Effect of Postweaning Handling Strategies on Welfare and Productive Traits in Lambs

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Postweaning management strategies that include an element of social enrichment may reduce weaning stress and improve welfare and productive performance. We analyzed the effect of postweaning handling strategies on welfare and production traits in lambs. After weaning, 36 lambs were assigned to 3 experimental groups with 12 lambs each (control [C], fattening with gentle human female contact [H], and fattening with 2 adult ewes [E]). The average daily gain (ADG) was estimated. Blood samples were taken, and infrared thermography was used to estimate stress variables. There were significant differences among treatments (in favor of alternative strategies) regarding production and stress variables (cortisol, glucose, and creatine kinase). The results suggest that the lambs handled gently during the fattening were less reactive and better able to modulate their physiological stress. The E group adapted better to acute stress than the C group but was less efficient in modulating chronic stress. Both treatments showed higher slaughter live weights and better ADGs compared with the control. The use of social enrichment at weaning, especially to establish a positive human–nonhuman animal bond, alleviates lamb weaning stress and improves welfare and performance.

Keywords: *lambs, welfare, postweaning handling strategies, gentle human contact, ewe nurse*

Weaning is one of the most stressful events in a lamb's life because it involves both nutritional (milk replaced by solid food) and psychological changes (breakdown of the mother–young bond; Orgeur et al., 1998). In a natural social environment, mother–young relationships do not end abruptly after the end of suckling; they continue for several weeks or even months (Hinch, Lynch, Elwin, & Green, 1990). Progressive natural weaning has little apparent negative consequences on the welfare of ewes and lambs (Grubb & Jewell, 1966). Artificial weaning imposed by the breeder involves mother–young separation, modification of the lamb's feeding habits, and changes in his or her social and physical environment (Lansade, Bertrand, Boivin, & Bouissou, 2004; L'Heureux, Lucherini, Festa-Bianchet, & Jorgenson, 1995; Orgeur et al., 1998). This early disturbance can be an important stressor (Orgeur et al., 1998, 2001; Schichowski, Moors, & Gauly, 2008).

In sheep, both ewes and lambs express their distress by an increase in bleating and locomotor activity (Alexander, 1977; Torres-Hernandez & Hohenboken, 1979). Some stressors may suppress the immune system—with negative effects on nonhuman animal health, welfare, and performance—as the release of glucocorticoid hormones coincides with a decrease in growth hormones (Kuhn, Pauk, & Schanberg, 1990; Orgeur et al., 1999). Moreover, artificially weaned animals are more susceptible to multifactorial diseases, affecting their behaviors and physiologies, which can jeopardize the efficiency of the production system (Miranda-de la Lama, Villarroel, & Maria, 2012).

Management practices that minimize stress by making this transition less harmful can improve lamb health and weight gain. Handling strategies, proper facilities, and feed resources should be considered when deciding which weaning protocol is most likely to minimize stress on lambs while still preparing them for the next stage of production (Mathis & Carter, 2008). Different approaches have been considered when trying to reduce the undesirable effects of weaning. Under natural conditions, lambs develop in rich and varied social environments, so social facilitation by adults at weaning may be more appropriate than weaning only with same-age peers. Social facilitation appears to be a robust phenomenon that encourages animals to sample foods that they observe others eating and facilitates the ontogeny of natural behavior (Ralphs, Graham, & James, 1994; Ralphs & Provenza, 1999). Social facilitation alleviates weaning stress, limiting the occurrence of abnormal behaviors and encouraging positive social behavior (Henry et al., 2012; Schichowski et al., 2008).

In the same way, gentle handling and a positive human–animal relationship may modulate the animals' stress responses (Boivin, Nowak, & Terrazas-Garcia, 2001; Hargreaves & Hutson, 1990; Pearson & Mellor, 1976). The relationship between humans and farm animals can have a strong impact on their welfare, ease of handling (Boivin, Lensink, Tallet, & Veissier, 2003; Hemsworth, 2003), and productivity (Hemsworth & Coleman, 1998). For domestic species, it has been shown that animals can perceive humans as social support providers (Rault, Boissy, & Boivin, 2011). In sheep, the establishment of social relationships with members of other species has been previously observed (Cairns, 1966).

In some circumstances, the presence of a human can attenuate the behavioral and physiological consequences of separation distress, supporting the idea of the human being perceived as a social substitute (Boivin, Tournadre, & Le Neindre, 2000; Korff & Dyckhoff, 1997; Markowitz, Dally, Gursky, & Price, 1998). High affinity with the stockperson has often been associated with lower levels of physiological stress (Raussi, Lensink, Boissy, Pyykkonen, & Veissier, 2003). Although most of the studies on human–animal relationships are used to defend the idea of establishing relationships at an early age (e.g., during artificial rearing), it has been suggested that any period of reorganization associated with stress could be used because animals become especially sensitive to external stimuli (Lansade et al., 2004). Thus, young animals could be particularly susceptible to environmental influences at weaning time, and human contact during this period may result in a more efficient and friendly human–animal relationship (Boivin et al., 2000).

Sheep are gregarious by nature, and lambs prematurely separated from their dams try to compensate for the absence of maternal stimuli by interacting with other social stimuli such as conspecifics or members of other species (Cairns, 1966). We hypothesized that the establishment of postweaning management strategies that include an element of social enrichment (the presence of adult ewes or the establishment of a positive human–animal relationship) may reduce weaning stress and improve welfare and productive performance.

MATERIALS AND METHODS

The study was conducted at the Animal Experimentation Service of the University of Zaragoza in Spain (41°41′N). The area is located in the Ebro Valley, which is characterized by a dry Mediterranean climate with an average annual temperature of 15°C and an average annual rainfall of 317 mm. The study was approved by the Animal Experimentation Ethics Committee of the University of Zaragoza. The study lasted 60 days, including the milking period (30 days) and the fattening period (30 days). All animals belonged to the *Chamarita* breed native to La Rioja (Spain).

