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Valuation of immune response by using phagocytosis index and parameters associated as markers of animal stress in fattening lambs

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ABSTRACT

The aim of this study was to evaluate immune response function by using neutrophils phagocytic index and parameters associated to stress in order to analyse the effect of feedlot in the fattening lambs classification center (CC). Thirty six Merino lambs were randomly chosen with a live weight about 18–20 kg with 70–90 days of life. Two blood samples were taken in two moments of their period in CC, after the classification processes at the beginning of feedlot and one day before slaughter. High values compared to the reference values were found in red blood cell count (RBC) and haematocrit value, with statistically significant differences ($P = 0.003$, $P = 0.004$, respectively) between two moments. However, the increase in the neutrophil/lymphocyte ratio (N/L ratio), the decrease in phagocytosis index and high cortisol values point to stress conditions and a predisposition to develop diseases.

1. Introduction

In Spain, lamb meat are based on light lambs less than 3 month old and standardized by its live weight (8.5–13 kg carcass weight) (Miranda-de la Lama et al., 2010a). To supply high quality sheep meat products, lamb cooperative producers have developed the denominated classification centers (CC) (Miranda-de la Lama et al., 2010a) where lambs are fattening in feedlots and improve carcass homogeneity and develop a quality mark.

Several steps and processes are involved in this system of fattening lamb (weaning, transport, classification, regrouping) (Miranda-de la Lama et al., 2010a) which have been pointed as stressors (Knowles et al., 1995): between them, previous transport and several loading stops to the center and novel environment may be a main stress agent in lamb production systems.

The production system in which animals are raised has a major impact on their immune response (Bonnette et al., 1990; Niekamp et al., 2007). Extrinsic factors (like production system) and intrinsic factors (such as the animals' social status) are both involved in determining physiological status and, ultimately, defensive potential and health of the animals.

The aim of this study was to determine the possible effects of fattening period and its management practices on some indicators of stress and the effect of these on innate immune response.

2. Material and methods

2.1. Animal samples

This study was carried out in a CC in Extremadura (South-Western Spain). 36 male and female Merino lambs were randomly chosen after classification within of a pen in order to establish a homogeneity batch with a live weight of 21 ± 3 kg and between 90 ± 20 days. These data were expressed as mean ± standard deviation (SD). Animals were grouped with other animals in pens with a density of 0.5 m^2 per animal. Feeding (pellet concentrate and straw) and water were administered ad libitum. Animals are fed by a continuous feeding system from silos by using designated concentrate for fattening lambs. Straw is added ad libitum in mangers. These animals were transported by road from farms to the CC. Classification centers are built with pens of 60 m^2 where approximately 100–120 animals are kept in order to establish a density of 0.5 animals/m^2. Straw beds are replaced every 3 days by using more straw to apply a drying effect in order to avoid the accumulation of ammonia in pens. Ventillation is controlled by closing and opening doors and side windows. Twenty one days later the

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Table 1
Summary of results of studied parameters.

Variable	Start of fattening (mean ± SD)	End of fattening (mean ± SD)	*p*-Value
Phagocytosis (%)	64.36 ± 30.13	60.17 ± 29.51	0.557
Cortisol (nmol/ml)	61.86 ± 62.28	59.01 ± 62.38	0.833
RBC (10^6/mm^3)	14.06 ± 1.59	12.74 ± 2.13	0.003
WBC (10^3/mm^3)	8.46 ± 5.62	8.51 ± 4.28	0.943
HTO (%)	56.75 ± 5.81	51.62 ± 9.15	0.004
N/L Ratio	0.63 ± 0.37	0.81 ± 0.59	0.034

animals were transported until slaughterhouse when their live weight was 24 ±4 kg.

Animals are transported in a truck. The loading are organized by the proximity of the farms to the classification center. Used truck had compartments of 0.25 m^2/animal. These compartments have a lateral dividers and non-slip floors.

2.2. Blood samples

Two samples per animal were collected in pens one day after the arrival and classification process at the CC and other at the end of the feedlot period before slaughtering. Three 10 ml tubes (without anticoagulant, with EDTA-K_3 and with heparin), within the pen, these animals were restrained by the head and by using a venucupunture system (Vacutainer®) blood samples were recorded. were extracted using venepuncture with Vacutainer® extraction system. These blood samples were kept on ice and taken to the laboratory for routine haematological measurements. Tubes without anticoagulant were centrifuged at 3000 rpm for 10 min and aliquots were frozen at −21 °C until be analyzed.

