

Finishing feedlot lambs in enriched pens using feeder ramps and straw and its influence on behavior and physiological welfare indicators

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Abstract

Sixty lambs were placed in enriched (EE) or conventional (CO) pens (3 pens per treatment or 10 lambs per pen), in which the EE pens had a wooden platform with ramps giving access to a concentrate hopper, straw as bedding and forage, and a further ramp for play. The CO pen was barren without any enrichment. The general behavior of the lambs and the use of space were similar for both treatments; however, CO lambs developed significantly more stereotypies ($P < 0.05$). The EE lambs resolved a T-maze more quickly ($P < 0.05$), and their physiological adaptation response to the feedlot environment was more efficient. The CO lambs mobilized more body reserves and had lower levels of immunity (i.e., increased nonesterified fatty acid and neutrophil/lymphocyte ratio, respectively, $P < 0.05$) than EE lambs at the end of the fattening period, which indicates chronic stress, probably associated with the barren environment. The EE lambs had a higher ($P < 0.05$) average daily gain, heavier carcasses and higher fattening scores, as well as lower pH_{ult}. This study shows that enrichment can improve the welfare of feedlot lambs by reducing stereotypies and enhancing the physiological adaptation response to a novel environment.

Keywords: Transport; full environmental enrichment finishing lambs; behavior; animal welfare; T-maze task

1. Introduction

As in other animal production industries, sheep production has become more intensive and increasingly takes place indoors, with high densities and in environments that lack complexity (Fraser et al., 2013). The finishing phase of fattening lambs in some Mediterranean countries (e.g., Spain) is as intensive as pig production (Miranda-de la Lama et al., 2010b). Lamb feedlotting externalizes the final stage of fattening to off-farm units (Aguayo-Ulloa et al., 2013; Miranda-de la Lama et al., 2009), stratifying production in 2 specialized parts, flock breeding on the farm (under the farmer's responsibility) and fattening at feedlots, also called classification centres (CCs). It is assumed that this approach simplifies the finishing process for the farmer and improves carcass homogeneity (Miranda-de la Lama et al., 2010a; Miranda-de la Lama et al., 2010b). However, farmers and animals face other problems, such as dependency on external resources, multiple live transports, social mixing and frequent handling in novel, and barren environments (Aguayo-Ulloa et al., 2013; Miranda-de la Lama et al., 2012).

Adjusting to newly formed groups can entail difficulties, particularly with regard to competition for food or access to other resources, which in turn can lead to decreased fitness (Estevez et al., 2007). Stress in animals can have a psychological origin related to the novelty of the situation, social separation, the mixing of unfamiliar animals, and/or handling (Terlouw et al., 2008). This situation can be further worsened if the environment is not stimulating, encouraging the development of stereotypies, abnormal behavior, frustration, and stress (Fraser, 1980; Lawrence and Rushen, 1993; Wood-Gush and Beilharz, 1983).

Because of increasing public concern about the conditions in which animals are produced, over the years pressure has been exerted on government authorities to establish new regulations to control the quality of the livestock industry (Garnier et al., 2003;

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María, 2006; Lusk and Norwood, 2008; Winter et al., 1998). Many scientific studies have been carried out to improve the welfare of farm animals, mostly referring to poultry and pigs. Less is known about how to improve the well-being of lambs under similarly intensive indoor systems, about which the public is less aware (María, 2006).

Environmental enrichment is a subject that in recent years has attracted much scientific and media attention because of its link to animal well-being (Schetini de Azevedo et al., 2007; Young, 2003). Enrichment devices and substrates may have a positive effect on weight gain, decrease morbidity (Flint and Murray, 2001), and reduce abnormal behavior (Mason et al., 2007). They may also help to facilitate adjustment to feedlots in ruminants (Wilson et al., 2002) by reducing negative emotional states such as fear and stress associated with the adaptation to a novel environment (e.g., the CC). This will reduce the frustration that animals may experience when unable to express their behavioral needs (Hughes and Duncan, 1988a; Nicol, 1992; Wood-Gush and Vestergaard, 1989). The current trend in European legislation for various species is to develop regulations that increase environmental enrichment, making it important to identify the most adequate elements for each species. The study that follows is based on the hypothesis that full enrichment (multiple items, Abou-Ismaïl, 2011) during the finishing period of fattening may improve the adaptation process of lambs to a novel environment at the CC and hence optimize their welfare. This study was conducted to evaluate the influence of environmental enrichment (ramps and substrate) on use of space, behavior, cognitive abilities, physiology of stress, and performance traits in lambs fattened in feedlots.

