 \pm 1$ °C pending analysis.

### 2.4. Physiological indicators

Hematocrit (HEM) was measured, in duplicate, using whole blood, whereas cortisol (CORT), urea nitrogen (PUN), total protein (TP) and creatine kinase (CK) were measured in the plasma also in duplicate. Hematocrit (expressed as a percentage) was determined using micro-hematocrit capillary tubes (Tecnon, Ciudad Autónoma de Buenos Aires, BA, Argentina) and a micro-capillary reader (catalogue number 2201, International Equipment Co., Norfolk, MA, USA). Both PUN and TP were colorimetrically determined using commercially available enzymatic kits according to manufacturer's instructions. The UREMIA test kits (code number: 1810058) used to measure PUN (g/L) and the PROTI2 test kits (code number: 1690001) used to measure TP (g/dL) were manufactured by Wiener Laboratories S.A.I.C. (Rosario, SFE, Argentina) and absorbance was measured using the Spectronic 20D+ spectrophotometer (model number: 33183-000, Thermo Fisher Scientific Inc., Middlesex, MA, USA). Plasma CORT (μg/dL) was determined using the Active Cortisol EIA assay kit (DSL-10-2000; Diagnostic Systems Laboratories, Inc., Webster, TX, USA), and absorbance was measured using a Multiskan PLUS spectrophotometer (Type 314, Labsystems, Helsinki, Finland). Finally, CK (UI/L) was quantified using the CK-NAC UV AA test kit (code number 1009309; Wiener Laboratorios S.A.I.C., Rosario, SFE, Argentina) and a Metrolab spectrophotometer (model number: 2300 Plus, Metrolab, Ciudad Autónoma de Buenos Aires, BA, Argentina).

HEM, CORT and PUN were measured in samples collected on all four days ($n = 64$), but TP and CK were only measured in blood samples collected on the last two days of the experiment ($n = 32$).

### 2.5. Slaughtering and sample collection

At the conclusion of each stressor treatment and immediately after blood sampling, kids were slaughtered at an experimental abattoir, and carcasses were chilled at $4 \pm 1$ °C for 5 h, followed by storage at $2 \pm 1$ °C for 24 h.

Temperature and pH were measured 45 min (Ti and pHi, respectively) and 24 h post-slaughter (Tu and pHu, respectively). Then, the entire *Longissimus thoracis et lumborum* muscle (LTL) was removed from the left carcass sides and refrigerated at $2 \pm 1$ °C for colour and water holding capacity measurements. A portion of the LTL between the 5th and 13th ribs was removed, vacuum-packaged, aged an additional two days at $2 \pm 1$ °C, and subsequently frozen.

### 2.6. Meat quality traits

#### 2.6.1. pH and colour

Muscle pH and temperature were measured according to the methodology suggested by Garrido et al. (2005) in the LTL between the 4th and 5th lumbar vertebra using a Testo pH meter (model number 230, Testo, Ciudad Autónoma de Buenos Aires, BA, Argentina) equipped with a glass pH electrode and a temperature probe.

Meat colour was measured according to the methodology suggested by Albertí et al. (2005). The cross-section of the LTL at the region of the 1st lumbar vertebra was allowed to bloom for 30 min at $2 \pm 1$ °C before instrumental colour ($L^*$, $a^*$ and $b^*$) 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 each LTL, avoiding areas of connective tissue or intramuscular fat.

#### 2.6.2. Water holding capacity

Water holding capacity (WHC) was determined in duplicate on LTL samples removed from the area of the 6th rib according to the compression method described by Pla Torres (2005). A 2.5 g sample was placed on a sheet of filter paper (type 585, Schleicher & Schulle, Dassel, Germany), and compressed for 2.5 min. The area of the moisture ring was measured. This procedure assumes that this area is related to the amount (weight) of the meat free juice. Results are expressed as a percentage of released juice.

