Effect of a screen with flaps and straw on behaviour, stress response, productive performance and meat quality in indoor feedlot lambs

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A b s t r a c t

We analysed the effect of a modified pen using a wooden screen with flaps and cereal straw as forage and bedding, on behaviour, stress response, performance and meat quality variables of lambs housed in feedlots. Sixty male lambs were placed in enriched (ESF) or conventional (CO) pens (3 pens/treatment, 10 lambs/pen). The CO envi-ronment was barren. The ESF lambs showed a great preference for the provided items, which encouraged more natural and richer behaviour, reducing stereotypies and lamb aggressions, and increasing affiliations ($P \le 0.05$), which improves group cohesion. However, ESF lambs also developed a more natural coping style to the handling, evidenced by the higher cortisol levels (65.4 vs. 43.8 nmol/L) and a higher eye temperature as response to the reactivity test (38.1 vs. 37.8 °C). The ESF lambs had a higher ($P \le 0.05$) slaughter weight (27.2 vs. 26.3 kg), confor-mation score (7.38 vs. 6.07) and pH 24 (5.63 vs. 5.56) but lower cooking losses (12.9 vs. 14.9%) than CO lambs.

Keywords: Enriching items; Indoor housing; Intensive system; Lamb welfare; Meat quality

1. Introduction

In Europe there is a growing public concern for the welfare of farm animals, which is considered a priority for an increasing number of peo-ple (European Commission, 2006). Current sheep production systems should pay more attention to these more informed consumers and their increasing demands. A recent study of meat retailers' attitudes to-wards animal welfare in Spain concluded that according to their percep-tion, one of the reasons why consumers buy welfare-friendly products is the improved well-being of the animals and the associated organoleptic properties of the meat (Miranda-de la Lama, Sepúlveda, Villarroel, & María, 2013a).

In several Mediterranean countries lambs are kept indoors for inten-sive fattening (Bernués, Ruiz, Olaizola, Villarba, & Casasús, 2011; De Rancourt, Fois, Lavín, Tchakérian, & Vallenard, 2006). In Spain, Ternasco type lambs are light lambs that are weaned at approximately 45 days-old and fattened until less than 100 days-old (Ripoll, Joy, Muñoz, & Albertí, 2008). The intensification of management programmes for this type of lamb has several advantages for the production chain. The final stage of fattening is externalised to off-farm units (cooperative feedlots, CCs) so that the farmer is only responsible for the breeding stage, which simplifies the process and improves the homogeneity of the product (Miranda-de la Lama et al., 2009). However, previous stud-ies have found that lambs face new problems such as multiple trans-ports, social mixing, and novel and barren environments with frequent handling, which may compromise animal welfare (Miranda-de la Lama, Villarroel, & Maria, 2012; Miranda-de la Lama et al., 2009; Miranda-de la Lama et al., 2010a,b; Miranda-de la Lama, Villarroel, Liste, Escós, & María, 2010). These difficulties could also have negative effects on production yield and the quality of the product delivered to the consumers, as a consequence of the insertion of a new link in the logistic chain of the process (Ferguson & Warner, 2008; Miranda-de la Lama et al., 2009).

In the intermediate step from farm to slaughter lambs are fattened at feedlots (CCs) in barren and uninspiring environments, often even deprived of straw. Recent studies in young ruminants have shown that straw is an important source of fibre and stimulation that promotes the welfare of these animals through a healthy rumen (Webb et al., 2014) and by encouraging natural and richer behaviours and reducing abnormal ones (calves, Webb et al., 2014; lambs, Teixeira, Miranda-de la Lama, Villarroel, Escós, & María, 2014). A barren environment lacking in stimulating elements leads to boredom which may result in animals developing stereotypies, frustration and stress (Wood-Gush & Beilhartz, 1983). Environmental enrichment programmes can be an important tool to considerably improve the life of confined animals (Mason, Clubb, Latham, & Vickery, 2007; Pascual-Alonso et al., 2015; Young, 2003).

Considering the positive effect that straw can have on lambs, the present study provides a full functional enrichment using several items (including straw) to analyse the effect from a broader perspective,

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evaluating a combined set of variables. The hypothesis is that providing full enrichment for lambs improves their adaptation processes to new environments, benefiting animal welfare through a reduction in abnor-mal behaviours and by encouraging more diverse behaviour, this in turn having a positive effect on the final product quality. The aim of this study was to analyse the effect of furnished pens, equipped with a screen with flaps and provided with cereal straw, on the behaviour, stress response, performance and meat quality variables of finishing lambs.