Animals and Housing

Preweaning period. Thirty-six lambs were used (18 male and 18 female) and were delivered naturally after estrus synchronization of their mothers with intravaginal progestogen sponges (30 mg fluorogestone acetate; Sincropart, Ceva Salud Animal S.A., Barcelona, Spain). Birth weight was recorded within 24 hr of birth. During the milking period, all lambs had ad-libitum access to hay and water and were kept together in one group with their mothers (indoor pen, 2 m^2/ewe and lamb) until weaning (30 days old). The ewes were fed twice per day, in the morning between 08:00 hr and 08:30 hr and in the afternoon between 15:00 hr and 15:30 hr. All of the dams were fed pellet concentrate (11.5 megajoules [MJ] metabolic energy/kg dry matter [DM] and 15.5% crude protein; approximately 0.3 kg per ewe) and lucerne chaff (*Medica gosativa*) ad libitum. The pen was equipped with a metallic water trough (1.5 m × 0.60 m), two metallic feeders (4.5 m × 0.80 m, 27 cm per ewe), and a lick stone for minerals.

Postweaning period. After weaning (30th day), lambs were separated from their mothers and were assigned to three experimental groups with 12 lambs in each group (control [C], fattening with gentle human female contact [H], and fattening in the presence of two adult dry ewes [E]). After weaning and regrouping, visual contact and vocal communication with the mothers were not allowed. Only vocal communication between the lambs of different treatment groups was permitted. Each group of 12 lambs was housed in 3 m × 4 m pens (stocking density 1 m^2 per lamb).

All lambs were fed with commercial pellet concentrate (Ovirum) containing barley, wheat, calcium carbonate, sodium chloride, a vitamin supplement corrector (18% crude protein and 11.5 MJ metabolizable energy/kg DM), and straw rations (3.5% crude protein and 5.02 MJ metabolizable energy/kg DM). Feed and water consumption were ad libitum. Pens had a concentrate hopper to allow all lambs to eat concentrate simultaneously. The forage was offered in a fodder rake that also allowed lambs to eat simultaneously. Water was provided with a float drinker installed in each pen.

After weaning, weights and sex were balanced across treatments, and twin lambs were never included in the same experimental treatment. During fattening, the C group was raised following the conventional postweaning strategy, with minimum contact with the stockperson and other adult animals. The E group was penned together with two unknown adult dry ewes (for social and environmental enrichment), with minimum contact with the stockperson. Finally, the H group spent 1 hr daily (16:00 hr to 17:00 hr) with the same female stockperson during the whole fattening period (30 days). During this hour, the stockperson sat in the same position in the middle of the pen and stroked and talked softly to the lambs, who approached her on their own free will.

Productive Traits

Lambs were weighed at birth (BW), at weaning (WW), and at the end of the fattening period (SW), when they averaged 60 days of age. Preweaning average daily gain (ADG1) was estimated by the difference between WW and BW divided by the total milking period (30 days). Postweaning average daily gain (ADG2) was estimated by the difference between SW and WW divided by the total fattening period (30 days). The total ADG was estimated by the difference between SW and BW divided by the total raising period (60 days).

Physiological Measurements

Blood samples were taken at the end of the study (two 10-mL tubes were collected per animal, with and without anticoagulant, EDTA-K3). The sampling was at 08:00 hr, and the lambs from each group were randomly sampled. Animal handling and venepuncture required less than 1 min per lamb. Samples were kept on ice for less than 1 hr and were taken to the laboratory for routine hematological measurements. The EDTA plasma and serum were centrifuged at 3,000 rpm for 10 min, and aliquots were frozen and kept at −30°C until they were analyzed.

An automatic particle counter (Vet-ABC, Divasa Farmavid S.A.) was used to count red blood cells (RBC) and white blood cells (WBC; number permm3), hemoglobin (g/dL), hematocrit (%), platelets, mean corpuscular volume, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration. The leukocyte formula was estimated from blood swabs on clean slides. Staining was performed by the rapid panoptic method using dyes from Química Clinica Aplicada Inc.

Using an optic immersion microscope, we counted and identified 100 leucocytes per sample (neutrophils, lymphocytes, eosinophils, basophils, and monocytes). The neutrophil/lymphocyte ratio (N/L) was used as a chronic stress indicator (Lawrence & Rushen, 1993). In the serum samples, with a Multichannel Technicon Analyser (RA-500) and using reagents from Bayer diagnostics (SA) for RA technicon systems, we determined the concentration of glucose (mg/dL; Ref. T01-1492-56) and the activity of creatine kinase (CK; IU/L; Ref. T01-1885-01).

The concentration of cortisol was determined from plasma (EDTA-K3) by enzyme immunoassay using an "in-home kit" (validated by Chacón, Garcia-Belenguer, Ilguera, & Palacio, 2004). Each sample was determined in duplicate from 50 μl of plasma, and the results were expressed in nmol l^{-1}, with the corresponding controls. Variation coefficients of the analysis, interassay and intra-assay, were 7% and 8%, respectively. The concentration of lactate was determined using a Sigma Diagnostic kit (lactate no. 735-10) and spectrophotometer (Lambda 5, Perkin Elmer). Serum concentrations of nonesterified fatty acid (NEFA) levels were analyzed by a multianalyzer ACE (Clinical Chemistry System of the Alfa Wasserman), with commercial kits (NEFAC Ref. 994-75409 of the Wako).

Each lamb was subjected to a "reactivity-to-handling test" at the end of the fattening period and 2 days after the collection of blood samples. The test consisted of the random capture and restraint of an animal on the ground by a handler for 1 min. All the animals were tested in their home pen in one session, and lambs within experimental groups were randomly tested. The whole test required less than 2 min per lamb. An infrared thermography (IRT) camera (Testo 880 Thermal Imaging Camera; Testo AG, Lenzkirch, Germany) was used to take images of the eye during restraint to evaluate the acute stress (Stewart et al., 2007).

IRT has been applied to detect surface body temperature in animals (Luzi, Mitchell, Costa, & Redaelli, 2013; Stewart, Webster, Schaefer, Cook, & Scott, 2005). This technique is based on the detection of thermal energy emitted by a body, with the signal being converted into an electronic signal, which in turn is processed by software to produce digital images. The imaging is produced using different color scales to highlight the areas that have different temperatures (accuracy of 0.1°C). IRT is a completely noninvasive technique that allows recording measurements to be used to assess stress during animal handling (Schaefer, Jones, Tong, & Vincent, 1988).

All images were taken from the left sides of the lambs (approximate distance of 0.5 m). The built-in lens (24°) was used, and the camera was calibrated to the temperature and relative humidity of the room. The emissivity value used was 0.98, which is recommended by the camera manufacturer for biological tissues. A good-quality infrared image (precise location and perfect focus) was chosen for each animal. Image analysis software (IRSoft software, Testo AG, Lenzkirch, Germany) was used to determine the maximum temperature within an oval area traced around the eye, including the eyeball and approximately 1 cm surrounding the outside of the eyelids (Stewart et al., 2007).

