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Feedlot conditions and their influence on blood parameter values in Merino lambs in Extremadura, Spain over a specific time-period

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ABSTRACT. It has been suggested that the evaluation of specific blood parameters can be used as indicators of animal health and welfare. The aim of this study was to determine possible differences in these parameters at the beginning and at the end of the feedlot period and to analyse the influence of feedlot conditions on these animals. Blood parameter ranges can vary widely depending on factors such as transport, handling and feedlot. Four hundred and ten fattening lambs were studied to establish average values of different blood parameters. For data analysis, descriptive statistics and paired t-tests were used. The results of the current study indicate that mean values of Haematocrit, Cellular Haemoglobin Concentration and Cortisol levels were outside of the normal range, both at the beginning of the feedlot period and at upon leaving the feedlot. Higher mean values of MCHC, Urea, Total Proteins and Cortisol levels were observed at the end compared to the beginning of the feedlot period. On the other hand, Haematocrit, Mean Corpuscular Volume (MCV), Eosinophils and Creatine phosphokinase (CPK) were at higher mean values at the beginning of the feedlot period. Elevations in some blood parameters that are associated with acute stress states were raised in slaughter lambs during the feedlot period, transport and handling.

Keywords: Blood parameters, feedlots, Merino, lambs, welfare.

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1. INTRODUCTION

Ovine production is of great economic and cultural importance for rural populations in developed countries such as Spain. In the Spanish region of Extremadura, agriculture is the main economic drive and sheep production plays an important role in the regional economy (Marsal et al., 2009).

In Spain, lambs produced for meat are of low body weight and are slaughtered when they are less than 100 days-old. They are fed milk and a concentrate-based diet until weaning and then, until slaughter, fed by concentrates and straw (Sañudo et al., 1998). Animals are usually raised indoors to produce pale pink meat with white fat, with highly appreciated sensorial qualities (Miranda-de la Lama et al., 2009a). In addition, extra handling procedures in the pre-slaughter logistic chain often exacerbate stress and reduces animals' welfare, as indicated by altered behavioural or immune response (Miranda-de la Lama et al., 2009a).

Nowadays, there are increasing societal concerns regarding high standards of farm animal welfare. According to María, (2006) animal welfare concern in Spain is considered important, but still lower than that observed in northern European countries or USA. Management practices should be orientated to minimize stressful events in the different phases of animal production. Stress factors (handling and transport) can result in changes of haematological, biochemical and hormonal blood parameters (Knowles and Warris, 2007).

The Merino lamb is the most common sheep breed in Extremadura. Known values of haematological, hormonal and biochemical reference intervals are limited. Several published sources of reference intervals in lambs do not specify details such as age, breed, sex or geographic location, methodologies used or even the use of automatic analysers, which provide their own reference range and differed according the analysers (Avellanet et al., 2007; Lepherd et al., 2009). Therefore, it is difficult to be certain that the use of these reference intervals is appropriate for all breeds of sheep (Lepherd et al., 2009).

The objective of this study was to estimate the values of blood parameters (haematological, biochemical and hormonal) in fattening Merino lambs during the feedlot period, to determine possible differences in

these parameters at the beginning and at the end of the feedlot period and to correlate possible variations that could be found between these two time periods.

2. MATERIALS AND METHODS

2.1 Animals and experimental design

Four hundred and ten Merino Spanish breed lambs of both sexes, were randomly selected to be studied from four different classification centres in Extremadura (Southern Spain) from February to November 2014. The animals were brought to the feedlot in three periods (February, June and October) and they were slaughtered approximately one month later. The environmental conditions were measured using Data loggers 175^a (Testo AG, Titisee, Germany) with the objective of having similar conditions during the different periods. The unique differences in management was that lambs were offered a commercial concentrate and cereal straw in addition to a protein supplement for feed intake and digestibility during the warmer month (June). We considered these actions throughout the study and accounted for their interferences in our measurements.