Methods

The study was carried out at the Animal Experimentation Service of the University of Zaragoza in the Autonomous Community of Aragon, Spain (41° 41'0 N). The area is located in the Ebro river depression, characterized by a dry Mediterranean climate with an average annual temperature of 15 °C and mean annual rainfall of 317 mm. Experimental protocols were approved by the Animal Experimentation Ethics Committee of the University of Zaragoza (ES 50 297 0012 006).

Sixty entire Rasa Aragonesa male lambs (65 days old) with an average live weight of 17.13 (± 0.18) kg were allocated to 2 treatments (weights were balanced across treatments) according to their pen environment during the finishing phase of fattening (5 weeks). Lambs were housed indoors in 6 pens with 10 lambs each (2.9 x 3.3 m, animal density of 0.95 m² per lamb) and 3 replicates per treatment. Lambs from the enriched group (EE) were kept in pens with a wooden platform with slatted ramps that provided access to a concentrate hopper (Figure 1). The dimensions of the platform and ramps were 2.35 m long x 1.55 m wide

0.35 m high. The platform area was 1.67 m². The ramp slope was approximately 20°. The platform in the pen was attached to the fence below the food hopper, allowing the lambs to feed, explore or to lie down. The EE lambs were provided with cereal straw (72% neutral detergent fiber: 38% cellulose, 25% hemicellulose, 8% lignin, and 0.2% cuticle) as forage in the fodder rake (fresh straw was added every morning, *ad libitum*) and bedding on the floor. Additionally, a small slatted ramp (0.90 m long x 0.50 m wide x 0.35 m high and 0.08 m² surface area) was placed near to the opposite solid fence but away from the food hopper and fodder rake, to allow lambs to play. Lambs from the control group (CO) were kept in a barren pen that had the same dimensions as enriched lambs, but without any furniture or cereal straw as bedding or forage, mimicking CC conditions. For hygienic reasons, a thin layer of sawdust was added at the beginning of the experiment to the CO pens only. All lambs were fed with commercial concentrate (Ovirum Alta Energía) containing barley, wheat, calcium carbonate, sodium chloride, and a vitamin supplement corrector (18% crude protein and 11.5 MJ metabolizable energy/kg dry matter). 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. Lighting was natural, and pens were ventilated with a system of windows and lintels to provide a chimney effect that ensured temperature and air quality were kept within normal ranges for lambs.

Lambs were weighed individually at the beginning of the experiment (W1) and just before transport to slaughter (W2). The total consumption of concentrate (CCo) was estimated as the difference between the concentrate added (weighted and registered each time) and the concentrate remaining at the end of the experiment. Average daily gain was the difference between W2 and W1 (WG) divided by the total fattening days (35). The concentrate conversion index (CCI) was estimated as CCo/WG. After overnight lairage, lambs were electrically stunned, slaughtered, and dressed according to standard commercial procedures, and carcasses were kept in a cold room at a temperature of 2 °C-3 °C for 24 hours. At the end of this period, the pH 24 of the *Musculus longissimus* was determined with a portable pH meter (fitted with a penetration electrode 52-00 from Crison Instruments, Allela, Barcelona, Spain). Carcass conformation score and carcass fatness were graded (15-point scale) according to the European classification system (European Union, 1993).

Observations: home pen behavior

All lambs were individually identified by numbers or letters painted on their sides and rump with washable spray for animal marking. A videorecording device (model VDVR-9; Circontrol S.A., Terrassa, Spain) was set up in a room close to the pens to record maintenance behavior, use of space, abnormal behaviors (stereotypies), and social behavior. A camera was placed in front of each pen, 2.2 m above the ground. Recordings were made for 12 hours/day during the fourth week, between days 21 and 27 (inclusive) of the fattening period. All videos were analyzed by the same trained observer. We recorded the use of space in CO pens tracing the same (albeit imaginary) divisions of the same areas in EE pens.