Statistical Analysis

Data were analyzed using the least squares methods of the general linear models procedure using Statistical Analysis System (SAS/STAT; 9.1 SAS Inst Inc., Cary, NC) by Statistical Analysis System (1998), fitting a one-way model with a fixed effect of postweaning strategy (three levels). The general representation of the model used was: $y = Xb + e$, where y was an $N \times 1$ vector of records, b denoted the fixed effect in the model with the association matrix X, and e was the vector of residual effects. Least square means and standard errors were computed and listed for the effect analyzed. Probability of difference request of all possible probability values for the hypothesis H_0, $LSM(i) = LSM(j)$, was printed to assess multiple comparisons between the three treatment groups. A probability of $p < .05$ was considered statistically significant.

TABLE 1
Least Square Means (±*SE*) for Productive Traits of Lambs During the Preweaning and Postweaning Periods

Variables	*Control Group*	*Human-Nursed Group*	*Ewe-Nursed Group*
Preweaning			
BW (kg)	3.84 ± 0.17^{a}	3.35 ± 0.19^{a}	3.68 ± 0.19^{a}
WW (kg)	11.90 ± 0.62^{a}	12.72 ± 0.65^{a}	13.18 ± 0.65^{a}
ADG1 (g)	226 ± 13^{a}	243 ± 15^{a}	250 ± 15^{a}
Postweaning			
SW (kg)	16.05 ± 1.04^{a}	19.12 ± 1.09^{b}	18.88 ± 1.09^{b}
ADG2 (g)	154 ± 21^{a}	229 ± 22^{b}	204 ± 22^{b}
ADG (g)	204 ± 14^{a}	223 ± 15^{a}	213 ± 15^{a}

Note. Different superscript letters (a, b) represent significant differences ($p < .05$) between treatments.
BW = birth live weight; WW = weaning live weight; SW = slaughter live weight; ADG1 = average daily gain during the milking period; ADG2 = average daily gain during the fattening period; ADG = total average daily gain.

RESULTS

Overall, there were significant differences ($p \leq .05$) among treatments regarding production traits (e.g., SW and ADG) and physiological stress variables (cortisol, glucose, and CK). All animals were healthy and received approval from the local veterinarian for transport to the abattoir, and there were no mortalities during the study.

Productive Traits

No significant differences were found between groups in BW or WW. However, SWs in the H and E groups were significantly higher (+19% and +18%, respectively) than in the C group. There were no significant differences between groups in ADG during the preweaning period. However, ADG during the fattening period was higher in the H and E groups than in the C lambs (+49% and +32%, respectively). Nevertheless, ADG for the whole productive period (from lambing to slaughtering) was not significantly different among groups (Table 1).

Physiological Measurements

The results indicated significant differences ($p \leq .05$) in the levels of cortisol, N/L ratio, glucose, and CK. Cortisol levels were higher in the C group compared with the H group (−38%) and the E group (−32%). Glucose levels were also higher in the C group compared with the H and the E groups (−9% and −18%, respectively). The N/L ratio was lower in the H group than the E and C groups (+71% and +77%, respectively). In the case of CK, the E group scored the lowest levels and the H group scored the highest (+53%), but neither of them were significantly different from the C group, which had intermediate values (Table 2).

The complete blood count was significantly different between groups in WBC, RBC, platelets, and MCH values ($p \leq .05$). The E group had more WBC than the H group (+23%).

TABLE 2
Least Square Means (±*SE*) for Effect of Postweaning Handling Strategies on Physiological Welfare Indicators Assessed in *Chamarito* Lambs

Variables	*Control Group*	*Human-Nursed Group*	*Ewe-Nursed Group*
Cortisol (nmol/L)	56.07 ± 4.23^{a}	40.57 ± 4.23^{b}	42.44 ± 4.23^{b}
CK (UI/L)	124.50 ± 12.7^{ab}	141.80 ± 13.9^{a}	92.70 ± 13.9^{b}
Glucose (mg/dL)	110.33 ± 3.3^{a}	100.80 ± 3.6^{b}	93.50 ± 3.6^{b}
Lactate (mg/dL)	8.94 ± 3.1^{a}	16.82 ± 3.4^{a}	13.84 ± 3.4^{a}
NEFA (nmol/L)	0.23 ± 0.1^{a}	0.04 ± 0.1^{a}	0.17 ± 0.1^{a}
Ratio N/L	0.62 ± 0.08^{a}	0.35 ± 0.08^{b}	0.60 ± 0.08^{a}
WBC (10^3/mm^3)	10.13 ± 0.7^{ab}	8.87 ± 0.8^{a}	10.91 ± 0.8^{b}
RBC (10^6/mm^3)	10.31 ± 0.2^{a}	10.77 ± 0.3^{ab}	10.94 ± 0.3^{b}
HG (g/dL)	11.10 ± 0.2^{a}	11.32 ± 0.2^{a}	11.57 ± 0.2^{a}
HCT (%)	32.76 ± 0.7^{a}	34.04 ± 0.8^{a}	34.21 ± 0.8^{a}
Platelets	709 ± 53^{a}	974 ± 58^{b}	836 ± 58^{ab}
MCV	31.92 ± 0.3^{a}	31.60 ± 0.4^{a}	31.20 ± 0.4^{a}
MCH	10.80 ± 0.1^{a}	10.51 ± 0.1^{b}	10.58 ± 0.1^{ab}
MCHC	33.91 ± 0.3^{a}	33.29 ± 0.4^{a}	33.87 ± 0.4^{a}
IRT	NA	38.61 ± 0.15^{a}	39.08 ± 0.15^{b}

Note. Different superscript letters (a, b) represent significant differences ($p < .05$) between treatments.

CK = creatine kinase; NEFA = nonesterified fatty acid; ratio N/L = neutrophil/lymphocyte ratio; WBC = white blood cells; RBC = red blood cells; HG = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; IRT = maximum infrared temperature.

However, both groups were similar to the C animals. The RBC count was higher in the E group and significantly different from the C group (−6%). However, none of them was different from the H group, which had intermediate values. Platelet count was significantly higher in the H group than in the C group (+37%), while the lambs from the E group had intermediate values (not different from the H or C groups). The MCH values were significantly higher in the C group (+3%) compared with the H group, but neither was different from those in the E group. Finally, the lambs fattened in the E group had slightly higher temperatures (assessed by IRT) compared with the H group (+1%; $p < .05$). Unfortunately, for technical reasons, the IRT of the C group was not available.