**Table 1**
Least squares means of post-treatment values corrected by covariance model of physiological indicators of stress from kids exposed to control, fasting, exercise and fear treatments.

| Item | *n* | Treatments | | | | s.e.m[a] |
|---|---|---|---|---|---|---|
| | | Control | Fasting | Exercise | Fear | |
| Urea (g/L) | 64 | 0.26 | 0.29* | 0.26 | 0.25 | 0.02 |
| Cortisol (μg/dL) | 64 | 9.5 | 7.8 | 13.1* | 14.2* | 0.92 |
| Hematocrit (%) | 64 | 30 | 34* | 31 | 33* | 0.8 |
| Total protein (g/dL) | 32 | 6.3 | 6.8* | 6.3 | 6.7 | 0.12 |
| Creatine kinase (UI/L) | 32 | 276 | 216 | 225 | 206 | 46 |

[a] Standard error of least-squares means.
* For each treatment significant differences between it and the Control (Dunnett test, $\alpha = 0.05$).

#### 2.6.3. Instrumental tenderness

Frozen samples taken from LTL between 5th and 13th ribs were thawed at 4 ± 1 °C for 24 h and then cooked to an endpoint temperature of 71.5 ± 0.5 °C on an electric grill (Philips, Ciudad Autónoma de Buenos Aires, BA, Argentina). Internal temperature was monitored with a T-type thermocouple inserted in the geometric centre of each LTL sample. WBSF was determined following the general guidelines established by AMSA (American Meat Science Association, 1995) guidelines. Eight 1.27-cm diameter cores were removed parallel to the muscle fibre orientation of a 2.0-cm thick steak from the middle portion of each cooked LTL, and each core was cut once through the centre with a Warner-Bratzler shear force device (G-R Electric Manufacturing Co., Manhattan, KS 66502, USA). Results are expressed in Newtons.

### 2.7. Statistical analyses

Data were analysed as a randomized complete block design, with each day as a random block. Treatment effects were evaluated through the analysis of variance (ANOVA) using a mixed model. A covariance structure of compound symmetry was used to model the correlation between animals of the same group (16 groups with 4 animals each, subjected the same day to the same treatment). For physiological indicators, basal levels were used as covariates in the model. When significant differences were detected with the ANOVA analysis, the differences between the mean values of each treatment vs control were analysed by Dunnett's test ($\alpha = 0.05$). The statistical analysis was carried out using PROC MIXED, SAS version 8, SAS Institute Inc., 2002, Cary, NC, USA.

## 3. Results and discussion

### 3.1. Physiological indicators

Table 1 shows the concentration of physiological indicators after the application of treatments corrected by the covariance model. According to the results of ANOVA, the overall treatment *P*-value was significant for physiological indicators except for CK (0.027, <0.001, 0.001, 0.015, and 0.09 for PUN, CORT, HEM, TP, and CK, respectively).

#### 3.1.1. Plasma urea nitrogen

The overall mean ± standard error of the basal level of PUN was 0.24 ± 0.05 g/L. According to Dunnett's test results, only animals subjected to fasting showed greater levels of PUN ($P = 0.013$) than control kids. Fasting increased protein catabolism, resulting in an increase in PUN. In the present study the PUN of fasted animals was within the range values reported by Kannan et al. (2000) in mature Spanish goats after 18 h of lairage. High PUN concentrations in response to nutritional stressors were previously reported in goats by Kouakou et al. (1999).

#### 3.1.2. Cortisol

The values of CORT concentration obtained in this experiment were greater than values reported by Sanhouri et al. (1989), Kannan et al. (2000, 2002) and Greenwood et al. (2010). However, they were similar to those reported by Nwe et al. (1996) and by Greenwood and Shutt (1992). The overall mean ± standard error of the basal level of CORT was 6.8 ± 2.86 ug/dL. According to Dunnett's test results, exercise and fear caused an increase in CORT concentration ($P = 0.005$ and $P < 0.001$, respectively) compared to the non-stressed controls.

