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Mixing unfamiliar lambs upon arrival at the abattoir affects their social behavior and meat traits

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The aim of the study was to determine the effects of mixing unfamiliar lambs when they arrive at the abattoir 28-29 h before slaughtering on social behavior and meat quality. Forty Texel x Corriedale male lambs were transported together in the same truck for 5 h to a slaughterhouse where they were separated into two experimental groups homogeneous according their body weight: twenty lambs were allocated in a single resting pen (1.13 m^2/animal) together with other ten familiar male lambs (CON group), while other twenty lambs were allocated together in a resting pen with similar characteristics, but mixed with ten resident unfamiliar male lambs (MIX group). Animals were kept in the resting pens for 28-29 h before slaughter and were slaughtered on the same day following standard procedures. Lambs' behavior was recorded during the premortem period. The *longissimus thoracis* muscle pH, and temperature were recorded 45 min and 24 h after slaughter. Meat traits were measured in 24-h and 7-day-aged meat from the *longissimus lumborum* muscle. The MIX lambs displayed a greater number of both sexual behaviors and the sum of agonistic and sexual behaviors than the CON lambs ($P < 0.001$ for all). The MIX lambs had lower carcass pH ($P = 0.04$) and temperature ($P < 0.001$), and meat was lighter ($P = 0.016$), redder (higher a* values; $P = 0.003$) and more tender (lower Warner Bratzler shear force values; $P = 0.048$) than those from CON lambs. The MIX lambs tended to have more glycogen content than CON lambs ($P= 0.057$). However, no differences were detected regarding sarcomere length or lactate residual content. In summary, mixing unfamiliar eight-month-old male lambs during a 28-29 h resting period before slaughter affected the lambs' social behavior and induced changes in meat quality. Lambs exposed to MIX treatment showed carcass with lower values of pH and temperature, meat lighter, redder, and more tender, with normal sarcomere lengths and residual lactate content. The findings of the present study have potential implications for lamb meat industry since they demonstrate that pre-slaughter social mixing affects animal welfare and induces changes in meat quality characteristics.

KEYWORDS

meat tenderness, muscle fiber, glycogen, aggressiveness, social behavior, stress, welfare, sheep

1 Introduction

During the pre-slaughter period, meat-producing animals are often subjected to various stressful situations, including loading and unloading from vehicles, transport, and confinement in unfamiliar resting pens (Ferguson and Warner, 2008). In several countries, unfamiliar lambs are grouped at classification centers before slaughtering according to their body weight to ensure consistent quality and standardized meat products (Miranda-de La Lama et al., 2009). Besides, in regions with limited sheep abattoirs, such as southern Brazil, ovine slaughtering is concentrated in a few slaughterhouses (Matte and Waquil, 2021). Consequently, the resting period at the abattoir frequently involves grouping animals from different origins, which heightens stress due to social interactions among unfamiliar individuals.

Mixing unfamiliar lambs increases aggression, display of stereotypes, and blood cortisol concentration levels, raising welfare concerns (Ruiz-de-la-Torre and Manteca, 1999; Sevi et al., 2001; Miranda-de La Lama et al., 2012). In ruminants, grouping unfamiliar males into stable groups often increases the display of aggressive behaviors. For this reason, the European Committee on Legal Co-operation (1988) recommends against this practice. Furthermore, it is widely accepted that animal perimortem stress affects muscle metabolism and meat quality (Gregory, 2003). Indeed, the lapse (one day, one week, or four weeks) in which recently mixed unfamiliar lambs remain at classification centers before being transported to the abattoir affects meat texture (Miranda-de La Lama et al., 2009). Although there was not an unmixed group in the said study, this effect was likely associated with establishing a new social hierarchy and reducing social stress before transporting the animals to the abattoir (Ruiz-de-la-Torre and Manteca, 1999; Sevi et al., 2001).