DISCUSSION

The establishment of postweaning management strategies that include positive human–animal relationships, or the presence of adult ewes during the fattening process, reduces weaning stress and improves productive performance and lamb welfare. Weaning is a critical point in a lamb's life that may compromise productive performance, thereby affecting the efficiency of the production system (Kelly, 1992). It can also elicit numerous negative effects on animal health and welfare (Cañeque et al., 2001; Schichowski, Moors, & Gauly, 2010). Our results indicate that the alternative weaning strategies proposed alleviate certain effects of postweaning stress, as suggested by improved productive traits and lower values of physiological stress variables.

Productive Traits

Weaning weight is strongly affected by birth weight, the milk supply of the dam, and individual growth potential. In our study, the lack of differences in body weight between treatments at birth and at weaning ensured balanced groups. In addition, birth weights were within the normal range for the breed. Similarly, no significant differences were found in ADG during the preweaning period. The choice of when to wean depends on animal age, live weight, and the ability to eat solid food (Napolitano, De Rosa, & Sevi, 2008). If chosen correctly, lambs will perform well during their lifetimes. However, in Mediterranean sheep breeds, lambs are slaughtered early and consequently weaned early, at approximately 45 days old. In the case of the *Chamarita* breed, lambs are weaned even earlier (30 days of age), making the postweaning phase more critical (Lyford, 1988; Pryce, 1992).

Early weaning can inhibit growth rates because animals are slower to adapt to solid food consumption as a consequence of the physiological delay in rumen development (Langlands & Donald, 1975). During the fattening period, the lambs subjected to a less stressful social environment grew faster than if we had used conventional strategies, which may be because weaning in barren environments decreases plasma levels of growth hormones, decreases the quantity of ingested food, and impairs digestion (Dantzer & Mormède, 1983; Kuhn et al., 1990). Between the weaning strategies proposed, the H group showed the best performance. Gentle handling during the postweaning period could alleviate stress and some of its undesirable effects on productive performance. Gentling is a form of positive physical attention that serves to calm the animals and promotes a healthy human–animal bond, with positive effects on body weight, behavior ontogeny, and health (Hemsworth, Coleman, Barnett, & Borg, 2000).

Our results are consistent with those of Napolitano et al. (2006), who found that weight gain during artificial rearing in lambs tended to be affected by gentling, as gentle contacts induced the animals to gain more weight. Similar results have been found in other species such as goats (Lyons, 1989), heifers (Hemsworth, Barnett, Coleman, & Hansen, 1989; Hemsworth, Barnett, & Hansen, 1987), and poultry (Jones & Hughes, 1981; Thompson, 1976). However, Caroprese et al. (2006) found that growth rate was not significantly different between gentled and nongentled artificially reared lambs, as Lensink et al. (2000) found in calves. In our study, lambs were gentled during the fattening period after a natural milking. To our knowledge, this is one of the first studies analyzing the effect of human gentling during the fattening process in naturally reared lambs. The study confirmed the importance of human contacts as indicators of a stockperson's positive attitude toward animal welfare, which are critical aspects of selection and training programs.

The presence of unrelated conspecific adults with the young, weaned animals may alleviate postweaning stress (Henry et al., 2012). In our study, the presence of a nurse ewe mitigated the undesirable effects of postweaning stress, but not as decisively as in the H group. Social facilitation increases the occurrence of a particular response when it is shown in the presence of others engaged in the same behavior at the same time (Clayton, 1978). In our study, animals from the E group had significantly higher slaughter weights than those in the C group, and they had higher ADGs during the fattening period, which may be related to a better transition from liquid to solid feeding. However, we cannot exclude other possible physiological or cognitive explanations.

One interesting issue is whether the social context contributes to the efficiency of food adjustment, and in particular to the ability to eat solid food. Social facilitation is an extremely strong force influencing the efficiency of switching the animals to sample diets (Ralphs et al., 1994). In the context of complex social groups, learning—and specifically learning about and within the social context—appears to be essential to survival for animals during their life spans (Box & Gibson, 1999; Provenza & Balph, 1987). When sheep are in a situation of conflict between feeding and social motivations, group size and distance between the two attractants interact to determine their feeding efficiency (Dumont & Boissy, 2000). In our study, the two attractants were in the same pen, reducing the possibility of conflict between social motivation and feeding.

Physiological Measurements

Physiological and hematological variables are valid to assess animal welfare status when animals are subjected to short- or long-term stressors (Barnett & Hemsworth, 1990; Caroprese, 2008; Mendl, 1991). Our results show differences in stress variables between treatments. Control lambs demonstrated higher cortisol and glucose values and a higher N/L ratio, suggesting there was a higher biological cost during the postweaning stage. The lambs fattened under socially enriched environments were more efficient at coping with the challenges and novelty of the fattening phase. However, the results obtained fall within normal ranges for sheep during routine handling procedures (Hargreaves & Hutson, 1990).

Cortisol levels are a good indirect measure of the stress experienced by an animal exposed to adverse conditions, such as restraint, isolation, or an unsuitable rearing environment (Baldock & Sibley, 1990; Burton, Kennedy, Burnside, Wilkie, & Burton, 1989; Fisher et al., 1997). In our study, lambs from the E group and the H group had lower cortisol levels than lambs in the C group, suggesting better modulation of physiological stress associated with both social enrichments. Many authors have found an increase in plasma or total serum cortisol following weaning (Mears & Brown, 1997; Rhind et al., 1998). By contrast, Lensink et al. (2000) found no effects on physiological stress indicators in gentled calves. Our results support the hypothesis that recovery from conditions of poor animal welfare may be attained through the administration of gentle contacts by humans, which may supply an additional social bond with subjects of a different species (Boivin et al., 2000).

The decrease in plasma cortisol observed in the lambs fattened in a socially enriched environment may be related to higher oxytocin secretion associated with the establishment of positive social bonds (Gordon, Martin, Feldman, & Leckman, 2011; Uvnäs-Moberg, 1997). In our study, the social bond between lambs and humans or nurse ewes may have attenuated the high levels of cortisol expected during the postweaning period, as was observed in the control animals. Stroking animals in a manner similar to intraspecific allogrooming can elicit calming responses including relaxed body postures, increases in the frequencies of approaching humans, and reductions in heart rates (Feh & deMazieres, 1993; Schmied, Waiblinger, Scharl, Leisch, & Boivin, 2008). As far as we know, there have not been previous studies that analyze the effects of the presence of an adult nurse ewe on postweaning physiological stress in lambs, but the study in horses by Henry et al. (2012) supports our findings.