2. Methods

The study was carried out using the facilities of the Animal Experi-mentation Service of the University of Zaragoza in the Autonomous Community of Aragon, Spain (41°41′ N). All the lambs were raised, transported and slaughtered according to the current regulations of the European Commission (1986) for Scientific Procedure Establish-ments. Experimental protocols were approved by the Animal Experi-mentation Ethics Committee of the University of Zaragoza (ES 50 297 0012 006).

2.1. Study description

Sixty Rasa Aragonesa intact male lambs (65 days old) with an aver-age live weight of 17.35 ± 0.23 kg, were allocated to two treatments (weights were balanced across treatments) according to the pen envi-ronment during the finishing phase of fattening, which lasted five weeks (35 days). Lambs were housed indoors in six pens with 10 lambs/pen (2.9 × 3.3 m, 0.95 m^2 per lamb) and three replicates per treatment. Lambs from the enriched group (ESF) were kept in pens with items of furniture that consisted of a screen (wooden panel) with wooden flaps placed in the middle of the pen, parallel to the concentrate hopper (Fig. 1). The screen dimensions were 1.6 m high by 1.8 m wide and the flap dimensions on each side of the screen were 0.5 m high by 0.5 m wide by 1.8 m long. The furniture allowed lambs to hide from po-tential aggressors (as alternative environments are created) to explore, to rest lying down (on the upper or ground level), to play and shelter themselves. The ESF lambs were also provided with cereal straw (72%NDF, 38% cellulose, 25% hemi cellulose, 8% lignin and 0.2% cutine) as for-age in a fodder rake (fresh straw was added every morning, ad libitum) and bedding on the floor. Lambs from the control group (CO) were kept in a barren pen of the same dimensions as that of the enriched lambs, but without any enrichment, mimicking CC conditions. For hygienic rea-sons a thin layer of sawdust was added to CO pens at the beginning of the experiment. All lambs were fed a commercial concentrate (Ovirum Alta Energía®) containing barley, wheat, calcium carbonate, sodium chloride and a vitamin supplement corrector (18% crude protein and 11.5 MJ metabolisable energy/kg DM). Feeding and water consumption were ad libitum. In both treatments the concentrate hopper was wide enough to allow all lambs to eat simultaneously. Water was provided using a float drinker installed in a corner of each pen. Feed consumption (concentrate) was recorded to estimate the conversion index during the fattening period. The animals were weighed individually at the begin-ning of experimental period (W1) and just before slaughter (W2). Lighting was natural and pens were ventilated with a system of win-dows and lintels to provide a chimney effect that ensured that the tem-perature and air quality were kept within normal ranges for lambs.

2.2. Behaviour and cognitive test

All lambs were individually identified by numbers or letters painted on their sides and rump with a washable animal marking spray. A video-recording device (model VDVR-9, Circontrol S.A., Terrassa, Spain) was set up in a room close to the pens to record animal mainte-nance behaviour, use of space, stereotypies and social behaviour. One camera was placed in front of each pen, 2.2 m from ground level. The an-imals were recorded by video for 12 h/day during the 4th week, be-tween days 21 and 26 (inclusive) of the fattening period. All videos were analysed by the same trained observer.

Two kinds of sampling were carried out: instantaneous, every 10 min (8 am to 8 pm for 6 days) with a total of 1296 scan samples per treatment, recording in each sample the use of space (12,960 obser-vations per treatment) and maintenance behaviour and stereotypies (12,960 observations per treatment). Recorded behaviours included resting (RT, lamb lying down), standing (ST, lamb standing on all 4 legs), walking (WK, lamb on all 4 legs and in motion), feeding on con-centrate (FC, lamb searching for feed concentrate in the hopper and eat-ing it), foraging in the straw (FS, lamb searching for forage straw in the fodder rake and eating it), drinking (DK, lamb drinking water from the drinker), and stereotypes (frequent and non-functional oral manipula-tion of objects, an abnormal behaviour commonly seen in ungulates in stressful situations, Mason et al., 2007). The recording of stereotypes by scan sampling was only a general approximation and allowed to identify the time of day when they were more common. To measure the use of space (placements), four areas were defined: the screen with flap (SFA, lamb using the area of the furniture with half or more than half of its body on it, also differentiating two subzones in ESF lambs: upper and lower), concentrate hopper area (CHA, lambs with their heads inside of the feed hopper or resting on it with half or more than half of their body supported in that area), straw rake area (SRA, lambs foraging or resting on the rake with half or more than a half of their body supported in that area) and remaining area (RMA, rest of the pen). We recorded the use of space in CO pens tracing the same (al-beit imaginary) divisions of the areas defined in ESF pens. To identify the time of the day in which lambs commonly displayed certain behaviours, daily observations were divided into three blocks: morning (am, from 8 h to 12 h), afternoon (noon, from 12 h to 16 h) and evening (pm, from 16 h to 20 h). Continuous sampling was performed to record social interactions, i.e. the number of agonistic (aggressive) and affiliative in-teractions per animal (described in Table 1) and stereotypies, i.e. the number of times that an animal repeated an abnormal behaviour, if per-ceived as such (as defined by Mason et al., 2007). We used scan sam-pling to detect considerable behavioural changes during the course of the day.