While the effects of mixing unfamiliar animals immediately before slaughter on meat quality attributes have been intensely studied in pigs (Guise and Penny, 1989; Faucitano, 1998; Terlouw, 2005), to the best of our knowledge, there is scarce research on ruminants. Warriss et al. (1984) reported that mixing unfamiliar young bulls immediately before slaughter produced high pH and dark meat color carcasses. Similarly, Sanz et al. (1996) reported that mixing unfamiliar bulls overnight before slaughtering led to both physical and emotional stress, which reduced the muscle glycogen concentration and increased physical and emotional stress, reducing muscle glycogen concentration and increasing dark beef cutting. More recently, Colditz et al. (2007) reported that regrouping steers one week before slaughter diminished instrumental meat tenderness. Much more recently, López-Pedrouso et al. (2020) reported that pre-slaughter social mixing impacts the texture of the longissimus muscle from cattle, decreasing the tenderness and increasing the fibrousness of meat without affecting meat color. These authors also observed that pre-slaughter social mixing reduced drip loss and tended to diminish shear force values in 14-day-aged meat. Overall, mixing unfamiliar sheep triggers behavioral and stress responses (Sevi et al., 2001; Miranda-de La Lama et al., 2012; Ungerfeld et al., 2023). However, to our knowledge there are no studies demonstrating the consequence of mixing unfamiliar lambs at the abattoir on meat quality. We hypothesized that mixing unfamiliar male lambs before slaughtering increases the display of social interactions, influencing meat quality of the longissimus muscle. The aim of the study was to determine the effects of mixing unfamiliar lambs when they arrive at the abattoir 28-29 h before slaughtering on social behavior and meat quality.

2 Materials and methods

The Comissão de Ética de Pesquisa e do Uso em Animais of Universidade Federal de Santa Catarina (Brazil) approved all the experimental procedures (number 1613210823).

2.1 Location, animals, and pre-slaughter mixing stress treatment

The experiment was performed at a commercial abattoir in Fraiburgo, Santa Catarina, Brazil (27°01' SL, 50°55′ WL), in April 2023 (early autumn), using 40 eight-month-old Texel x Corriedale male lambs. Fraiburgo is located at an elevation of 1019.98 m above sea level and has a humid subtropical, no dry season climate (Classification: Cfa). The average annual air temperature and relative humidity are 15.5 °C and 82%, respectively (Wrege et al., 2012). All the animals came from the same farm located at Ibiraiaras, Rio Grande do Sul, Brazil (28°22' SL, 51°38' WL), where they were reared and maintained together from weaning (4.5 months old; weaning age) to shipment when they were transported together in the same truck to the abattoir. The truck (Ford, model 1119, Brazil) was designed for sheep transport with two inside floors and a loading ramp door. Loading density was 0.3 m^2 per lamb, and no food or water was provided during the 5-h transportation period (230 km). From weaning until the day of transportation, the lambs were raised in confinement, receiving a commercial ration (humidity: 12%; crude protein: 36%; minerals: 22%; dry matter: 10%; crude fiber: 10%; ether extract: 3%) mixed with crushed corn and ryegrass hay at 20%, 65% and 15%, respectively. Feeding was offered daily at 3% of the lambs' body weight. Animals had free access to water.

Lambs were loaded at the farm and unloaded at the slaughterhouse, where they were weighted, identified with numbers on the body, and separated into two experimental groups: (a) 20 lambs (13 castrated and seven entire; body weight: 40.6 ± 2.9 kg) were allocated in a single resting pen of 34 m^2 (1.13 m^2/animal) together with ten other familiar male lambs (one castrated and nine entire; body weight: 39.9 ± 3.6 kg), raised together at the same farm and transported in the same pen in the truck (CON group); (b) the other 20 lambs (13 castrated and seven entire; body weight: 40.6 ± 3.4 kg) were allocated together in another resting pen with similar characteristics as those mentioned above, but mixed with ten resident unfamiliar 8-month-old Texel x Corriedale male lambs (one castrated and nine entire; body weight: 39.5 ± 5.9 kg) (MIX group). Resident lambs came from a different farm and remained in a pen adjacent to the

slaughterhouse for 12 days before the experiment. They had free water access and were fed the same diet mentioned above (commercial concentrate ration mixed with crushed corn). All animals from CON and MIX groups were kept in the resting pens for 28-29 h before slaughter. During the resting premortem period, all animals had free access to water and received 300 g/lamb of crushed corn 1 h after allocating animals to the resting pens and seven hours later. The feeding was offered in standard feeders with enough space to allow all the lambs to access the feeding simultaneously. Approximately 12 h before lambs were slaughtered, the air temperature and relative humidity were 16.2°C and 77%, respectively. Rainfall was not observed during the experiment.