The results for cortisol are consistent with the glucose levels observed. During stress, the increase in plasma glucose is preceded by an increase in cortisol (Miranda-de la Lama et al., 2010), which can be related to the gluconeogenic effect of this hormone (Steffens & Boer, 1999). A stress response increases circulating adrenaline, inducing catabolism of muscle glycogen to be used in the liver for gluconeogenesis, which increases glucose and lactate levels (Apple et al., 1995). Therefore, energy-related variables should be included to assess animal welfare in stressful situations such as handling and poor quality facilities. Although we found similar results in cortisol and glucose levels (both of them increased in the C group when compared with the other treatments), there were no significant differences between treatments in lactate levels in our study.

Few studies have been conducted to analyze the effect of gentle handling on energy status variables in lambs. As occurs with glucose, an increase in the serum concentration of NEFA indicates a breakdown of fat (lipolysis) caused by an increase in energy demand (Stewart et al., 2007). Thus, NEFA is generally accepted as a biomarker of negative energy balance, when the supply of glucose is insufficient to meet energy needs (Adewuyi, Gruys, & van Eerdenburg, 2005). There were no significant differences in NEFA levels between treatments, suggesting that no lambs had a negative energy balance. However, we have to take into account that our lambs may be considered pre-ruminants (Faichney, 1992), which reduces the importance of NEFA in measuring their energy statuses.

The CK values observed are within normal ranges for this species (Dubreuil, Arsenault, & Bélanger, 2005), and a decreasing tendency was also observed in the lambs from the E group. Kannan et al. (2000) and Miranda-de la Lama et al. (2010) report that CK activity increases significantly in lambs in very stressful situations. In cases of muscular damage and fatigue or vigorous exercise, the CK enzyme is released into the blood (Knowles, Warriss, Brown, & Edwards, 1998; Van de Water, Verjans, & Geers, 2003), so plasma CK is generally considered a chemical index of physical stress due to intense activity. Miranda-de la Lama et al. (2012) found a correlation between aggressive interactions and higher plasma CK activity. In our study, a possible explanation for this increase in CK values observed in the H group could be the possible increase in locomotor activity.

The N/L ratio is generally used as an indicator of immunity status when animals undergo chronic stress (Lawrence & Rushen, 1993). Some situations, such as transport or poor management practices, may increase the amount of neutrophils and decrease the amount of lymphocytes and eosinophils, thereby increasing the N/L ratio (Kannan et al., 2000). In our study, the N/L ratio did not indicate immunodepression in any animals, nor did any lambs show clinical signs of illness.

Nevertheless, lambs in the C and E groups had significantly higher N/L ratios than lambs in the H group, suggesting that the former may be under chronic stress, which could be alleviated by the establishment of a positive human–animal bond. The inconsistency between the acute stress and the chronic stress of the E group may be due to the presence of unfamiliar ewes, who may interact immediately after mixing. However, we verified (with one observer situated in a covered place) that there were no important aggressive interactions between lambs and ewe nurses (who were themselves selected from a flock for their low aggressive temperament).

Hematological variables are useful for assessing chronic stress related to housing and management (Radostits, Blood, & Gay, 1994; Ramos & Mormède, 1997). In our study, the WBC and RBC were significantly higher for the lambs in the E group, in accordance with the N/L ratio. The high RBC values may be due to excitement or strenuous exercise during handling (Gartner, Callow, & Granzien, 1969). This leads to the release of adrenaline, which contracts the spleen, releasing more RBC into circulation. The increased WBC may also be attributed to excitement during handling (Coles, 1980) and barren housing conditions (Miranda-de la Lama et al., 2010).

The platelet counts observed in our study were higher in lambs from the H group. From a welfare point of view, it is difficult to explain the increase in platelets in the less-stressed lambs. Some studies indicate that platelets increase in humans when they feel emotionally stressed (Levine et al., 1985). However, Wallen (1997) concluded that platelet secretion seems to invariably increase with physical exercise, suggesting that responses of platelets to mental stress are highly variable between individuals and situations. In our study, the higher levels of platelets in animals from the H group are in accordance with higher CK values. Nevertheless, all values were within normal reference ranges for weaning lambs of the same age (Kaneko, Harvey, & Bruss, 1997; Lepherd, Canfield, Hunt, & Bosward, 2009; Lepherd, Canfield, Hunt, Thomson, & Bosward, 2011). The hematocrit was not affected by treatment, indicating that there was no apparent dehydration in our lambs.

The handling practices can cause fear, anxiety, and reactivity to humans (Nordquist et al., 2011). The impact of these practices on the stress response can be evaluated using noninvasive techniques such as IRT. This technique can determine heat loss gradients in the ocular area caused by blood kinetics, as part of the sympathetic-adrenal response during acute stress (Stewart et al., 2007). As a result, the IRT camera is a useful tool for measuring handling reactivity in a noninvasive way.

Our results indicate that physical restraint by a handler provoked higher infrared temperatures in the lambs in the E group compared with the H group, showing that the lambs in the former were more reactive to handling. Cook et al. (2001) found a significant correlation between maximum eye temperature and cortisol. In contrast, Stewart et al. (2007) did not find changes in eye temperature in response to exogenous stimulation of hypothalamic–pituitary–adrenal axis activity in cows measured using the same technique. Our data showed that the H group was more familiar with human handling than the E group, suggesting lower fear and reactivity during IRT (Lyons & Price, 1987; Waiblinger et al., 2006).

CONCLUSION

This study demonstrated that lambs handled gently and raised with daily contact with a female human during the fattening phase are less reactive and better able to modulate their physiological stress during the postweaning period, thereby improving lamb health and weight. Using a ewe-nurse strategy also helps lambs adapt better to the fattening phase, but its effect was less evident. Both strategies improved productive performance and farmer income, compared with control lambs. In summary, providing social enrichment at weaning, especially involving a positive human–animal bond, is a good strategy to alleviate postweaning stress and its undesirable effects on welfare and productive performance. The study also confirms the importance of the need for positive attitudes in stockpersons, who could be taught and included in training programs to increase animal welfare.