The lambs underwent cognitive testing using a T-maze (Fig. 2). A mirror (70 × 30 cm) and loudspeakers were placed in the target zone in the left arm and an observation platform was located on a 3 m-high platform adjacent to the T-maze apparatus so as not to influence animal movements. Further details of the structural design of the maze can be found in Aguayo-Ulloa, Villarroel, Pascual-Alonso, Miranda-de la Lama, and María (2014b).

The stimulation sounds used in the experiment were a playback of a computer random selection of calls from lambs from all of the pens (same breed, sex and age). Recordings were made at a distance of 2 m from the noise source using a Handy Recorder H1 (Zoom Corporation, Tokyo, Japan) numeric recorder (sampling rate: 44.1 kHz). Sounds were then imported to a computer at a sampling rate of 44.1 kHz and saved in WAV format, at 16-bit amplitude resolution. We used Audacity® 2.0 (General Public License) audio software to prepare the sound sequences that were played back. A sample of each of the sounds was combined into a 5-minute segment and a random portion of this segment was played back during each trial. The intensity of the noise was measured using a Bioblock Scientific Sound Level Meter type 50517 (Thermo Fisher Scientific Inc.). The sounds were played back at a set volume, ensuring that the lambs were exposed to an intensity of 81 dB through most of the T-maze, using a Handy Recorder H1 connect-ed to a loudspeaker located at floor level in left arm of the target zone.

Each lamb underwent the cognitive test during the last week of the experiment, on two consecutive days, once each day of the test, without receiving prior training. Each animal stayed in the start box for 10 s before a guillotine door was lifted to allow it to enter the maze. After the lambs left the start box, the guillotine door was quietly closed. At the same time the recording was played and the test began. The test was successfully passed if the individual found the target zone (which was always located in the left arm) where there was a social clue: vi-sual source (mirror) and sound source (bleating sound from loud-speaker). Each animal was given a maximum time of 5 min to solve the T-corridor and any lambs that did not solve the challenge were assigned the maximum time. Each test was filmed and the time taken by each lamb to solve the T-corridor was recorded. For each lamb and exposure we recorded the total time employed to solve thetest, thetimethat thelamb spentinthefirst chamber (isolation time) and the number of crossed areas (NQUAD) by the lamb during the trial (as an estimation of the locomotor behaviour).