2.2 Premortem recordings

2.2.1 Social behaviors

Lambs' behaviors were recorded from 1100 h to 1400 h, and from 1600 h to 1900 h (8 to 11 h and 13 to 16 h after being grouped or not, respectively). Locomotion, posture, and feeding behaviors [standing up, lying down, walking, eating, and ruminating; see description in Freitas-de-Melo et al. (2019)] were recorded using 10 min scan sampling, totaling 39 scans/lamb. The frequency of posture, locomotion, and feeding behaviors were calculated and expressed as a percentage of observations/day. Also, the occurrence of the following social behaviors categorized as sexual (A) and agonistic behaviors (B) was continuously recorded during the same observation periods: (A) anogenital sniffing, flehmen, lateral approaches, mount attempts, mounts (Ungerfeld et al., 2014), (B) push, butt-push, head-push and kicking (Erhard et al., 2004). The display of sexual and agonistic behaviors increases the chance of animals moving and making physical contact, which can raise the risk of injury and affect meat quality (Fisher and Matthews, 2001; Miranda-de La Lama et al., 2009; 2012). The pair of lambs (CON-CON, CON-familiar lambs, MIX-MIX, or MIX-resident lambs) and the type of social behavior were recorded. The total number of sexual and agonistic behaviors in which each CON or MIX lamb was involved was summed, considering the total number of interactions displayed or received by CON and MIX lambs. All the behaviors were recorded by four trained observers from a distance enough not to disturb the lambs' normal behavior.

2.3 Slaughter procedures

All lambs were slaughtered at Bel Borrego Frigorifico Ltda (Fraiburgo, Santa Catarina, Brazil) the day after premortem recordings, between 7:00 h and 8:00 h. Slaughter and dressing were undertaken following standard Brazilian industry practices. Lambs were stunned with a captive bolt pistol with a penetrating dart (11.4 mm in diameter and 121 mm in length) (Jarvis Model PAS, 22R Caliber, USA). After that, lambs were exsanguinated on an immobilization table before evisceration and skinning. Each carcass was identified and, approximately 60 min after slaughter, was conventionally hanged (by both Achilles tendons) and cooled at 1-2°C for 24 h. Hot and cold carcass weight were record.

2.4 Post-mortem recordings

2.4.1 Muscle determinations and meat sampling at the abattoir

The temperature and pH of the *longissimus thoracis* muscle from the right half-carcass were registered 45 min and 24 h after slaughter. Muscle pH and temperature were measured using a portable meat pH meter with a FC232D combined pH and temperature probe (HI99163, Hanna Instruments, Italy). The probe was inserted into the muscle at the level of the last rib (at the 13th thoracic vertebra). Additionally, after 24 h of carcass cooling, the carcasses were split down the dorsal midline. The *longissimus lumborum* muscle was dissected from all carcasses by trained personnel by cutting between the 13th thoracic vertebra and the last lumbar vertebra. The left and the right *longissimus lumborum* muscles were removed from the carcass (boneless and fat untrimmed). The left side was used to determine the subcutaneous fat thickness with the aid of a caliper at a point 4 cm from the carcass midline according to Cañeque et al. (2004).

Each right-side *longissimus lumborum* muscle was divided into three portions that were separately vacuum-packaged and stored in the dark at -20°C until further measurements. The samples were packaged in nylon and polyethylene multilayer film bags of 200-micron thickness, and high oxygen barrier permeability (SC SHA 200 MI - 18 X 25 F/RT, Praembalar, Rio Grande do Sul, Brazil). The bag complies with the Agência Nacional de Vigilância Sanitária (ANVISA, Brazil) standards for packaging in contact with food. The first portion, a 20 g sample was obtained from the most cranial portion of the muscle to determine the sarcomere length and glycogen and lactate residual contents. The rest of the muscle was divided into two different equal-size portions: 1) the most cranial portion was used for meat quality determinations in 24-h-aged meat; 2) the remaining caudal portion of the muscle was aged in a chiller at 4°C for seven days to determine meat quality traits in 7-day-aged meat.