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REFERENCES

Adewuyi, A. A., Gruys, E., & van Eerdenburg, F. J. (2005). Non esterified fatty acids (NEFA) in dairy cattle. A review. *Veterinary Quarterly*, *27*, 117–126.

Alexander, G. (1977). Role of auditory cues in mutual recognition between ewes and lambs in Merino sheep. *Applied Animal Ethology, 3*, 65–81.

Apple, J. K., Dikeman, M. E., Minton, J. E., McMurphy, R. M., Fedde, M. R., Leith, D. E., & Unruh, J. A. (1995). Effects of restraint and isolation stress and epidural blockade on endocrine and blood metabolite status, muscle glycogen metabolism, and incidence of dark-cutting longissimus muscle of sheep. *Journal of Animal Science*, *73*, 2295–2307.

Baldock, N. M., & Sibley, R. M. (1990). Effects of handling and transportation on the heart rate and behaviour of sheep. *Applied Animal Behaviour Science*, *28*, 15–39.

Barnett, J. L., & Hemsworth, P. H. (1990). The validity of physiological and behavioural measures of animal welfare. *Applied Animal Behaviour Science*, *25*, 177–187.

Boivin, X., Lensink, B. J., Tallet, C., & Veissier, I. (2003). Stockmanship and farm animal welfare. *Animal Welfare, 12*, 479–492.

Boivin, X., Nowak, R., & Terrazas-Garcia, A. (2001). The presence of the dam affects the efficiency of gentling and feeding on the early establishment of the stockperson–lamb relationship. *Applied Animal Behaviour Science*, *72*, 89–103.

Boivin, X., Tournadre, H., & Le Neindre, P. (2000). Hand-feeding and gentling influence early-weaned lambs' attachment responses to their stockperson. *Journal of Animal Science*, *78*, 879–884.

Box, H. O., & Gibson, K. R. (1999). *Mammalian social learning. Comparative and ecological perspectives*. Cambridge, UK: Cambridge University Press.

Burton, J. L., Kennedy, B. W., Burnside, E. B., Wilkie, B. N., & Burton, J. H. (1989). Dinitrochlorobenzene contact hypersensitivity as a marker trait for selection to improve disease resistance in calves. *Journal of Dairy Science*, *72*, 2351–2361.

Cairns, R. B. (1966). Development, maintenance, and extinction of social attachment behavior in sheep. *Journal of Comparative and Physiological Psychology*, *62*, 298–306.

Cañeque, V., Velasco, S., Díaz, M., Pérez, C., Huidobro, F., Lauzurica, S., . . . González, J. (2001). Effect of weaning age and slaughter weight on carcass and meat quality of Talaverana breed lambs raised at pasture. *Animal Science*, *73*, 85–95.

Caroprese, M. (2008). Sheep housing and welfare. *Small Ruminant Research*, *76*, 21–25.

Caroprese, M., Napolitano, F., Albenzio, M., Annicchiarico, G., Musto, M., & Sevi, A. (2006). Influence of gentling on lamb immune response and human–lamb interactions. *Applied Animal Behaviour Science, 99*, 118–131.

Chacón, G., Garcia-Belenguer, S., Ilguera, J. C., & Palacio, J. (2004). Validation of an EIA technique for the determination of cortisol. *Spanish Journal of Agricultural Research*, *2*, 45–51.

Clayton, D. A. (1978). Socially facilitated behavior. *Quarterly Review of Biology, 53*, 373–392.

Coles, E. H. (1980). *Veterinary clinical pathology* (3rd ed.). Philadelphia, PA: W.B. Sanders.

Cook, N. J., Schaefer, A. L., Warren, L., Burwash, L., Anderson, M., & Baron, V. (2001). Adrenocortical and metabolic responses to ACTH injection in horses: An assessment by salivary cortisol and infrared thermography of the eye. *Canadian Journal of Animal Science*, *81*, 621.

Dantzer, R., & Mormède, P. (1983). Stress in farm animals: A need for reevaluation. *Journal of Animal Science, 57*, 6–18.
Dubreuil, P., Arsenault, J., & Bélanger, D. (2005). Biochemical reference ranges for groups of ewes of different ages. *The Veterinary Record*, *156*, 636–638.
Dumont, B., & Boissy, A. (2000). Grazing behavior of sheep in a situation of conflict between feeding and social motivation. *Behavioural Processes*, *49*, 131–138.
Faichney, G. J. (1992). Consumption of solid feed by lambs during their transition from pre-ruminant to full ruminant function. *Applied Animal Behaviour Science, 34*, 85–91.
Feh, C., & deMazieres, J. (1993). Grooming at a preferred site reduces heart rate in horses. *Animal Behaviour, 46*, 1191–1194.
Fisher, A. D., Crowe, M. A., Nualláin, E. M. O., Monaghan, M. L., Prendiville, D. J., Kiely, P. O., & Enright, W. J. (1997). Effect of suppressing cortisol following castration of bull calves on adrenocorticotropic hormone, in vitro interferon-γ production, leukocyte, acute-phase proteins, growth, and feed intake. *Journal of Animal Science*, *75*, 1899–1908.
Gartner, R. J. W., Callow, L. L., & Granzien, C. K. (1969). The concentration of blood constituents in relation to handling of cattle. *Research in Veterinary Science*, *10*, 7.
Gordon, I., Martin, C., Feldman, R., & Leckman, J. F. (2011). Oxytocin and social motivation. *Developmental Cognitive Neuroscience*, *1*, 471–493.
Grubb, P., & Jewell, P. (1966). Social grouping and home range in feral Soay sheep. *Symposia of the Zoological Society of London, 18*, 179–210.
Hargreaves, A. L., & Hutson, G. D. (1990). The effect of gentling on heart rate, flight distance and aversion of sheep to a handling procedure. *Applied Animal Behaviour Science*, *26*, 243–252.
Hemsworth, P. H. (2003). Human–animal interactions in livestock production. *Applied Animal Behaviour Science, 81*, 185–198.
Hemsworth, P. H., Barnett, J. L., Coleman, G. J., & Hansen, C. (1989). A study of the relationships between the attitudinal and behavioural profiles of stockpersons and the level of fear of humans and reproductive performance of commercial pigs. *Applied Animal Behaviour Science, 23*, 301–314.
Hemsworth, P. H., Barnett, J. L., & Hansen, C. (1987). The influence of inconsistent handling on the behaviour, growth and corticosteroids of young pigs. *Applied Animal Behaviour Science, 17*, 245–252.
Hemsworth, P. H., & Coleman, G. J. (1998). Human–animal interactions and animal productivity and welfare. In P. H. Hemsworth & G. J. Coleman (Eds.), *Human–livestock interactions: The stockperson and the productivity and welfare of intensively farmed animals* (pp. 39–61). Bristol, England: CAB International.
Hemsworth, P. H., Coleman, G. J., Barnett, J. L., & Borg, S. (2000). Relationships between human–animal interactions and productivity of commercial dairy cows. *Journal of Animal Science, 78*, 2821–2831.
Henry, S., Zanella, A. J., Sankey, C., Richard-Yris, M. A., Marko, A., & Hausberger, M. (2012). Adults may be used to alleviate weaning stress in domestic foals (*Equus caballus*). *Physiology & Behaviour, 106*, 428–438.
Hinch, G. N., Lynch, J. J., Elwin, R. L., & Green, G. C. (1990). Long-term associations between Merino ewes and their offspring. *Applied Animal Behaviour Science*, *27*, 93–103.
Jones, R. B., & Hughes, B. O. (1981). Effects of regular handling on growth in male and female chicks of broiler and layer strains. *British Poultry Science*, *22*, 461–465.
Kaneko, J. J., Harvey, J. W., & Bruss, M. L. (1997). *Clinical biochemistry of domestic animals* (Appendix VII). San Diego, CA: Academic.
Kannan, G., Terrill, T. H., Kouakou, B., Gazal, O. S., Gelaye, S., & Amoah, E. A. (2000). Transportation of goats: Effects on physiological stress responses and live weight loss. *Journal of Animal Science, 78*, 1450–1457.
Kelly, R. W. (1992). Lamb mortality and growth to weaning in commercial Merino flocks in Western Australia. *Australian Journal of Agricultural Research*, *43*, 1399–1416.
Knowles, T. G., Warriss, P. D., Brown, S. N., & Edwards, J. E. (1998). Effects of stocking density on lambs being transported by road. *The Veterinary Record*, *142*, 503–509.
Korff, J., & Dyckhoff, B. (1997). Analysis of the human animal interaction demonstrated in sheep by using the model of 'social support.' In *31st International Congress of the International Society for Applied Ethology* (pp. 87–88). Prague, Czech Republic: Wageningen Academic.
Kuhn, C. M., Pauk, J., & Schanberg, S. M. (1990). Endocrine responses to mother–infant separation in developing rats. *Developmental Psychobiology*, *23*, 395–410.