2.3. Physiological welfare indicators

Blood samples were taken by jugular venipuncture with vacuum tubes (before final weighing) to evaluate physiological responses to stress (two 4 ml tubes per animal, with and without anticoagulant, EDTA-K3). The samples were collected by the trained personnel that handled the animals and venipuncture was carried out on each lamb as quickly as possible as a necessary precaution to avoid sampling error. The samples were kept on ice for a maximum of 2 h and taken to the laboratory for routine haematological measurements. EDTA plas-ma and serum were centrifuged at 3000 rpm for 10 min and aliquots were frozen and kept at −30 °C until analysed. An automatic particle counter (Microcell counter F-800 and auto dilutor AD-260, both from Sysmex™) were used to count red blood cells (RBC) and white blood cells (WBC) (number per litre), and haematocrit (%). The leukocyte for-mula was estimated from blood swabs on clean slides. Staining was per-formed by the rapid panoptic method using dyes from Química Clinica Aplicada Inc. (QCA). 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 an indicator of chronic stress (Lawrence & Rushen, 1993). Serum samples were used to determine the concentra-tion of glucose (mg/dl, Ref. Glucose AE2-17), and the activity of creatinine kinase (CK) (UI/L) (Ref. CK.NAC AE1-13) using an ACE® multianalyser (Alfa Wasserman Clinical Chemistry System) and Alfa Wasserman reagents. Serum concentration of non-esterified fatty acid (NEFA) levels was analysed by an ACE® multianalyser (Alfa Wasserman Clinical Chemistry System), with commercial kits (Wako 994-75409 NEFA C test kit). The concentration of cortisol was determined from plasma (EDTA-K3) by enzyme immunoassay using an "in home-kit" (validated by Chacón, Garcia-Belenguer, Illera del Portal, & Palacio, 2004). Each sample was determined in duplicate from 50 µl of plasma. The mean of the duplicate was used as the result and expressed in nmol/L. Inter- and intra-assay coefficients of variation were 3.5–6%and 3.9–9.9%, respectively. The concentration of lactate was determined using a Sigma Diagnostic kit (lactate no. 735-10) and spectrophotome-ter (Lambda 5, Perkin Elmer).

Eye temperature was taken by infrared thermography (IRT) as a response to a reactivity test (Aguayo-Ulloa, Villarroel, Pascual-Alonso, Miranda-de la Lama, & María, 2014b; Pascual-Alonso et al., 2013). The lambs were randomly captured and restrained by a trained handler for 1 min during which a photograph of the left eye was taken (approx-imate distance 20 cm) with an IR camera (Testo 880 Thermal Imaging Camera; Testo AG, Lenzkirch, Germany) to evaluate acute stress re-sponse produced by handling (Stewart et al., 2007). The built-in lens (24°) was used and the camera was calibrated for the current room temperature and relative humidity. The emissivity value used was 0.98, which is that recommended by the camera manufacturer for bio-logical tissues. A clear infrared image (precise location and perfect focus) was taken of each animal and image analysis software (IRSoft™ software, Testo AG, Lenzkirch, Germany) was used to determine the maximum temperature within an oval area traced around the eye, in-cluding the eyeball and approximately 1 cm around the outside of the eyelids (Stewart et al., 2007).

2.4. Productive performance and meat quality

The amount of concentrate added to the feeder and feed remains (at the end of experiment) was recorded. We estimated the total consump-tion of concentrate (TCC) as the difference between concentrate added and concentrate remaining in the feeder hopper. Average daily gain (ADG) was estimated as the difference between W2 − W1 (WG) divid-ed by the total fattening period (35 days). The concentrate conversion index (CCI) was estimated as TCC/WG. The animals were slaughtered within the weight range of the Ternasco-type category (Sañudo, Sánchez, & Alfonso, 1998; Sañudo, Santolaria, María, Osorio, & Sierra, 1996) at an EU-approved abattoir located in the city of Zaragoza. After overnight lairage, the lambs were electrically stunned and dressed using standard commercial procedures.

After slaughter, carcasses were stored in cold rooms at 2 °C for 24 h. Cold carcasses were weighed (CW) at 24 h (at 1–2 °C) in the cold room. The extent of bruising on the carcasses was estimated visually using an adapted scale from Miranda-de la Lama et al. (2009) with a score of 0 (no bruises), 1 (slight bruising), 2 (moderate bruising) or 3 (high bruis-ing). The carcass conformation (CS) and carcass fatness (FS) scores were graded according to the European classification system (EEC, 1993), the EUROP conformation scale (converted to a 15-point scale) and the car-cass fatness scale (converted to a 15-point scale). After chilling for 24 h, the left rack was removed from T1 to L6 vertebrae (standard lamb cut as described by Colomer-Rocher, Morand-Fehr, Kirton, Delfa, & Sierra, 1988). The pH 24 h (pHult) of the M. longissimus was assessed using a portable pH meter (fitted with a penetration electrode, model 52-00 Crison Instruments), which was inserted into a small incision on the left loin (L2–L3 vertebrae). The pH meter was re-calibrated after every five samples, using two standard buffer solutions at pH 7.02 and 4.00. Subsequently the left rack was transferred to the Meat Laboratory at the Faculty of Veterinary Medicine of the University of Zaragoza without cold chain interruption.