2.4.2 Determinations of glycogen and lactate residual content in meat samples

A 3 g meat sample was homogenized and extracted with 8 mL of HCl (4.0 N) for 2 h at 100°C for glycogen and lactate determinations. The homogenate was then filtered and neutralized with NaOH (4.0 N) until the pH reached 6.5–7. Glycogen was determined as glucose total equivalents according to Bergmeyer and Bernt (1974) using colorimetric diagnostic kits (1,001,201, Spinreact, Spain) and expressed as g of glucose/100 g of fresh meat. Lactate was measured in the same hydrolyzed slurry with a commercially available enzymatic colorimetric diagnostic kit (LO-POD; 1,001,330; Spinreact, Spain) and expressed as g of lactate/kg of fresh meat, according to Ithurralde et al. (2021).

2.4.3 Sarcomere length determinations

Sarcomere length was histologically determined according to the methodology described by Ithurralde et al. (2017). Cubic, 5 mm-side meat samples were immersed-fixed in 2.5% glutaraldehyde. An image analysis software (Infinity analyze, Toronto, Canada) was used to measure sarcomere length by counting the number of A-bands along an arbitrary distance (70–140 mm) in each of the 30 myofibrils within the muscle sample. Thus, the mean sarcomere length was calculated by dividing the measured distance by the number of A-bands in the measured segment. The measurement of sarcomere length in the longissimus lumborum muscle from lamb carcasses is presented in Figure 1.

2.4.4 Meat quality determinations

Meat quality determinations were performed in 24 h and 7 day-aged meat samples. Instrumental color determinations were performed using a colorimeter device (Minolta CR-10, Osaka, Japan). The aperture was 8 mm. Illuminant D65 and 10 Standard Observer were calibrated against a standard white tile in the CIE system. CIE (Commission Internationale de l' Éclairage) lightness (L*), redness (a*) and yellowness (b*) were measured in triplicate after a 1-h exposure to the air (blooming) for each sample, and each value was expressed as the mean value of the three determinations.

Tenderness was determined in cooked meat through Warner-Bratzler (WB) shear force. Meat samples were tested with a WB shear device mounted on an Instron series 3342 after cooking in a thermostatic water bath at 75°C until the internal temperature reached 71°C. After cooking in plastic bags, samples were dried with a paper towel and re-weighed to determine cooking losses. At least six Cores, 1.27 cm in cross-section, were cut with muscle fibers parallel to the longitudinal axis of the sample. Each core was evaluated in the shear device, and WB shear force was expressed as the mean value of the six determinations.

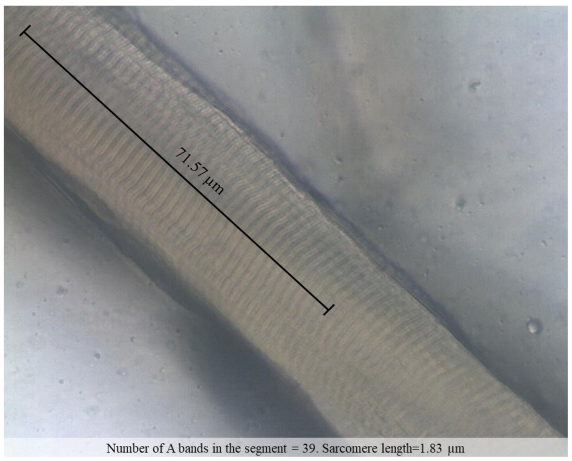

FIGURE 1
A micrograph showing the measurement of sarcomere length in the longissimus lumborum muscle from lamb carcasses.

2.5 Statistical analyses

The normal distribution of the data was tested with the Shapiro-Wilk test, and those variables not normally distributed were normalized by logarithm transformation. All data were compared using a mixed model (proc MIXED of SAS on Demand for Academics). The statistical model used for analyzing the behaviors, fat thickness, sarcomere length, and glycogen and lactate content included the treatment (MIX vs. CON) as the main effect and the category of male lambs (entire or castrated) as a random effect. All the data were analyzed, including the category of male lambs in the model as main factor, but as no significant effects were observed, it was included as random factor. The model used for muscle temperature, pH, and meat quality determinations (WB shear force, cooking loss, and meat color) included the treatment, the time (aging time), and their interaction as the main effects, and the category of the lambs as a random effect. Time was included as a repeated measure. A first-order autoregressive covariance structure (AR1) was used to adjust the time difference between recording moments. Data are presented as LSmean ± s.e.m. Differences were considered significant when $P \leq 0.05$, and as tendencies when $0.05 < P \leq 0.1$.