Two kinds of sampling were carried out: instantaneous, every 10 minutes (8 AM to 8 PM for 7 days) with a total of 1512 scan samples per treatment, recording, in each sample, use of the space (15,120 observations per treatment), and maintenance behavior and stereotypies (15,120 observations per treatment). The behaviors recorded 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 concentrate (FC, lamb searching for feed concentrate in the hopper and eating it), eating straw (ES, lamb searching for forage straw in the fodder rake and eating it), drinking (DK, lamb drinking water from the drinker), and stereotypies (frequent and nonfunctional oral manipulation of objects, an abnormal behavior commonly seen in ungulates in stressful situations, Mason et al., 2007). Recording stereotypies by scan sampling only provided a general approximation (number of lambs performing stereotypies) that allowed us to identify the time of the day when they were most common. Scan sampling is not the most appropriate way to sample stereotypies, but it is useful to detect differences in the time of the day when animals displayed abnormal behaviors. Three areas were defined to measure use of space: the feeder ramp area (FRA, lamb using the fixtures on it or with more than half of its body on it), the play ramp area (PRA, lamb on the ramp or with more than half of its body on it), and the remaining area (RM, lambs in the remaining area of the pen). To identify the time of day in which lambs commonly displayed certain behaviors, day observations were divided into 3 blocks: morning (AM, from 8 to 12 hours), afternoon (noon, from 12 to 16 hours), and evening (PM, from 16 to 20 hours). Continuous sampling was used to record social behavior, that is, the number of agonistic (aggressive) and affiliative interactions per animal (Table 1), and stereotypies, that is, the number of times that an animal repeated an abnormal behavior, if perceived as such (as defined by Mason et al., 2007). We used scan sampling to detect considerable behavioral changes during the course of the day.

Cognitive test, T-maze

We used a T-maze built with 1.40-m high plastic panels adapted from Pascual-Alonso et al. (2013) (Figure 2). The T-maze used had 5 areas (3 chambers and 2 corridors). It consisted of a start box (0.8 x 0.5 m) and an isolation chamber joined on one of its sides to a T corridor (B and C areas). The start box was fully closed but large enough to enable an individual to move around. The T corridor consisted of a 2 x 0.80 m path linked to 2 perpendicular arms (1 x 0.8 each) that connected with 2 chambers (D and E). A mirror (70 x 30 cm) and loudspeaker were located in the target zone on the left arm. An observation platform was located on a 3-m high platform so as not to influence animal movements, adjacent to the T-maze apparatus. The apparatus was kept in a sound proof room (9 x 6m) at constant temperature and humidity during the trial.

The sound used in the experiment was a playback of callings from congeners. The stimulation sound was a computer random selection from lambs from all pens (same breed, sex, and age). The recording was 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 into a computer at a sampling rate of 44.1 kHz and saved in Waveform Audio Format at 16 bit amplitude resolution. Audacity 2.0 (General Public License) audio software was used to prepare the sound sequences that were played back. A sample of each sound was combined into 5-minute segments, and a random portion of this segment was played back during each trial. The noise was played, and its intensity was measured using a Bioblock Scientific Sound Level Meter type 50517 (Thermo Fisher Scientific Inc., India) at a set volume to ensure that lambs were exposed to 81 dB of intensity throughout most of the T-maze. For each trial, the sounds were played back by a Handy Recorder H1 connected to a loudspeaker located at floor level on the left arm in the target zone.

Each lamb underwent the cognitive test once on 2 consecutive days during the last week of the experiment, without prior training. Each animal remained in the start box for 20 seconds before a guillotine door was lifted to allow entrance into 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 animal found the target zone (which was always located in the left arm) where there was a mirror (social clue) and the sound source (sound clue). Each animal was given a maximum time of 5 minutes to solve the T corridor. If an animal did not solve the challenge, it was 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 registered the total time taken to solve the test, the time that the lamb spent in the first chamber (isolation time), and the number of areas crossed (NQAD) by the lamb during the assay (as an estimation of the locomotor behavior).