2.3. Haematological parameters

An automatic particle counter (Sysmex F-620®, Norderstedt, Germany) was used to count red blood cells (RBC), white blood cells (WBC) and haematocrit (%). Leukocyte formula was estimated from blood swabs on clean slides, using the rapid panoptic method from Química Clinica Aplicada Inc.® (QCA). 100 leucocytes per sample were counted and identified as neutrophils, lymphocytes, eosinophils, basophils and monocytes in order to calculate the N/L ratio (Table 1).

2.4. Cortisol measurements

To quantify the level of plasmatic cortisol, Immulite® 1000 (Munich, German) was performed. One tube with a barcode label is needed for the assay and each barcode-labelled unit contain one bead coated with polyclonal rabbit anticortisol antibody. For this purpose, 10 μl of serum sample was added to the unit test that incubated at 37 °C in a persistent agitation. After incubation, the tube with barcode-labelled unit was centrifuged about its vertical axis. After centrifuged, samples were washed to remove supernatant not united to bead. The chemiluminescent substrate was added and light emission was read with a high sensitivity photo counter. The sensitivity of the test was 0.2 μg/dl. Results were expressed in μg/dl and converted to nmol/L by using a correction factor.

2.5. Assessment of phagocytosis function

One tube (10 ml with heparin) was used by this test. Briefly, a 3% dextran solution in PBS was added to the blood at a 1:1 ratio, followed by incubation for 30 min at room temperature. The supernatant was collected and centrifuged (650 g for 40 min) on a density gradient (Histopaque-1077 together with Histopaque-1119, Sigma®). The neutrophil halo was harvested, washed twice with PBS, and centrifuged at 420 g for 10 min. The supernatant was discarded and the pellet was re-suspended in 1.5 ml Hanks' medium. The number of neutrophils was counted with Neubauer chamber, and the suspension was adjusted to a concentration of 1×10^6 neutrophils/ml. Cell viability, determined by trypan blue, was more than 95%. This method was adapted from García-Torres et al. (2011) in Iberian pigs.

Phagocytosis was evaluated using the method described by De la Fuente (1985). 200 μl aliquots of the neutrophil suspension (10^6 cells/ml) were incubated at 37 °C in 100% R. H. and 5% CO_2 on MIF plates for 30 min. The adherent cell monolayer was washed with PBS, and 200 μl of Hanks' medium was added to the plates, together with 20 μl of a suspension of latex beads (1.09 μm, diluted to 1% in PBS (Sigma®)). After 30 min of incubation, the plates were washed with PBS at 37 °C, fixed and stained, and the number of particles phagocytised per 100 neutrophils (phagocytosis index) was calculated under a microscope (100×). A total of 100 neutrophils were counted and for each cell, phagocytized beads were counted as 0–6 or more. The percentage of cells that phagocytized at least one bead and the average numbers of phagocytized beads were determined (Rudine et al., 2007).

2.6. Data analysis

Dependent sample *t*-tests were applied to find statistically significant difference between the means of each variable at the beginning and the end of the feedlot period. The experimental unit is the animal.

The dependent-sample *t*-test was applied to compare the difference in the means between pre and post scores of the haematological, hormonal and immune response parameters. In order to apply this hypothesis test, the applicability conditions were checked prior to the analysis. This test may be applied to all the pairs of pre and post scores in this study. The results were considered as significant when *P*-values were less than 0.05. Statistical software SPSS® 19.0 was used.

3. Results

Several parameters were analyzed at the beginning and end of the feeding period. Elected blood parameters were red blood cells, white blood cells count and hematocrit value and; physiological parameters were neutrophilo/lymphocito ratio, serum cortisol level and phagocytosis function of neutrophils.

Red series and WBC were within normal ranges. When red serie was analyzed, we observed a decrease in red blood cells and haematocrit, with significant difference between the two values obtained in an animal ($P = 0.003$; $P = 0.004$). Finally, the white blood cells showed no changes, just seeing a close significance to 1. An interesting measure stress indicator is the ratio N/L, in which an increase in the value analyzed in the two sampling in this fattening period was observed and statistically significant differences were observed. For cortisol, very similar results were obtained between two samples, recording high values compared to normality; a mild decrease although without statistically significant differences ($P = 0.833$). Isolated neutrophils are shown in Fig. 1. In the case of phagocytosis index, a low value together a mild decrease