3 Results

3.1 Behaviors recorded premortem

The treatment did not affect locomotion, postures, or feeding behaviors (Table 1). However, MIX lambs displayed a greater number of both sexual behaviors and the sum of agonistic and sexual behaviors than CON lambs ($P < 0.001$ for all) (Table 1).

3.2 Postmortem recordings: carcass, muscle, histological and biochemical traits

Muscle pH and temperature measured in MIX and CON lambs varied with treatment ($P = 0.04$ and $P < 0.001$, respectively) and time ($P < 0.001$ for all), but there was no interaction between treatment and time. The LSmean ± s.e.m. of pH and temperature at 45 min and 24 h for each group is shown in Figure 2. Social mixing reduced the mean muscle pH (6.11 ± 0.03 vs. 6.17 ± 0.03, for MIX and CON muscle, respectively; $P = 0.04$) and temperature (17.8 ± 0.1°C vs. 18.4 ± 0.1°C, for MIX and CON muscle, respectively; $P < 0.001$). Meat residual glycogen content tended to be greater in MIX than in CON lambs ($P= 0.057$; Table 2). There were no effects of social mixing on hot carcass weight, cold carcass weight, subcutaneous fat thickness, lactate content or sarcomere length (Table 2).

3.3 Postmortem recordings: instrumental meat quality determinations

Mixing unfamiliar lambs reduced the WB shear force ($P = 0.048$), enhanced meat lightness (L*) and redness (a*) ($P = 0.016$ and

TABLE 1 Locomotion, postures, feeding, and social behaviors of eight-month-old Texel x Corriedale male lambs mixed with unfamiliar male lambs (MIX group) or not 28-29 h before slaughtering (CON group) (LS mean ± SEM).

Behavior	MIX	CON	P values
Standing up (%)	22.7 ± 2.1	22.5 ± 2.1	0.941
Lying down (%)	74.9 ± 2.2	74.1 ± 2.2	0.794
Walking (%)	2.5 ± 0.8	3.5 ± 0.8	0.220
Eating (%)	5.8 ± 1.5	3.9 ± 1.5	0.370
Ruminating (%)	17.5 ± 1.9	16.6 ± 1.9	0.742
Agonistic behaviors (number)[1]	2.8 ± 2.8	3.9 ± 3.9	0.441
Sexual behaviors (number)[2]	29.4 ± 5.4	3.5 ± 0.6	< 0.001
Agonistic + sexual (number)	32.2 ± 5.2	7.4 ± 1.2	< 0.001

[1]The agonistic behaviors included the sum of the number of pushes, butt-pushes, head-pushes and kicking; [2]The sexual behaviors included the sum of anogenital sniffing, flehmens, lateral approaches, mount attempts and mounts.

P= 0.003), and tended to increase meat yellowness (b*) (P = 0.1) (Table 3). The WB shear force decreased from 24-h to seven days of aging (5.72 ± 0.18 vs. 2.71 ± 0.18; P < 0.001). Lightness (L*) (38.39 ± 0.41 vs. 37.10 ± 0.41; P < 0.001), redness (a*) (19.11 ± 0.38 vs. 17.79 ± 0.38; P < 0.001) and meat yellowness (b*) also decreased from 24-h to seven days of aging (7.71 ± 0.17 vs. 7.32 ± 0.17; P < 0.001). Treatment and time did not affect cooking losses (Table 3). There were no interactions between treatment and time for any of the instrumental meat quality variables (Table 3).

