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Short communication

The effect of pre-slaughter stressors on physiological indicators and meat quality traits on Merino lambs

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ABSTRACT

Merino lambs of 90 days of mean age (standard deviation – s.d. – 6 days) and 22.0 kg of mean live weight (s.d. 2.7 kg) were used to explore the effects of pre-slaughter stressors on physiological characteristics and meat quality attributes. Three stressors were studied in a controlled experiment: fasting (food deprivation for 24 h before slaughter), physical exercise (keeping animals walking for 30 min at approximately 3 km/h) and fear stress (exposing animals to barking dogs for 5 min). A fourth treatment was kept as a control. Fasted lambs had greater ($P < 0.05$) urea and cortisol concentrations than control. Exercise had no effects ($P > 0.05$) in physiological indicators and lambs exposed to barking dogs had greater ($P < 0.05$) cortisol concentration compared with control. The stressor treatments studied did not affect meat quality parameters. Therefore, even though the stressors imposed on the lambs induced changes in blood constituents typically associated with the stress response, the intensity and (or) duration of these stressors had no effect on meat quality traits.

1. Introduction

There are three modern production aspects that presently attract consumers concern: integral food quality, environmental protection and animal welfare. The third aspect can be approached in two ways. One of them is focussed on the ethical aspects of production, due to consumers demand the avoidance of unnecessary suffering to farm animals. The other refers to the use of animal welfare as a market tool. There is a particular segment of consumers that is willing to pay more for products that are produced following animal welfare protocols. Meat animals are inevitably exposed to handling procedures, including isolation and/or feed deprivation prior to slaughter that could affect animal welfare (Kannan et al., 2000). Stressors produce a perturbation on animal's homeostasis; consequently, an adaptive response is triggered to restore balance. Knowles and Warriss (2000) proposed some blood parameters such as urea, total protein, creatine kinase, cortisol and vasopressin to evaluate the effects of food deprivation, dehydration, physical exertion, fear and motion sickness, respectively.

Several studies have been carried out to assess the effects of pre-slaughter handling on meat quality of sheep. Apple et al. (1993) studied the effects of isolation; Daly et al. (2006) the effect of fasting; Bond et al. (2004), Bond and Warner (2007), Daly et al. (1995) and Warner et al. (2005) the effects of pre-slaughter exercise, whereas Jacob et al. (2006) studied the effects of management inside abattoirs.

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Lambs produced in Argentinean Patagonia are reared in extensive systems and herded for long distances before being trucked. Hence, total transportation time may last many hours followed by a variable lairage period imposing not only physical stress but also a long period of fasting. Furthermore, lambs are usually herded by dogs imposing additional stress. Therefore, the aim of the present study was to determine the effects of different controlled short-term pre-slaughter stressors on indicative blood parameters associated with stress and meat quality traits in Merino lambs.

2. Materials and methods

The experiment was conducted in the Pilcaniyeu Experimental Farm of INTA (Instituto Nacional de Tecnología Agropecuaria) in the Río Negro Province of Argentina (70°35′21″W and 41°01′42″S) at 970 m above sea level.

2.1. Animals and stressors treatments

Sixty four Merino lambs with a mean age of 90 days (s.d. 6 days) and mean live weight of 22.0 kg (s.d. 2.7 kg) were used. Animals came from the same flock and were reared with their dams under an extensive rangeland production system. 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 the live weight of lambs was recorded. The experiment was carried out in four different days and 16 lambs 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 lambs were penned in an open paddock and deprived of food for 6 h with free access to water.

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

2.2. Blood sampling and physiological measurements

Blood samples were collected immediately after stressor treatment, via jugular venipuncture and processed as described by Zimerman et al. (2011). Samples were collected at 10:00, 15:00, 16:30 and 17:30 h for lambs in the fasting, control, exercise and fear treatment groups, respectively.

Plasma cortisol concentration (CORT) was determined using the Active Cortisol EIA assay kit (DSL-10-2000; Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Plasma Urea Nitrogen (PUN) was colorimetrically determined using commercially available test kits (code number: 1810058, Wiener Laboratorios S.A.I.C., Rosario, SFE, Argentina). In both cases the procedures described by Zimerman et al. (2011) were followed.