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). Samples were taken by trained personnel who performed the venipuncture in less than 1 minute per lamb, as a necessary precaution to avoid sampling error. Samples were kept on ice for a maximum of 2 hours and taken to the laboratory for routine hematological measurements. The EDTA plasma and serum were centrifuged at 3000 rpm for 10 minutes, and aliquots were frozen and kept at -30 °C until analyzed. The leukocyte formula was estimated from blood swabs on clean slides. Staining was performed by the rapid panoptic method using dyes from Química Clínica Aplicada Inc, Tarragona, Spain. 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 an indicator of chronic stress (Lawrence and Rushen, 1993). Serum samples were used to determine the concentration of glucose (mg/dL, Ref. Glucose AE2-17) and the activity of creatinine kinase (CK; UI/L, Ref. CK.NAC AE1-13) using an Alfa Wasserman ACE Clinical Chemistry System multi analyser and reagents. Serum concentration of nonesterified fatty acid (NEFA) levels was analyzed by an Alfa Wasserman ACE Clinical Chemistry System multi analyser, with Wako 994-75409 NEFA-C commercial test kits. Cortisol concentration was determined from plasma (EDTA-K3) by enzyme immunoassay using an “in home-kit” (validated by Chacón et al., 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 nanomoles per liter. Inter- and intra-assay coefficients of variation were 7% and 8%, respectively. The concentration of lactate was determined using a Sigma Diagnostic kit (lactate no. 735-10) and spectrophotometer (Lambda 5, PerkinElmer).

Eye area temperatures were taken by infrared thermography (IRT) as a response to a reactivity test (Pascual-Alonso et al., 2013). Lambs were randomly captured and restrained by a trained handler.

Restraint continued for 1 minute during which a photograph of the left eye was taken (approximately in the middle of the restraint period) with an infrared camera (Testo 880 Thermal Imaging Camera; Testo AG, Lenzkirch, Germany) to evaluate acute stress response produced by handling (Stewart et al., 2007). All images were collected from the left side of the lamb (approximate distance of 20 cm). The built-in lens (24 mm) was used, and the camera was calibrated for the room temperature and relative humidity. The emissivity value used was 0.98, which is recommended by the camera manufacturer for biological tissues. A clear infrared image (precise location and perfect focus) of each animal was chosen. Usually, the camera was easily calibrated to take a photo, and normally, only 1 photo was taken per animal. Exceptionally, if the photo was not clear (out of focus), it was not kept, and a second photograph was quickly taken. Image analysis software (IRSoft software; Testo AG) was used to determine the maximum temperature within an oval area traced around the eye, including the eyeball and approximately 1 cm around the outside of the eyelids.

Statistical analysis

Data were analyzed using SAS/STAT (9.1 SAS Inst. Inc., Cary, NC) by SAS (1988). Behavior data were transformed by the square root function. Social behavior (average per animal per day) was analyzed using PROC MIXED with repeated measurements (day), treatment as the fixed effect, and lambs as the random effect. For maintenance behavior and stereotypies, we added time of day to the model as the fixed effect. T-maze variables were subjected to repeated measures analyses of variance that examined the

main effects of treatment (conventional and enriched), T-maze trial (first and second T-maze exposure; the repeated measure), and their interaction.

Data regarding physiology and productive traits were analyzed using the least squares methods of the GLM procedure of SAS (SAS, 1988), fitting a 1-way model with a fixed effect of enrichment (2 levels). The general representation of the model used was as follows: $y = Xb + e$, 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. The original full model included the effect of replicate (fitting animals nested within pens), which was found to be nonsignificant and consequently was dropped from the model. A P-value of ≤ 0.05 was considered to represent significant differences.

Results

Final live weight was significantly higher in EE lambs (27.40 kg) versus CO lambs (26.30 kg). The EE lambs gained 56 g more per day than CO lambs. The EE carcasses were 710 g heavier and had a higher fattening score than CO lambs. The ultimate pH was also lower ($P \leq 0.05$) in EE lambs (5.53 vs. 5.59 in CO lambs). There were no significant differences in the CCI between treatments.