It is difficult to explain changes in CORT levels due to exercise and most probably these changes were the consequence of the fear caused by the act of a human chasing the animals during the exercise. CORT concentrations vary according to circadian rhythms showing the highest levels in the morning and the lowest values in the afternoon (Fulkerson and Tang, 1979; Parraguez et al., 1989; Dickmeis, 2009). According to these findings the lowest CORT levels should have been found in samples taken at 17:30 h (fear stressor) compared to samples collected at 15:00 h (control). The results of this study would confirm that the increase in CORT concentrations should be attributed to fear treatment.

#### 3.1.3. Hematocrit and total protein

The overall means ± standard errors of the basal level of HEM and TP were 31 ± 3.8% and 6.3 ± 0.33 g/dL, respectively. According to Dunnett's test results, exercise failed to cause a change in HEM and in TP, while fasting caused an increase in HEM ($P = 0.002$) and in TP ($P = 0.041$) and fear caused an increase in HEM ($P = 0.008$) when compared to controls.

An increase in HEM can be due to dehydration that in this case it is also associated with an increase in TP concentration (Stull and Rodiek, 2000; Broom and Fraser, 2007; Ferguson and Warner, 2008). Mitchell et al. (1988) attributed the increase in packed cell volume and in TP concentration observed in stressed animals to the movement of fluids out of the cardiovascular compartment. In the present experiment, animals exposed to fasting showed an increase in both, HEM and TP that could be due to dehydration produced by keeping the animals for an extended period (24 h) in an unfamiliar environment where they may have had limited water intake.

An increase in hematocrit also can be due to splenic contraction induced by sympathetic nerve activity or

**Table 2**
Least-squares means of meat quality traits from kids exposed to control, fasting, exercise and fear treatments.

| Item | Treatments | | | | s.e.m.[a] | *P*-value[b] |
|---|---|---|---|---|---|---|
| | Control ($n=16$) | Fasting ($n=16$) | Exercise ($n=16$) | Fear ($n=16$) | | |
| pHi | 6.18 | 6.25 | 6.24 | 6.46* | 0.09 | <0.001 |
| Ti (°C) | 33.7 | 34.9 | 36.5 | 36.1 | 1.4 | 0.315 |
| pHu | 5.62 | 5.61 | 5.66 | 5.64 | 0.11 | 0.599 |
| Tu (°C) | 7.2 | 7.4 | 7.5 | 7.6 | 1.6 | 0.182 |
| $L^*$ | 43.13 | 41.45 | 42.91 | 42.22 | 0.94 | 0.146 |
| $a^*$ | 18.83 | 18.55 | 19.43 | 18.96 | 0.43 | 0.387 |
| $b^*$ | 7.44 | 7.48 | 7.84 | 7.40 | 0.41 | 0.561 |
| WHC (%) | 29.9 | 30.1 | 28.7 | 29.7 | 1.3 | 0.775 |
| WBSF (N) | 34.2 | 34.8 | 32.8 | 34.7 | 2.4 | 0.752 |

[a] Standard error of least-squares means.
[b] *P*-value for the global model.
* For each treatment significant differences between it and the Control (Dunnett test, $\alpha = 0.05$).

circulating catecholamines caused by stressor factors. As it was previously shown, animals exposed to fear also showed an increase in CORT which would explain the HEM increase. Parrott et al. (1987) reported that high plasma cortisol concentration was associated with a low level of the antidiuretic hormone arginine vasopressin, therefore, the vasoconstriction and water-retentive effects of the antidiuretic hormone are inhibited, and an increase in the glomerular filtration rate occurs (Parker et al., 2003). According to Rang and Dale (1991), cited by Parker et al. (2003), in the presence of *ad libitum* water, glucocorticoids would promote diuresis by increasing the glomerular filtration rate.