2.3. Slaughtering, sample collection and meat quality measurements

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

Muscle pH and temperature were measured 45 min (pHi and Ti, respectively) and 24 h post-slaughter (pHu and Tu, respectively) according to the methodology suggested by Garrido et al. (2005) using a Testo pH meter (model number 230, Testo, Ciudad Autónoma de Buenos Aires, BA, Argentina). Then, *longissimus thoracis et lumborum* muscle (LTL) was removed from the left carcass sides and refrigerated at 2 °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 period at 2 °C, and subsequently frozen. Instrumental colour (L^*, a^* and b^*) was measured according to Albertí et al. (2005) using Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Bergen, NJ, USA), D65 illuminant and an 8-mm aperture. Water-holding capacity (WHC) was determined according to the compression method described by Pla Torres (2005). Frozen samples taken from LTL were thawed at 4 °C for 24 h and then cooked to an endpoint temperature of 71.5 °C on an electric grill (Philips, Ciudad Autónoma de Buenos Aires, BA, Argentina). Instrumental tenderness was determined by Warner Bratzler shear force (WBSF) following the general guidelines established by AMSA (American Meat Science Association, 1995) guidelines with a Warner-Bratzler shear force device (G-R Electric Manufacturing Co., Manhattan, KS 66502, USA). Results are expressed in Newtons.

All procedures used to evaluate meat quality traits were curried out as described by Zimerman et al. (2011).

2.4. 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). 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 MIXED procedure, SAS version 8, SAS Institute Inc., 2002, Cary, NC, USA.

3. Results and discussion

3.1. Physiological indicators

Table 1 shows average values of the concentration of physiological indicators after the application of treatments. According to the ANOVA results, the overall treatment *P*-value was significant for PUN ($P_{PUN} < 0.001$). According to Dunnett's test results, only animals subjected to fasting showed greater mean level of PUN ($P < 0.004$) than control lambs. According to Knowles and Warriss (2000) any process that increases protein catabolism such as a long fasting will increase PUN concentration. PUN concentration is also related to protein intake and to an increased catabolism as it can occur in a stress situation (Kanelo, 1980, cited by Castañeda, 2010). Similar results were found by Zimerman et al. (2011) in kids: only fasted kids showed greater levels of PUN compared with control kids. In the present study the values of PUN in control lambs were within the range of those reported by Apple et al. (1993) in non stressed lambs, only the PUN values of scared animals (those subjected to the fear stress) were within the range of stressed lambs reported by these authors, but not the values of fasted or exercised lambs.

The overall treatment *P*-value was also significant for cortisol ($P_{CORT} < 0.006$). According to Dunnett's test results, fasting and scared animals caused an increase in CORT concentration ($P < 0.02$ and $P < 0.04$, respectively) compared to the non-stressed controls. In most stress studies the concentration of glucocorticosteroids (cortisol and corticosterone) were used as indicators of the status of the hypothalamic-pituitary-adrenal axis (Moberg, 2001). This

Table 1
Least-squares means of post-treatment values of physiological indicators of stress and meat quality traits from lambs exposed to control, fasting, exercise and fear treatments.

Item	Treatment				s.e.m.[a]	*P*-Value[b]
	Control ($n=16$)	Fasting ($n=16$)	Exercise ($n=16$)	Fear ($n=16$)		
Cortisol, μg/dL (CORT)	6.81	10.44*	6.99	9.51*	0.76	0.001
Plasma Urea Nitrogen, g/L (PUN)	0.15	0.19*	0.14	0.13	0.01	0.006
pH 45 min (pHi)	6.29	6.26	6.25	6.27	0.07	0.97
pH 24 h (pHu)	5.65	5.71	5.74	5.78	0.06	0.11
Minolta L^*	41.33	40.35	39.95	38.79	0.87	0.10
Minolta a^*	19.40	19.15	18.19	17.60	0.76	0.14
Minolta b^*	7.89	7.90	6.77	6.54	0.56	0.05
Water holding capacity, % (WHC)	33.97	33.76	33.47	34.40	3.60	0.98
Instrumental tenderness, N (WBSF)	23.31	21.83	26.42	29.81	2.81	0.14

[a] Standard error of least-squares means.
[b] *P*-value for the global model.
* After mean values indicate significant differences between that treatment and the control (Dunnett test, $\alpha=0.05$).

shows the role of central nervous system on cortisol secretion.