The M. longissimus was removed from the rack to prepare the sam-ples. Colour was estimated at 24 h post mortem and after 15 min of blooming using a Minolta CM 200 calibrated chromameter with a stan-dard illuminant D65 and a 10° observer with an aperture size of 2.54 cm, following the CIE L*a*b* system, to measure the colour of fresh meat on the cut surface of the T13 vertebra of the M. longissimus. Chroma (C*) and hue (H*) indices were calculated as $C^* = (a^{*2} + b^{*2})^{0.5}$ (related to the quantity of pigment) and hue $H^* = 1/\tan(b^*/a^*)$ (attribute of col-our perception). Final values were the average of three measurements. A section of meat from T10 to T13 vertebrae was weighed (FMW)(mean 118 g), vacuum-packed at −900 mbar of pressure in polyethyl-ene–polyamide bags with an ethyl vinyl acetate sealant layer (30 × 25 cm¸ 90 µm thickness, water vapour transmission rate of 2.8 g/m^2 day at 23 ± 1 °C and 85 ± 2% RH), frozen, and stored at −20 °C after 72 h of aging, to evaluate cooking losses (CL%) and to per-form the Warner–Bratzler test. The samples were thawed for 24 h in a refrigerator (2–4 °C) in their vacuum-sealed plastic bags before testing.

The thawed samples were weighed (TMW) (mean 113.2 g) and cooked for approximately 35 min in plastic bags at 75 °C in a water bath (GLF-D3006) until the internal temperature of the meat (measured with a penetration thermometer) reached 70 °C and then cooled for 30 min under cold running water. After the samples were cooled to room tem-perature, they were blotted dry using paper towels and weighed (CMW). The CL% was [CL% = 100 − (CMW × 100) / TMW]. The texture of the cooked meat was measured with a Warner–Bratzler device, using an Instron 4301 equipped with a Warner–Bratzler shear. A MITUTOYO Series 500 (Mitutoyo Corporation, Aurora, IL, USA) digital calliper was used to cut 1 cm^2 pieces (in the direction of the muscle fibres). Three measurements were taken for each animal. Shear force (kg/cm^2), max-imum stress (kg/cm^2), and toughness (kg) were measured as described by Campo et al. (2000). The gauge and the gauge length of the sample were 10 mm and 30 mm, respectively. The samples were sheared per-pendicularly to the grain. The load cell was 100 kg (minimum load level 0.001 kg), crosshead speed was 150 mm/min (high extension limit = 30 mm), and the sampling rate was 20 points/s.

2.5. Statistical analysis

Data were analysed using SAS/STAT (9.1 SAS Inst. Inc., Cary, NC, USA) by SAS (1988). Production, physiology and meat quality data were analysed using least squares methods of the GLM procedure of SAS (SAS, 1988) fitting a one-way model with a fixed effect of environmen-tal enrichment (two levels). The general representation of the model used was as follows: $y = Xb + e$, where y was an N × 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. The original full model included the effect of replicate (fitting animals nested within pens), which was found to be non-significant and consequently was dropped from the model. Meat and carcass quality variables were co-varied with cold car-cass weight.

Behaviour data were transformed by the square root function. Social behaviour (average per animal per day) was analysed using PROC MIXED with repeated measurements (day), treatment as the fixed ef-fect and the lamb as the random effect. For maintenance behaviour and stereotypies we added time of the day as the fixed effect to the model. T-maze variables were subjected to repeated-measures ANOVAs that examined the main effects of treatment (control and enriched), T-maze trial (1st and 2nd T-maze exposure; the repeated measure), and their interaction. A probability value of $P \leq 0.05$ was considered statis-tically significant.

3. Results

3.1. Behaviour and cognitive test

The lambs spent a great deal of their time resting (mean, 75%) main-ly in the afternoon (mean, 86%) and evening (mean, 85%), but the ESF lambs rested less and spent more time standing ($P \leq 0.05$) in the morn-ing than CO lambs (Table 2). The lambs fattened in the enriched envi-ronment ate more straw during the morning than in the afternoon or in the evening ($P \leq 0.05$). The CO lambs ate more concentrate ($P \leq 0.05$) in the morning and evening, than the ESF lambs. The CO lambs also walked more than the ESF lambs, but only in the morning ($P \leq 0.05$). The stereotypic behaviour recorded by continuous and scan sampling is shown in Fig. 3 (A and B, respectively). The CO lambs performed significantly more stereotypies ($P \leq 0.05$) than ESF lambs (Fig. 3A) and most of these stereotypies were recorded during the morning (Fig. 3B).