Langlands, J. P., & Donald, G. E. (1975). The intakes and growth rates of grazing Border Leicester × Merino lambs weaned at 21, 49 and 77 days. *Animal Production*, *21*, 175–181.

Lansade, L., Bertrand, M., Boivin, X., & Bouissou, M. F. (2004). Effects of handling at weaning on manageability and reactivity of foals. *Applied Animal Behaviour Science*, *87*, 131–149.

Lawrence, A. B., & Rushen, J. (1993). *Stereotypic animal behaviour: Fundamentals and applications to welfare*. Bristol, England: CAB International.

Lensink, B. J., Fernandez, X., Boivin, X., Pradel, P., Le Neindre, P., & Veissier, I. (2000). The impact of gentle contacts on ease of handling, welfare, and growth of calves, and quality of veal meat. *Journal of Animal Science*, *78*, 1219–1226.

Lepherd, M. L., Canfield, P. J., Hunt, G. B., & Bosward, K. L. (2009). Haematological, biochemical and selected acute phase protein reference intervals for weaned female Merino lambs. *Australian Veterinary Journal*, *87*, 5–11.

Lepherd, M. L., Canfield, P. J., Hunt, G. B., Thomson, P. C., & Bosward, K. L. (2011). Assessment of the short-term systemic effect of and acute phase response to mulesing and other options for controlling breech flystrike in Merino lambs. *Australian Veterinary Journal*, *89*, 19–26.

Levine, S. P., Towell, B. L., Suarez, A. M., Knieriem, L. K., Harris, M. M., & George, J. N. (1985). Platelet activation and secretion associated with emotional stress. *Circulation*, *71*, 1129–1134.

L'Heureux, N., Lucherini, M., Festa-Bianchet, M., & Jorgenson, J. T. (1995). Density-dependent mother–yearling association in bighorn sheep. *Animal Behaviour*, *49*, 901–910.

Luzi, F., Mitchell, M., Costa, L. N., & Redaelli, V. (2013). *Thermography. Current status and advances in livestock animals and in veterinary medicine*. Brescia, Italy: Fondazione Iniziative Zooprofilattiche E Zootecniche.

Lyford, S. J. (1988). Growth and development of the ruminant digestive system. In D. C. Curch (Ed.), *The ruminant animal* (pp. 44–63). Upper Saddle River, NJ: Prentice-Hall.

Lyons, D. M. (1989). Individual differences in temperament of dairy goats and the inhibition of milk ejection. *Applied Animal Behaviour Science*, *22*, 269–282.

Lyons, D. M., & Price, E. O. (1987). Relationships between heart rates and behaviour of goats in encounters with people. *Applied Animal Behaviour Science*, *18*, 363–369.

Markowitz, T. M., Dally, M. R., Gursky, K., & Price, E. O. (1998). Early handling increases lamb affinity for humans. *Animal Behaviour*, *55*, 573–587.

Mathis, C. P., & Carter, B. (2008). *Minimizing weaning stress on calves* (Cooperative Extension Service Report, Guide B-221). Las Cruces: New Mexico State University.

Mears, G. J., & Brown, F. A. (1997). Cortisol and beta-endorphin responses to physical and psychological stressors in lambs. *Canadian Journal of Animal Science*, *77*, 689–694.

Mendl, M. (1991). Some problems with the concept of a cut-off point for determining when an animal's welfare is at risk. *Applied Animal Behaviour Science*, *31*, 139–146.

Miranda-de la Lama, G. C., Rivero, L., Chacon, G., Garcia-Belenguer, S., Villarroel, M., & María, G. A. (2010). Effect of the pre-slaughter logistic chain on some indicators of welfare in lambs. *Livestock Science*, *128*, 52–59.

Miranda-de la Lama, G. C., Villarroel, M., & Maria, G. A. (2012). Behavioural and physiological profiles following exposure to novel environment and social mixing in lambs. *Small Ruminant Research*, *103*, 158–163.