Observations: home pen behavior

Overall, the general pattern of behavior and use of space was similar for the 2 treatments. There was a significant effect of time of day on maintenance behaviors (Table 2), but this effect was not related to treatment, with the exception of stereotypies, which were significantly higher in the morning in the case of the CO lambs (Figure 3A). Lambs spent most of the day resting (RT; mean, 78%), but less so during the morning (mean, 68.5%), when they were significantly ($P \leq 0.05$) more active (higher FC%, WK%) than in the afternoon or evening (PM). However, EE lambs were significantly more active than CO lambs. The latter rested 14.5 percentage points (pp) more than EE lambs ($P \leq 0.05$). There was a peak of rest in the afternoon in both treatments (mean, 85.5%), which decreased in the evening in both treatments (5.11 and 5.62 pp for CO and EE lambs, respectively). Lambs in both groups walked (WK) and fed more (FC) in the morning than in the afternoon and evening, but WK was higher in CO lambs in the evening ($P \leq 0.05$). EE lambs spent more time standing (ST) in the morning than CO lambs, but this behavior pattern was reversed in the afternoon (i.e., ST higher in CO lambs). Foraging behavior was higher in EE lambs in the morning ($P \leq 0.05$). Drinking (D) was quite low in both treatments but higher ($P \leq 0.05$) in EE lambs in the morning.

Regarding use of space (Table 3), the RM was the most used and the PRA the least used in both treatments. The FRA was used similarly in both treatments in the morning (mean, 27.1%) but significantly less by EE lambs ($P \leq 0.05$) in the afternoon and evening. Use of the PRA was different ($P \leq 0.05$) between treatments and within treatments ($P \leq 0.05$). The CO lambs used the PRA more than the EE lambs, during the whole day, with increasing use as the day wore on. However, EE lambs used the PRA sporadically (<6%) and more in the afternoon. In both treatments, RM was used the most, being significantly higher ($P \leq 0.05$) in EE lambs and increasing significantly ($P \leq 0.05$) throughout the day. There were no significant differences in RM use for CO lambs during the day.

A significant interaction effect was observed between treatment and time of day for stereotypic behaviors recorded by scan sampling (Figure 3A). Stereotypies were higher in CO ($P \leq 0.05$) in the morning. The results of stereotypies obtained by continuous sampling (Figure 3B) reveal that the general pattern (significantly higher in CO, $P \leq 0.05$) was sustained throughout the observation period.

The social behavior of the lambs is summarized in Figure 4. Initially, there were significant differences ($P \leq 0.05$) between treatments with EE lambs having more agonistic and affiliative interactions than CO lambs. As the study progressed, CO lambs had significantly more ($P \leq 0.05$) affiliative interactions on days 4 and 6. During the week, EE lambs decreased their social interactions significantly ($P \leq 0.05$), reaching a minimum on days 6 and 4, for agonistic and affiliative interactions, respectively. However, by the end of the observation period, the affiliative interactions tended to increase. For CO lambs, agonistic interactions tended to be similar during the observation period but decreased significantly on day 6. Affiliative interactions in CO lambs tended to increase during the week, being significantly higher on day 6.

Cognitive test, T-maze

The least square means of time taken to exit the isolation chamber, total time to resolve the T maze, and the number of areas the lambs passed through to solve the maze are shown in Table 4. There were no significant differences between treatments in the overall time taken to exit the isolation area; however, EE lambs showed a significant decrease ($P \leq 0.05$) in the time taken to leave the area on their second exposure. All lambs took less time to reach the target location on the second exposure ($P \leq 0.05$). There were significant differences between treatments in the overall time taken and areas passed through to solve the T-maze. The EE lambs solved the challenge 29 seconds faster ($P \leq 0.05$) and passed through almost 6 quadrants less than CO lambs ($P \leq 0.05$). There were no significant differences between treatments in the total time to solve the maze during the first or second exposure. There was, however, a significant difference between treatments in the number of quadrants passed through during the first exposure ($P \leq 0.05$). The EE lambs crossed 8 quadrants less than CO lambs. However, CO lambs significantly decreased ($P \leq 0.05$) the number of quadrants they passed through during the second exposure.