The overall rate of occupancy of space was different between treat-ments (Fig. 4). The most used area in ESF pens was the SFA, while in the CO pens it was the rest of the area. The highest occupancy rate of the SFA in ESF pens was during the afternoon and evening ($P \leq 0.05$, Table 3) and more than half of the lambs used the part underneath the flaps. The occupancy rate of the CHA and SRA were higher in CO ($P \leq 0.05$) than in ESF pens, with the exception of SRA in the morning. The upper zone of the flaps had a low occupancy rate (less than 3%throughout the day).

The social behaviour of lambs is shown in Fig. 5. The ESF lambs per-formed fewer aggressive interactions ($P \leq 0.05$) than CO lambs (Fig. 5A). It was only on day 5 that ESF lambs showed a similar level of aggression to that of CO lambs. The ESF lambs also showed higher affil-iations ($P \leq 0.05$) than CO lambs throughout the observed period (Fig. 5B).

Regarding the T-maze test (Table 4), there were no significant differ-ences between treatments in the time taken to leave the isolation cham-ber, in the total time to solve the maze or in the number of quadrants crossed to reach the target. The CO lambs significantly ($P \leq 0.05$) reduced the total time to solve the maze in the second exposure, while the ESF lambs significantly ($P \leq 0.05$) reduced the number of quadrants crossed during the second exposure.

3.2. Physiological welfare indicators

There were significant differences between treatments in the physi-ological and haematological variables analysis (Table 5). Cortisol and glucose levels were higher ($P \leq 0.05$) and lactate and CK were lower ($P \leq 0.05$) in ESF lambs compared to CO lambs. There was no significant difference between treatments in N/L ratio. Haematocrit, RBC and haemoglobin were higher in lambs from enriched pens. Eye tempera-ture was slightly higher (+0.32 °C) in ESF lambs ($P \leq 0.05$).

3.3. Production and meat quality

There were significant differences ($P \leq 0.05$) in slaughter weight and carcass conformation scores (Table 6). The ESF lambs weighed more (+3.6%) and had higher conformation scores (+1.3 points) than the CO lambs. In relation to meat quality, Table 7 shows that there were sig-nificant differences between treatments in ultimate pH at 24 h post mortem and cooking losses. The ESF lambs had higher meat pH (+0.07) and lower cooking losses (1.93 percentage points) than the CO lambs.

4. Discussion

The proposed enriched system allowed lambs to behave more natu-rally, to be more affiliative and it reduced stereotypies. However, the higher level of cortisol and glucose in these animals could be related to a certain degree of stress associated with increased reactivity, which was probably the origin of differences in meat pH.

4.1. Behaviour and the cognitive test

The difference found in total feeding time during the morning was due to an increase in the time that ESF lambs spent consuming straw rather than concentrate only (as was the case in CO lambs). Greter et al. (2013) found that when cattle were provided with long particle vs. short particle straw they preferred the longer option and spent more time feeding. As in the former study, our lambs provided with entire straw spent more time eating it and manipulating it orally, lead-ing to an increase in their total feeding time. This increase could be due to the longer time needed to consume the entire fibre of straw that requires more chewing and salivation than concentrate. Lindström and Redbo (2000) demonstrated in cattle that oral manipulation of feed was a behavioural need, whether the rumen was full or not. These authors defined two types of “hunger”: one that is more physio-logically determined and one that is more closely linked to a psycholog-ical need to orally manipulate feed. The relationship between feed-searching behaviours and oral stereotypies suggests the occurrence of a displacement behaviour process as a consequence of the frustration of feed-searching behaviour (Redbo, 1992 cited by Lindström & Redbo, 2000). This possible explanation and the fact that lambs were kept busi-er longer could be the reason why ESF lambs had less stereotypic behav-iours than CO lambs, as was found by Aguayo-Ulloa et al. (2014a); Aguayo-Ulloa, Villarroel, Pascual-Alonso, Miranda-de la Lama, and María (2014b) (comparable experiments) and Teixeira, Miranda-de la Lama, Villarroel, Escós, and Maria (2014) working with the same type of lambs, and Webb et al. (2014), Greter et al. (2013) and Lindström and Redbo (2000) in cattle. It is important to remark that stereotypies have been used as an indicator of a sub-optimal animal environment and welfare problems such as stress (Mason, 1991). Providing a feeding regimen including concentrate and straw, increases the overall time spent feeding and helps to restore the feeding patterns observed in more natural environments, which may improve animal welfare. For technical reasons, in the present study we were unable to measure rumination, but the time spent ruminating is often similar to that spent feeding (Greter et al., 2013) and it is likely that the lambs that were fed straw would spend more time ruminating as well, with a pos-sible overlapping of ruminating and resting behaviour.