4 Discussion

Mixing unfamiliar eight-month-old male lambs during the rest period at the abattoir enhanced their social interactions, impacting on meat color, tenderness, pH and temperature. Mixing unfamiliar lambs increased the display of social behaviors within the group, likely enhancing muscle activity before slaughter, thereby

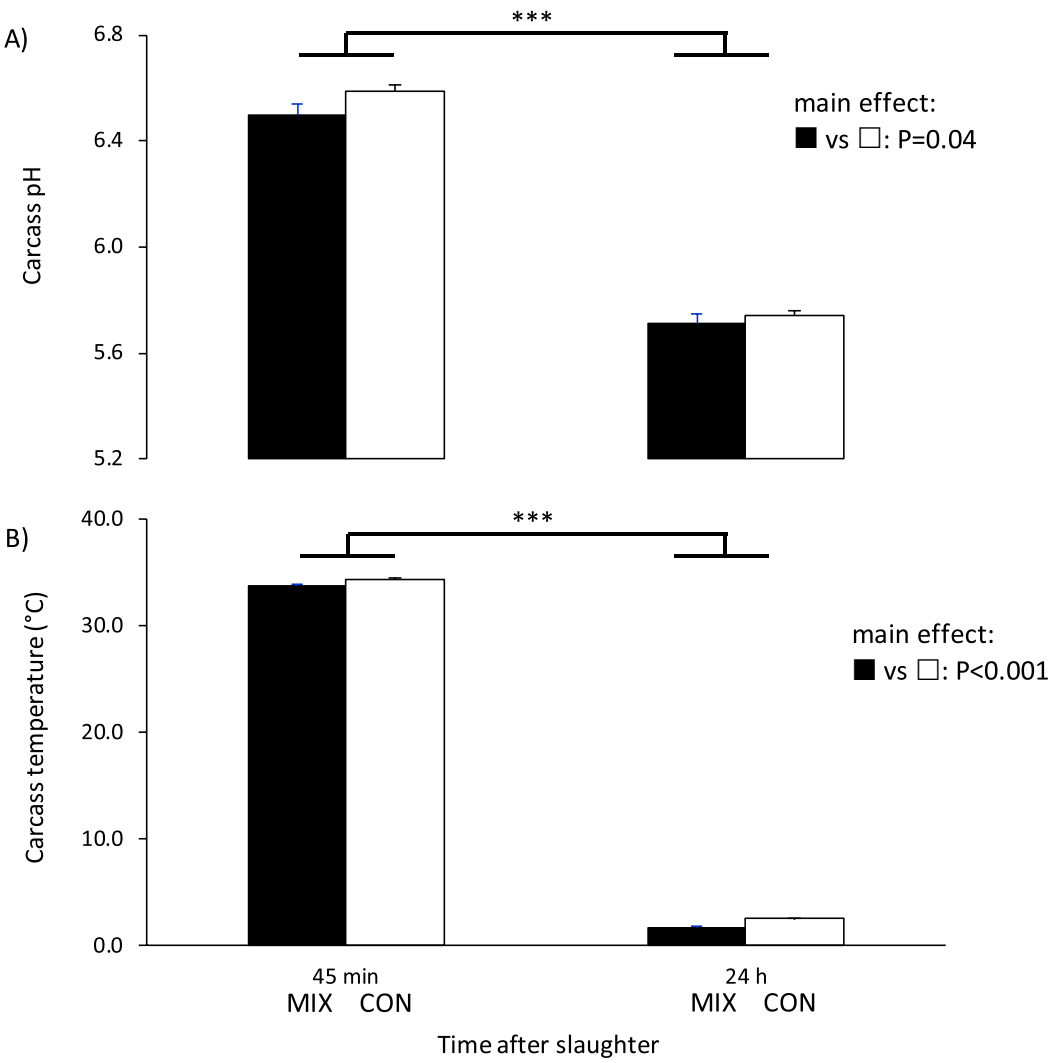

FIGURE 2
Carcass **(A)** pH and **(B)** temperature of eight-month-old Texel x Corriedale male lambs mixed with unfamiliar male lambs (MIX group; black bars) or not 28-29 h before slaughtering (CON group; white bars). Carcass pH and temperature were measured in the *longissimus thoracis* muscle at 45 min and 24 h *postmortem*. MIX lambs had lower carcass pH and temperature than CON lambs (P ≤ 0.04). *** P<0.0001: show significant differences between 45 min and 24 h.

TABLE 2 Mean effect of hot and cold carcass weight, subcutaneous fat thickness, sarcomere length, lactate and glycogen contents from the *longissimus lumborum* muscle of 8-month-old Texel x Corriedale cross-bred male lambs mixed with unfamiliar male lambs (MIX group) or not for 28-29 h before slaughter (CON group) (LS means ± SEM).