Physiological variables

The least square means (standard error) for plasmatic and hematological stress indicators are shown in Table 5. Glucose levels were significantly ($P \leq 0.05$) higher in EE lambs, and NEFA levels were significantly ($P \leq 0.05$) higher in CO lambs. No significant differences between treatments were detected for cortisol, lactate, or CK. The N/L ratio was significantly ($P \leq 0.05$) higher in CO lambs. No significant differences were found between treatments for the other hematological variables analyzed, nor did we find any significant differences in eye temperatures taken by IRT.

Discussion

Our results are consistent with the hypothesis that lambs adapt better to a new environment when provided with enrichment. Lambs without enrichment had more abnormal behaviors (stereotypies), lower cognitive abilities, lower performance indicators, higher meat pH, and blood parameters indicative of chronic stress. This hypothesis is in agreement with the general principles for the welfare of animals in production systems described by World Organisation for Animal Health, based on the idea that certain types of environments can produce welfare problems by being too barren, inducing boredom, and by being too restrictive or otherwise limiting the expression of natural behaviors (Fraser et al., 2013). Animals become frustrated when they are not permitted to perform behaviors which they are strongly motivated to perform (Fraser, 2008; Fraser et al., 2013). For these reasons, the full enrichment considered could be used as a way to improve the welfare of lambs that are fattened using intensive systems and could even promote faster growth and produce better carcasses.

Behavior and use of space

Total lying time was similar in both treatments but somewhat longer than in previous studies using the same type of lambs in 2 different feeding regimes (Aguayo-Ulloa et al., 2013) and much longer than indoor housed ewes provided with additional walls (Jørgensen et al., 2009) or different types of flooring (Færevik et al., 2005). The fact that the total time spent resting (lying) was similar in both EE and CO lambs coincides with the results found for cattle and sheep, indicating that rest is a high priority and an inelastic behavioral need (Jensen, 2005). However, the EE lambs were slightly more active in the morning (more standing up and drinking) which may be because of enrichment and the fact that they could eat fresh straw (the CO lambs could not). The underlying hypothesis to investigate the behavioral patterns of lambs in time blocks (morning, afternoon, and evening) was that we believe that there is a close relationship between the level of activities motivated by straw and the level of frustration that they undergo because of the absence of this source of sensory stimuli, which generates increased stereotypies. Ruminants are strongly motivated to eat fiber (Campion and Leek, 1996) and can alter their time budget to forage, if possible, as happened during the morning in our study. The CO lambs spent time eating concentrate only (7.84%), but the EE lambs spent time eating concentrate and straw, and both together (14.05%) involved a higher time budget in foraging during the morning. This means that although we cannot compare the time spent eating just straw (given that only 1 treatment included straw), we can compare the total time spent foraging and keeping busy, performing more natural behaviors that satisfied their ethological needs. In addition, it must be observed that fresh straw was provided every morning (8 AM), which may act as a powerful attractant to the lambs. On the other hand, when

access to roughage is eliminated, this may cause frustration and trigger oral stereotypies (Redbo, 1992; Redbo and Nordblad, 1997; Tuytens, 2005), as seen in the CO lambs, especially in the morning. Similarly, Teixeira et al. (2012) found that the same type of lamb developed more stereotypies when there was no straw in the pen. It has been described in cattle that a short feeding time may not quell feeding motivation and can trigger oral stereotypies (Redbo, 1992). Likewise, Cooper and Jackson (1996) suggest that abnormal oral activities by sheep on slats may be a substitution for foraging activities, such as eating roughage or nosing straw. The motivation of sheep to ingest fiber which is expressed as a “fiber appetite” may be the expression of a motivation to carry out true rumination (Campion and Leek, 1996). That could have been the case of our CO lambs in barren pens with a single diet of concentrate and no straw.