Napolitano, F., Caroprese, M., Girolami, A., Marino, R., Muscio, A., & Sevi, A. (2006). Effects of early maternal separation of lambs and rearing with minimal and maximal human contact on meat quality. *Meat Science*, *72*, 635–640.

Napolitano, F., De Rosa, G., & Sevi, A. (2008). Welfare implications of artificial rearing and early weaning in sheep. *Applied Animal Behaviour Science*, *110*, 58–72.

Nordquist, R. E., Heerkens, J. L. T., Rodenburg, T. B., Boks, S., Ellen, E. D., & van der Staay, F. J. (2011). Laying hens selected for low mortality: Behaviour in tests of fearfulness, anxiety and cognition. *Applied Animal Behaviour Science*, *131*, 110–122.

Orgeur, P., Bernard, S., Naciri, M., Nowak, R., Schaal, B., & Levy, F. (1999). Psychobiological consequences of two different weaning methods in sheep. *Reproduction Nutrition Development*, *39*, 231–244.

Orgeur, P., Hay, M., Mormède, P., Salmon, H., Le Dividich, J., Nowak, R., . . . Lévy, F. (2001). Behavioural, growth and immune consequences of early weaning in one-week-old Large-White piglets. *Reproduction Nutrition Development*, *41*, 321–332.

Orgeur, P., Mavric, N., Yvore, P., Bernad, S., Nowak, R., Schaal, B., & Lévy, F. (1998). Artificial weaning in sheep: Consequences on behavioural, hormonal and immunopathological indicators of welfare. *Applied Animal Behaviour Science*, *58*, 87–103.

Pearson, R. A., & Mellor, D. J. (1976). Some behavioural and physiological changes in pregnant goats and sheep during adaptation to laboratory conditions. *Research in Veterinary Science*, *20*, 215–217.

Provenza, F. D., & Balph, D. F. (1987). Diet learning by domestic ruminals: Theory, evidence and practical implications. *Applied Animal Behaviour Science, 18*, 211–232.

Pryce, C. R. (1992). A comparative systems model of the regulation of maternal motivation in mammals. *Animal Behaviour, 43*, 417–441.

Radostits, O. M., Blood, D. C., & Gay, C. (1994). *Veterinary medicine*. London, England: Balliere Tindall.

Ralphs, M. H., Graham, D., & James, L. F. (1994). Social facilitation influences cattle to graze locoweed. *Journal of Range Management, 47*, 123–126.

Ralphs, M. H., & Provenza, F. D. (1999). Conditioned food aversions: Principles and practices, with special reference to social facilitation. *Proceedings of the Nutrition Society*, *58*, 813–820.

Ramos, A., & Mormède, P. (1997). Stress and emotionality: A multidimensional and genetic approach. *Neuroscience & Biobehavioral Reviews*, *22*, 33–57.

Rault, J., Boissy, A., & Boivin, X. (2011). Separation distress in artificially-reared lambs depends on human presence and the number of conspecifics. *Applied Animal Behaviour Science, 132*, 42–50.

Raussi, S., Lensink, B. J., Boissy, A., Pyykkonen, M., & Veissier, I. (2003). The effect of contact with conspecifics and humans on calves' behaviour and stress responses. *Animal Welfare*, *12*, 191–203.

Rhind, S. M., Elston, D. A., Jones, J. R., Rees, M. E., McMillen, S. R., & Gunn, R. G. (1998). Effects of restriction of growth and development of Brecon Cheviot ewe lambs on subsequent lifetime reproductive performance. *Small Ruminant Research, 30*, 121–126.

Schaefer, A. L., Jones, S. D. M., Tong, A. K. W., & Vincent, B. C. (1988). The effects of fasting and transportation on beef cattle. 1. Acid-base-electrolyte balance and infrared heat loss of beef cattle. *Livestock Production Science*, *20*, 15–24.

Schichowski, C., Moors, E., & Gauly, M. (2008). Effects of weaning lambs in two stages or by abrupt separation on their behavior and growth rate. *Journal of Animal Science*, *86*, 220–225.

Schichowski, C., Moors, E., & Gauly, M. (2010). Influence of weaning age and an experimental Haemonchus contortus infection on behaviour and growth rates of lambs. *Applied Animal Behaviour Science*, *125*, 103–108.

Schmied, C., Waiblinger, S., Scharl, T., Leisch, F., & Boivin, X. (2008). Stroking of different body regions by a human: Effects on behaviour and heart rate of dairy cows. *Applied Animal Behaviour Science*, *109*, 25–38.

Statistical Analysis System. (1998). *User's guide: Statistics* (Release 6.0). Cary, NC: Author.

Steffens, A. B., & Boer, S. F. (1999). Impact of stress on animal intermediate metabolism. In P. H. M. Balm (Ed.), *Stress physiology in animals* (p. 108). Sheffield, England: Sheffield Academic.

Stewart, M., Webster, J. R., Schaefer, A. L., Cook, N. J., & Scott, S. L. (2005). Infrared thermography as a non-invasive tool to study animal welfare. *Animal Welfare*, *14*, 319–325.

Stewart, M., Webster, J. R., Verkerk, G. A., Schaefer, A. L., Colyn, J. J., & Stafford, K. J. (2007). Non-invasive measurement of stress in dairy cows using infrared thermography. *Physiology & Behaviour*, *92*, 520–525.

Thompson, C. I. (1976). Growth in the Hubbard broiler: Increased size following early handling. *Developmental Psychobiology*, *9*, 459–464.

Torres-Hernandez, G., & Hohenboken, W. (1979). An attempt to assess traits of emotionality in crossbred ewes. *Applied Animal Ethology*, *5*, 71–83.

Uvnäs-Moberg, K. (1997). Physiological and endocrine effects of social contacts with special reference to oxytocin. *Annals of the New York Academy of Science, 807*, 146–163.

Van de Water, G., Verjans, F., & Geers, R. (2003). The effect of short distance transport under commercial conditions on the physiology of slaughter calves; pH and colour profiles of veal. *Livestock Production Science*, *82*, 171–179.

Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M. V., Janczak, A. M., Visser, E. K., & Jones, R. B. (2006). Assessing the human–animal relationship in farmed species: A critical review. *Applied Animal Behaviour Science*, *101*, 185–242.

Wallen, N. H. P. (1997). Effects of mental and physical stress on platelet function in patients with stable angina pectoris and healthy controls. *European Heart Journal, 18*, 807–815.