	MIX	CON	P values
Hot carcass weight (kg)	20.8 ± 0.3	20.5 ± 0.4	0.500
Cold carcass weight (kg)	20.2 ± 0.3	19.9 ± 0.4	0.480
Subcutaneous fat thickness (cm)	0.77 ± 0.05	0.79 ± 0.04	0.754
Glycogen content (g/100g)	0.19 ± 0.02	0.15 ± 0.01	0.057
Lactate content (g/kg)	1.78 ± 0.33	1.81 ± 0.31	0.680
Sarcomere length (μm)	1.82 ± 0.03	1.80 ± 0.03	0.502

influencing meat quality. The heightened muscle activity in these mixed lambs could affect muscle metabolism before and after slaughter, ultimately influencing meat quality. This observation affirms and expands the Klont and Lambooy (1995) findings in pigs to lambs, who demonstrated that muscles exercised before slaughter can have elevated lactate levels at the time of killing, meaning the lower pH observed in such meat is not solely due to accelerated postmortem glycogen breakdown. Other potential adverse effects not measured in this study are also likely, such as increased bruising on various muscles, which would reduce the usable carcass yield. Notably, the season (autumn) in which the present study was conducted could have conditioned our findings. Indeed, Miranda-de La Lama et al. (2009) reported seasonal variations in the meat quality of lambs mixed before slaughtering. In summary, the practical benefits of mixing male lambs before slaughter should be weighed carefully against the significant impacts on both animal welfare and meat quality.

Mixing unfamiliar lambs before slaughter led to a slight reduction in meat pH, producing lighter and more tender meat, resulting in no significant sarcomere shortening. We speculate that MIX lambs already had lower pH levels immediately before slaughter due to their increased locomotor and muscle activity displayed during social interactions. This outcome deviates from the conventional association between pre-slaughter stress and dark-cutting (DFD) meat in ruminants (Ponnampalam et al., 2017). Dark-cutting meat is usually linked to glycogen depletion before slaughter (Lister, 1989), and high pH values, which affect color by altering myoglobin's oxygenation and its ability to confer a bright red color to the meat (Tarrant, 1989). Instead, the lighter color and lower pH observed in MIX lambs resemble pale, soft, and exudative (PSE) meat, a condition more commonly observed in pigs but that can also occur in ruminants (Kim et al., 2014). However, unlike typical PSE cases—which usually involve high temperatures before rigor in hot-boned or pre-rigor excised muscles—the MIX lambs' muscles did not show extreme pH or temperature conditions indicative of true PSE or heat-shortening. These findings suggest that the MIX lambs experienced mild, short-term stress, sufficient to

TABLE 3 Mean effect of meat traits from the *longissimus lumborum* muscle of 8-month-old Texel x Corriedale cross-bred male lambs mixed with unfamiliar male lambs (MIX group) or not for 28-29 h before slaughter (CON group) (LS means ± SEM).

	LS mean		Pooled SEM	P values		
	MIX	CON		Treatment	Time	Interaction between treatment and time
WB shear force (N)				0.048	< 0.001	0.274
24 h WB shear force	53.7	58.3	2.4			
7 d WB shear force	22.6	30.5	2.4			
Lightness (L*)				0.016	< 0.001	0.125
24 h	37.4	36.4	0.5			
7 d	39.4	37.6	0.5			
Redness (a*)				0.003	< 0.001	0.645
24 h	20.3	19.2	0.4			
7 d	17.9	16.4	0.4			
Yellowness (b*)				0.106	< 0.001	0.951
24 h	7.1	6.7	0.2			
7 d	8.3	7.9	0.2			
Cooking losses (CL)				0.378	0.598	0.854
24 h	21.1	21.6	0.7			
7 d	20.6	21.4				

WB, Warner Bratzler.
Meat traits were evaluated after 24-h and seven days (7-d) of aging.