The values of CORT concentration obtained in this experiment were greater than those reported by Barrientos et al. (2006) in rested Corridale lambs, by Radostits et al. (2000), cited by Tapia Corbett (2007), and by Tadich et al. (2009). In the study done by Barrientos et al. (2006) and Radostits (2000) blood samples were collected with a catheter previously inserted in the jugular vein. Therefore, possibly this procedure of sampling was less stressful than the one used in the present study which is similar to the procedure used by Tadich et al. (2009). In the above mentioned papers, the technique used to measure cortisol concentration was radio immunoassay (RIA), while in the present study enzyme immunoassay (EIA) was used. Values obtained in the present study were transformed using the regression equation suggested by kit's manufacturer. CORT values obtained by EIA method shown in Table 1 gave the following results when the above mentioned equation was applied: 5.19 μg/dL, 9.57 μg/dL, 5.41 μg/dL and 8.45 μg/dL concentrations for control, fasting, exercise and fear treatments, respectively.

CORT concentration varies 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 be 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.

The values of CORT concentration obtained in fasted and scared lambs were within the range of values reported by Apple et al. (1993) in isolated lambs, whereas values of control lambs were greater than those reported by the same authors in non-stressed lambs. Tadich et al. (2009) studied the effect of lairage and transport in lambs. Animals were fasted up to 58 h and the values of CORT reported were smaller than those founded in fasted lambs of the present study. Pearson et al. (1977) cited by Kannan et al. (2000) reported lower levels of CORT in sheep slaughtered in a quiet research abattoir than those slaughtered in a commercial plant.

3.2. Meat quality measurements

Values of pHi, pHu, colour parameters (Minolta L^*, a^* and b^*), water holding capacity (WHC) and instrumental tenderness (WBSF) of LTL muscle in lambs exposed to control, fasting, exercise and fear treatments are shown in Table 1.

Meat quality traits in lambs exposed to fasting showed no significant differences with the control group. Similar results were found by Zimerman et al. (2011) in kids. Many authors did not found fasting effects in sheep meat quality (Daly et al., 2006; Tarrant, 1989) in lambs (Warriss et al., 1987; Jacob, 2003) neither in beef (Tarrant, 1989; Ferguson et al., 2007). Nevertheless, 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, Fisher et al. (2011) reported that fasting reduce meat colour values in sheep.

Quality parameters measured in animals exposed to exercise showed no significant differences compared to control animals. Similar results were found by Zimerman et al. (2011) in kids. There is a diversity of opinions about the effect of physical activity on meat quality: Warner et al. (2005) reported that 15 min of physical exercise increased pHu and reduce L^*, a^*, b^* and WBSF values of LTL in lambs. These authors found greater values of pHu, smaller of $L^*a^*b^*$ and slightly greater WBFS than those founded in the present study. Bond et al. (2004) also reported increased values of pHu in exercised lambs; nevertheless, Bond and Warner (2007) did not found effects of exercise on pHu, colour and WBSF in lambs. Daly et al. (1995) did not found effects of exercise on WBSF values on lambs.

In the case of animals exposed to fear no significant differences were detected for any meat quality trait compared with lambs subjected to control treatment. Apple et al. (1993) found that isolated lambs shown a slight increase of pHu values and a small effect on meat colour. Bond et al. (2004) also reported an increase in pHu values of lambs exercised with dogs, although these values, such as those reported in the present study, were lower than 6.0. Geesink et al. (2001) also reported greater values of pHu in lambs swim washed in pre-slaughter and conduced with dogs, but as it was reported in the present study, no effects were

found in WBSF values. Devine et al. (1993) studied the effects of pre-slaughter sheared and swim washed and also found effects on meat pHu, but not in WBSF.

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 and CORT concentration. Exercise did not produce changes in any of these two metabolites while fear increased CORT concentration. 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 strong enough to generate substantial changes in meat quality as reflected by pH, colour, WHC and WBSF of lambs.

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