With regard to placements, the percentage of observations of lambs using the screen with flaps suggests that the lambs liked the furniture provided, possibly for two reasons: firstly, because the flaps acted as a shelter (as found previously in an experiment using double bunks, Aguayo-Ulloa, Pascual-Alonso, et al., 2014a), and secondly, because the screen operated as an additional wall that lambs could rest against. It has been described in domestic sheep and goats that they have a clear preference for lying against a wall when resting (Færevik, Andersen, & Bøe, 2005; Marsden & Wood-Gush, 1986). Studies by Jørgensen, Andersen, and Bøe (2009) and Ehrlenbruch, Jørgensen, Andersen, and Bøe (2010) support this finding since they found that when sheep and goats were provided with additional walls they preferred resting next to them. The aforementioned experiments tested different wall config-urations, but the parallel wall (comparable to the screen of our study) was used approximately by 25% of animals. Some authors argue that an-imals use walls to maximise the individual distance between them (Strickling, de Bourcier, Zhou, & Gonyou, 1998), however this would not be the case in our experiment with young lambs as most of them lay very close to each other, occupying most of the bottom area of the flaps. A possible reason for the tied-resting behaviour could be that our lambs belong to a gregarious Mediterranean breed subjected to an ancient shepherding-system, which could also be potentiated by a early weaning (b45 days-old), replacing the mother's companionship by other lambs.

The provision of the screen with flaps decreased aggression and favoured the development of affiliative behaviours among enriched lambs, compared with lambs from non-enriched environment. Unlike other studies that have not found any differences in social behaviour (Ehrlenbruch et al., 2010; Jørgensen et al., 2009), in our study we observed differences in social interactions between groups, which were probably related with the function that fulfilled the virtual division (screen), with the age of animals, and with the high contact among them while resting under the flaps (as mentioned above). The screen could have reduced aggressive behaviour by providing a hiding area to avoid clashes with potential aggressors. Providing animals with the means to exert control over some negative events in their environment is likely to reduce their negative emotional responses (Greiveldinger, Veissier, & Boissy, 2009) and thus improve their welfare. Furthermore, the lambs of our study were younger, which could have given rise to greater tolerance and fellowship when occupying the furniture during the period of observations (4th week), rather than competing for it.

On the other hand, the occupancy rates of control lambs were more associated with the preference to rest against the walls (as mentioned above). During afternoon and evening, when most of the lambs rested, the CO lambs mostly used the walls of the rest of the areas (fence of the pen) and the walls under the concentrate hopper and fodder rake, avoiding the central area (SFA imaginary area).

Regarding to the T-maze, the ability to solve novel problems at the first attempt or to take short-cuts in a maze, involves a reasoning ability such as associative learning which would allow individuals to adapt and be able to solve a challenge (Morton & Avanzo, 2011; Nicol, 1996). No significant differences were found between treatments in the times or quadrants registered. Similar findings have been described by De Jong et al. (2000) in enriched vs. barren-housed piglets. There was no effect of treatment on the time to solve the T-maze, probably because it varied widely. However, enriched lambs tended to have lower times.

Interestingly, the control animals that took a long time to solve the maze in the first exposure (21.5 s longer), demonstrated a sort of “com-pensatory” learning on the second exposure and caught up to the ESF lambs. The reduction in the number of quadrants crossed by the enriched lambs at the second attempt may be associated with some degree of learning and an improved coping style.