#### 3.1.4. Creatine kinase

The overall mean ± standard error of the basal level of CK was 188 ± 96.7 UI/L. There were no significant differences in CK activity between each treatment and control (Table 1). These results disagree with those of Warner et al. (2000), who found that an increased activity (related to the intensity and duration of stress) produced changes in muscle creatine kinase concentration. Kannan et al. (2000, 2003) also found that transportation in goats produced an increase in plasma CK activity. Nevertheless, these authors indicated that vigorous physical activity, such as herding, loading and unloading procedures, were more important in determining plasma CK activity than transportation or food deprivation.

There are two possible explanations of the disagreement between the present results and most published information since plasma CK activity has been considered a good indicator of muscular activity or injury (Wilson et al., 1990). One explanation is that the maximum of CK activity occurs after a lag time of approximately two hours as found by Kannan et al. (2000). In the present study blood samples were collected immediately after the exercise so it is possible that it was before CK activity reached its peak. The other possible reason may be the length and/or the intensity of the stressor since most of the published information focused on the effect of transportation as a physical stressor.

### 3.2. Meat quality traits

Values of pH, temperature, colour parameters ($L^*$, $a^*$ and $b^*$), water holding capacity and instrumental tenderness of LTL muscle in kids exposed to control, fasting, exercise and fear treatments are shown in Table 2. Meat quality traits in animals exposed to fasting showed no significant differences with the control group. Similar results were found by Daly et al. (2006) in sheep, by Tarrant (1989) in cattle and sheep and by Ferguson et al. (2007) in beef. In contrast, Greenwood et al. (2008) found that fasting young goats increased meat darkness. Apple et al. (1993) concluded that the stress produced in lambs by lairage and isolation resulted in a slight increase in pHu and had minimal effect on meat colour. Whereas, Jacob et al. (2005) reported that lairage time had little effect on pHu in lambs.

Quality parameters measured in animals exposed to exercise showed no significant differences compared to the control animals. Values of pHu in kids exposed to exercise were in the normal range values of pH for goat meat (Swan et al., 1998; Dhanda et al., 1999; Kannan et al., 2001; Argüello et al., 2005). Similar results were found by Daly et al. (1995) and by Bond and Warner (2007). On the other hand, Warner et al. (2005) found higher pHu and lower $L^*$, $a^*$, $b^*$ and shear force values in lambs after only 15 min of exercise. A possible explanation for these discrepancies is a different response to physical stress in the species studied.

In the case of animals exposed to fear no differences were found in pHu, colour and WBSF. These results are in disagreement with those found by Bond et al. (2004). These authors used dogs to study the effects of stress on the meat quality of lamb and reported differences in pHu, $L^*$, $a^*$ and $b^*$. Geesink et al. (2001) studied lambs stressed by dogs and found an increase in pHu values. No published information on goats stressed by dogs was found to be compared with our results.

It was shown above that dehydration occurs in animals exposed to fasting (significant increase in HEM and TP) but no effects were found in meat quality parameters as colour or WHC. These results are agreement with those reported by Warner et al. (2002) who did not find effects of dehydration on lamb meat quality. Whereas Jacob et al. (2006)

found that dehydration reduced the percentage of drip and cooking losses and reduced $L^*$ and $b^*$ parameters in LTL of lambs.

## 4. Conclusion

There are a variety of measurements that can be used to assess stress in animals subjected to short-term treatments, among them physiological parameters. The stressor treatments applied produced significant changes in some physiological parameters. Fasting increased PUN, HEM and TP but it did not change CK and CORT concentrations. Exercise only increased CORT concentration without modifying the levels of the other indicators studied. Finally, fear increased CORT and HEM concentration but it did not produce a change in PUN, TP and CK levels. Therefore, we can conclude that the use of a single indicator may not be enough to characterize a particular stressor. These changes in physiological parameters were not accompanied by changes in meat quality. A possible explanation for this is that the intensity and/or duration of stressors applied in the present study were not enough to generate substantial changes in meat quality as reflected by pH, colour, WHC and WBSF of crossbred kids.

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