The use of space was similar among treatments, but EE lambs spent more time in the RM area (and less in the FRA) which could be because of the fact that the RM had more straw and was more comfortable to rest on or the FRA was mainly used as an activity area (to feed, play, pass through, or explore). Gordon and Cockram (1995) report that sheep prefer to lie on straw than on wooden slats (like the feeder ramp platform in our study). Similarly, Færevik et al. (2005) found that housed ewes prefer to lie down on straw rather than wooden or spandex metal floors. Nevertheless, those studies used female adult sheep and the preferences of young lambs could be different, as reported by Teixeira et al. (2013) in a multiple choice test that included several types of bedding. The EE lambs could have used FRA and PRA as activity areas because of their structural features and the natural inclination of lambs to climb on objects (Gonyou et al., 1985). The CO lambs used the FRA and PRA areas in the same way as the RM area, mainly to rest. Using the feeder ramp did not have a negative effect on consumption of concentrate because both groups had similar occupancy of the feeding area in the morning (the most active time of the day). However, they became less interested in these areas of activity in the afternoon and evening when resting increased. The fact that PRA had a very low occupancy rate in EE pens may be associated with the monopolization of the furniture by lambs or/and to the fact that the small space did not allow use by more animals (by either climbing on it or just resting against it) compared with the imaginary PRA space of the CO pens.

Social interactions can be used to evaluate the stability of a group (Broom, 1991). Previous studies reveal that one of the major risks that could affect lamb welfare in feedlots is social mixing after weight classification on arrival (Miranda-de la Lama et al., 2010b). In our study, aggressive interactions were similar between treatments suggesting that the lambs were organized as a stable group and that enrichment may play a secondary role in the formation of hierarchies (Fraser and Rushen, 1987). Previous studies (with the same type of lambs and system) showed that after initial mixing (day 1), social interactions are high (aggressive and affiliative) and lower later on (days 7, 14, 21, and 28; Miranda-de la Lama et al., 2012; Teixeira et al., 2012), as was also the case in our study.

The differences in affiliative interactions are more difficult to explain because CO lambs were more affiliative on some of days observed. Affiliative behavior can be used as an indicator of positive experiences in farm animals under commercial conditions (Boissy et al., 2007), so it may appear contradictory that in our study, there were high levels of affiliative interactions in CO lambs. However, Miranda-de la Lama and Mattiello (2010) described affiliative interactions (i.e., licking) as behaviors that help reduce aggression. These authors suggest that aggression and affiliative interactions may arise from the overall pressure of mixing and novelty, the effects of which are difficult to isolate under commercial conditions.

Cognitive test

To measure how enrichment can help to prevent the negative effects that chronic stress has on spatial learning and memory (Wright and Conrad, 2008), we analyzed lamb behavior in a cognitive test. We interpreted the time taken by lambs to solve the T-maze as the ability to learn, from specific stimuli, actions, and results to be able to solve a challenge (Morton and Avanzo, 2011; Pascual-Alonso et al., 2013). The EE lambs solved the maze faster than the CO ones, suggesting that including stimuli in the home pen improves cognitive abilities and, to a certain degree, it prevents the negative effects that the intensive fattening system (i.e., CCs) could have. The presence of stimuli that motivate lambs to perform more types of behavior could be a relevant factor in developing improved cognitive abilities (Mendel et al., 1996).

Ewes and lambs express distress by increased bleating and locomotor activity (Alexander, 1977; Torres-Hernandez and Hohenboken, 1979). Because of technical reasons (i.e., difficulty in distinguishing the sound source), we did not take bleating into account; however, the locomotor activity of the CO lambs was higher in the maze, crossing more areas than the EE lambs to get to the target. It is important to highlight that as gregarious species, this isolation task (maze) may be more stressful than in other species and their reaction and response times are related to their ability to solve challenges under high levels of stress. The differences between treatments in terms of overall time to solve the task and the number of crossed areas could indicate that lambs fattened without enrichment are less capable of adapting to stressful events (in this case the T-maze challenge).