4.2. Physiological welfare indicators

The activation of the hypothalamic–pituitary–adrenal axis (HPA) is an important and complex adaptive response to stress (Matteri, Carroll, & Dyer, 2000) and should be interpreted with a multidisciplin-ary approach of animal welfare involving data from behavioural obser-vation, production trait and disease incidence (Morméde et al., 2007). Glucocorticoids (cortisol) and catecholamine production can be used to monitor the physiological response to stress, and differences are seen between individual animals in regards to latency to production and peak levels (Dodd, Pitchford, Hocking Edwards, & Hazel, 2012). In our study, the differences in HPA activity levels between treatments could be due to a certain degree of reactivity in the enriched lambs. Sim-ilar findings were observed in a previous, comparable study using dou-ble bunks and straw as enrichment (Aguayo-Ulloa, Pascual-Alonso, et al., 2014a) and in a study using elevated areas as an enrichment fea-ture in goats (Miranda-de la Lama et al., 2013b). The ESF lambs spent most of the time using the screen with flaps which probably acted as "hide and shelter" furniture, reducing visual contact with the surround-ing environment (some undesirable companions and handlers). This sheltered position, with limited visual contact with the surrounding environment, probably provoked a greater response to handling proce-dures such as blood sampling. This was also supported by the higher eye (IR) temperature observed in reactivity to handling test. During acute

stress, an immediate behavioural or short-term physiological response from the animal is needed to avoid negative consequences (Moberg, 2000). Furthermore, age and behaviour response can have an influence on the activation of the HPA axis, as is observed in calves that are hyper-reactive and have higher levels of cortisol response after repeated mixing (Boissy, Veissier, & Roussel, 2001; Veissier, van Reenen, Andanson, & Leushuis, 1999). Differences in lactate and CK are more dif-ficult to explain. The higher level of lactate observed in control lambs may indicate a different source of stress with low intensity and prolonged stimulus. Differences in CK, even significant ones, were clear-ly within the normal values for penned animals. On the other hand, the higher haematocrit from enriched lambs compared to control lambs could be related to spleen contraction due to activity of the sympathetic system during their stress response (Mitchell, Hattingh, & Ganhao, 1988).

4.3. Performance and meat quality

Overall, the productive performance of lambs was similar between treatments but the final live weight and carcass conformation scores of enriched lambs were higher. Those differences could either be due to the presence of straw or directly attributable to the new furniture. Straw takes longer to be digested, increases rumen weight and im-proves the absorption of nutrients, but the fact that the ESF dressing yield was not significantly different from controls suggests that it was not responsible for the higher final weight. Both the final weight and conformation scores are probably more associated with the furniture than access to straw. Previous studies using straw (only) as enrichment did not find productive or meat quality differences between lambs with or without straw (Teixeira et al., 2012).

It is well know that pre-slaughter handling can cause stress which leads to a reduction in glycogen reserves and usually higher values of meat ultimate pH (Warris, 2010) especially in fearful animals. Ultimate pH can provide information on the commercial quality of the meat, since it can have a considerable effect on characteristics such as cooking loss, colour and texture (Miller, 2002). The enriched lambs had car-casses with a slightly higher ultimate pH but it was always within the normal range for quality meats. However, neither colour nor texture was affected by treatment. The higher ultimate pH in enriched lambs was probably related, as previously mentioned, to the increased reactiv-ity and fearfulness of these lambs, since the pre-slaughter procedures were the same for all the lambs. The cooking losses observed are in agreement with the pH results. There tends to be a denaturation of mus-cle proteins as the pH falls, leading to a reduction in their ability to bind water and thus to a lower water-holding-capacity (Warris, 2010). Water losses after cooking decrease linearly with an increased pH and decreased cooking losses have been associated with increased water-holding-capacity (Bouton, Harris, & Shorthose, 1971). Taking this into account, the muscle proteins of the enriched lambs could have had a higher water-binding capacity than the muscle proteins from the con-trol lambs. However, the ultimate pH of lambs in both treatments is in agreement with findings in similar lambs by Ripoll et al., 2008 and Díaz et al., 2002, and could be considered normal for light lambs, the same applies to cooking losses (Sañudo, Alfonso, Sánchez, Delfa, & Teixeira, 2000). No other characteristics were affected by the treatment.

5. Conclusion

The lambs showed a noticeable preference and motivation for the items provided in the fully enriched housing system. The proposed sys-tem encouraged more natural and richer behaviour, reducing stereoty-pies and social aggressions, and increasing affiliation, which improves group cohesion. The lambs provided with enriched housing showed a more natural coping style including higher reactivity/sensitivity to external stimuli such as handling. This fact may make handling more complex and require greater skills by handlers. Therefore, an efficient, enriched programme should include effective, well-organized training protocols. The lambs fattened under the enriched housing system showed efficient performance without any detrimental effect on meat quality. This study highlights the importance of a careful analysis of the most appropriate elements to enrich the environment of animals in intensive production systems.

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