alter meat color and tenderness without impacting ultimate lactate levels. This aligns with the stress categorization established for cattle (Immonen and Puolanne, 2000), highlighting that mild stress can lead to manageable changes in meat quality. Apparently, the lighter and redder color of MIX lambs' meat aligns with previous studies in ruminants that indicate that PSE meat typically has a low pH, resulting in a light red appearance (Roeber et al., 2000; Sammel et al., 2002). However, the said color improvement is typically temporary, as PSE meat has poor color stability and tends to discolor much before the seven days of aging (Kim et al., 2010; 2014; Sammel et al., 2002). In contrast, MIX lambs' meat maintained its lighter and redder coloration even after seven days of aging, suggesting that the stress endured was insufficient to produce PSE meat. The lower pH in MIX lambs' meat also correlated with reduced shear force, suggesting greater tenderness. The impact of pre-mortem stress on tenderness is mediated through mechanisms involving pH and temperature changes that influence sarcomere shortening and muscle proteolysis (Kim et al., 2014). Severe PSE meat typically shows a greater degree of denaturation of the myosin heads, causing lateral and longitudinal contractions, leading to sarcomere shortening and tougher meat (Tornberg, 1996). Contrarily, in less severe cases, the low pH primarily affects the denaturation of sarcoplasmic proteins, leading to lesser degrees of sarcomere shortening and, consequently, more tender meat (Tornberg, 1996). This concept is consistent with the current findings, reinforcing the hypothesis of a mild, short-term stress condition in MIX lambs, which exhibited normal sarcomere lengths (not differing from those in the CON group) and increased tenderness. Moreover, consistent with our findings, Aalhus et al. (1998) reported the occurrence of PSE-like meat in cattle and stated that PSE-like beef is more tender with lower shear force values. Additionally, faster pH decline can enhance postmortem proteolysis, as O'Halloran et al. (1997) found that rapid glycolysis increases proteolytic activity, contributing to tenderness. Therefore, the mild stress experienced by MIX lambs likely accelerated proteolysis, further explaining the observed reduction in shear force.

Surprisingly, mixing unfamiliar lambs did not affect the residual lactate content in meat, although MIX lambs tended to show slightly more glycogen content than CON lambs. This supports the idea that MIX lambs experienced only mild, short-term stress. Since MIX and CON lambs had normal ultimate pH values, no major differences in glycogen or lactate residual contents should be expected. Residual glycogen can vary widely and does not necessarily correlate with ultimate pH, particularly at low pH values (below 5.75) (Immonen and Puolanne, 2000). The stress associated with mixing unfamiliar lambs might not have been sufficiently intense to deplete glycogen reserves. Additionally, the lambs came from a confinement system with access to concentrated feed, which can support glycogen repletion (McVeigh and Tarrant, 1982). Postmortem glycogen breakdown stops if premortem glycogen is sufficient, regardless of residual levels (Bendall, 1973; Lawrie, 1955). The higher glycogen meat content in MIX than in CON lambs might have led to an earlier stop in glycolysis due to either the lower pH or lower carcass temperatures. Furthermore, the lack of differences in meat lactate content between experimental groups may suggest that, under the conditions of the present experiment, lactate residual content per se was not able to predict meat quality variations. This is generally consistent with previous findings in sheep meat in which despite the detection of significant effects of breed, sex, and lairage after transport on meat quality traits and pH, no effect of the aforementioned factors was detected for lactate meat content (Stempa et al., 2016; Xin et al., 2018). Overall, although highlighting the lack of experimental replications is important, the substantial impact of mixing unfamiliar lambs on individual meat characteristics leads to sound study insights.

In conclusion, mixing unfamiliar lambs during a 28-29 h resting period before slaughter was a short-term moderate stressor that affected meat quality, as it was lighter, redder, and more tender (with reduced WB shear force), with lower carcass temperature and pH values. However, the sarcomere lengths and the residual meat lactate content were unaffected. These results should be taken with caution since they might underestimate the consequences on muscles other than the *longissimus* as it cools more rapidly than deeper muscles, and therefore, greater effects could be expected in different muscles. Further research is required to determine additional potential adverse effects associated with mixing unfamiliar lambs before slaughter, including the incidence of bruising and, therefore, the usable carcass yield. The findings of the present study have potential implications for lamb meat industry since they demonstrate that pre-slaughter social mixing affects animal welfare and induces changes in meat quality characteristics.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Comissão de Ética de Pesquisa e do Uso em Animais of Universidade Federal de Santa Catarina (Brazil) approved all the experimental procedures (number 1613210823). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JI: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. RU: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. GM: Formal analysis, Investigation, Methodology, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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