Physiological variables

The hypothalamic-pituitary-adrenal axis activity in lambs fattened in feedlots under intensive conditions tends to be higher than that in nonconfined systems (Miranda-de la Lama et al., 2010a). In this study, EE lambs had similar cortisol levels to controls, indicating that the hypothalamic-pituitary-adrenal axis response of both groups was similar when faced with potentially stressful stimuli in these environments (e.g., handling). However, intensive feedlots can be a source of chronic stress, mainly due to high animal density, a poor environment, minimal sensory stimulation (Wood-Gush & Vestergaard, 1989), and a monotonous diet (Catanese et al., 2013). The higher N/L ratio found in CO lambs could indicate chronic stress, but the values were not accompanied by clinical signs of disease. The differences in glucose and NEFA between treatments could be because of several causes. In general, at rest, the skeletal muscle of sheep uses up considerable amounts of blood glucose. As exercise increases, the liver releases additional glucose which is used by the muscle (Maurya et al., 2012). Pethick (1993) indicates that the rate of glucose release by the liver and uptake by the muscle is sustained and matched during mild exercise (i.e., no hypoglycemia). This could explain the high levels of plasma glucose in EE lambs because they had more exercise (going up and down the ramp). The lower meat pH in enriched lambs suggests that they were less susceptible to stress, had more stored energy, and more efficient glycogen reposition during pre-slaughter handling, which coincides with Klont et al. (2001) and Essén-Gustavsson et al. (1988). However, the meat pH values observed in both treatments can be considered normal for light lambs (Ripoll et al., 2008). Additionally, Klont et al. (2001) note that an increased activity level may result in higher capillary densities in muscle during the rearing period, allowing animals to cope better with stressful events. Considering that these animals were very young when they began the fattening process, moderate exercise could influence the efficiency of energy use. However, the higher NEFA levels in controls may indicate the presence of underlying multi-factorial chronic stressors, including the absence of adequate substrate for behavioral or physiological needs (Hughes and Duncan, 1988b). In lambs, it has been shown that higher plasma NEFA concentrations indicate a breakdown of fat in response to elevated energy demand (Adewuyi et al., 2005). Thus, higher NEFA and lower plasma glucose levels in controls indicate a negative energy balance, which is suggestive of chronic stress, possibly linked to a psychological state of depression, as suggested previously by de Jong et al. (2000) and de Groot et al. (2000). The lack of difference between treatments for eye IRT values indicates that, although physiological stress indicators were affected, they did not represent a stress response with a high biological cost.

A useful measure of the sustained effect of stress is the N/L ratio (Blecha, 2000). As mentioned previously, these levels were higher in controls, indicating immune suppression. Distress can increase the amount of neutrophils and decrease lymphocytes and eosinophils (Kannan et al., 2000; Schaefer et al., 1997). Our results agree with the study by Miranda-de la Lama et al. (2010a) who reported immune suppression as a consequence of the cumulative effect of factors associated with a barren environment and poor handling at the CC. Catanese et al. (2013) found similar results in penned lambs subjected to a monotonous diet (without a source of fiber) compared with varied diet (with a fiber source). These authors suggest that a monotonous diet increases stress levels and hence immunosuppression. However, they also found higher levels of cortisol, which was not the case in our study. There were no significant differences in the remaining hematological variables between groups, and their values fell within the normal ranges for this type of animal (Kaneko et al., 1997).

Conclusions

Adding feeder ramps and cereal straw to lamb pens during the finishing period improves their adaptation to a novel environment, optimizing their welfare and improving productive performance. Enrichment did not affect the normal social stability attained by lambs at the end of the fattening period. The physiological indicators of the control lambs were indicative of chronic stress and immune suppression, probably related to the low level of sensory stimulation in a barren environment. The results should encourage feedlot managers to use enrichment to prevent negative welfare in lambs, achieving improved production performance and less stressed animals by preventing glycogen depletion, which may in turn result in better meat for consumers. Further studies are necessary to analyze the evolution in the social adjustment of the group throughout the fattening period under enriched conditions. From an economic point of view, the enrichment proposed for in-door feedlots could be an initial extra cost, but it is also an ethical benefit, which concerned consumers will be willing to pay for. Furthermore, in the short-term minimum standards such as the provision of forage straw could be made mandatory by European regulations.

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