Lambs were transported from different farms (management practices were similar between farms) to the feedlot (20-180 km taking approximately 30-260 min depending on the farms) in trucks adequately conditioned to avoid shocks, fractures and high densities. Animals were placed in pens with straw-bedding considering their body weight (7 m wide x 7.3 m long; 90 to 100 lambs per pen) and remained there for approximately 10 h, without access to food, although they received water ad libitum in buckets. During the fattening stage there are no manipulative materials available in the pens. At the beginning of this period, lambs weighed between 19 and 21 kg and were managed in the feedlot system for 20-40 days. After lairage, all lambs were slaughtered on the same day and assessed using a standard commercial procedure.

Blood samples were taken at the beginning (two days after transport) and at the end (one day before of slaughter), in all lambs on the same day. Two 10 ml tubes (without anticoagulant and with EDTA-K₃), were extracted using venepuncture with Vacutainer[®] extraction system. Animal conditions were the following:

- At the beginning of the feedlot period (pre-score was done two days after classification process), the liveweight was 20.5 ± 2.5 kg (mean \pm standard deviation (SD)) and the lambs were 60-70 days old.
- At the end of the feedlot period (post-score was done one day before pre-slaughter handling stress), the liveweight was 26.5 ± 0.5 kg and the lambs were 90-100 days old.
- The experiment was performed with the permission of the local bioethics committee (approval No. 19/2014, date 13/02/2014).

2.2 Blood sampling

Blood samples from each lamb were collected from the jugular vein by the same trained person at the beginning and at the end of the feedlot period. Approximately 20 sec were necessary for each extraction, using a 0.9 x 25 mm bevelled needle (Henke Sass Wolf, Tuttlingen, Germany), a 20 ml syringe (B. Braun Injekt®, Melsungen, Germany) and 10 ml tubes containing EDTA (Ethylene Diaminetetraacetic Acid) for haematological parameters determination, and 5 ml tubes without additive for hormonal and biochemical parameter measurements. Blood samples were maintained at 4°C in a portable refrigerator until they arrived at the clinical analysis laboratory. To collect serum from the blood, tubes stood at ambient temperature for 1 hour in an upright position to let clot formation. Tubes without anticoagulant were centrifuged at 3000 rpm for 10 min and aliquots were frozen at -21°C until analysis. Studied parameters were analysed five hours after blood samples were collected.

The following physiological indicators were analysed:

- Haematological: Red Blood Cell (RBC) (cells/ μ l), Haemoglobin (g/dl), Haematocrit (%), Mean Corpuscular Volume (MCV) (fl), Mean Cell Haemoglobin Concentration (MCHC) (g/dl), platelets (cells/ μ l) and White Blood Cell Distribution (WBC) (cells/ μ l) were measured with an automatic particle counter (Sysmex® F-620, Norderstedt, Germany). The leukocyte (cells/ μ l) count was estimated from blood smears prepared on clean slides. Staining (rapid panoptic method) was performed using dyes from Química Clínica Aplicada Inc.® (QCA). 100

leucocytes per sample (neutrophils, lymphocytes, eosinophils, basophils, and monocytes) were counted and identified by light microscopy (1000X).

- Hormonal parameters: In order to quantify the level of plasmatic cortisol (ng/dl), Cortisol Immulite® 1000 (Munich, Germany) was used. Barcode labels were used for the assay. Each barcode-labelled unit contained one bead coated with polyclonal rabbit anticortisol antibody. For this purpose, 10 μ l of serum samples were added to the unit test and incubated at 37°C in persistent agitation during 30 minutes. After incubation the sample was centrifuged about its vertical axis. After centrifugation, samples were washed with Milli-Q water to remove unbound and residual serum. The chemiluminescent substrate was added and light emission was read with a high sensitivity photo counter.
- Biochemical parameters: Urea (mg/dl), Creatinine (mg/dl), Aspartate Aminotransferase (AST) (UI/l), Creatine Phosphokinase (CPK) (UI/l), and Total Proteins (g/dl) were measured utilizing a clinical system Quantum Saturno 100 VET Wet Chemistry Analyser® (Woodley Equipment Company Ltd, United Kingdom).

2.3 Faecal examination

A faecal examination was performed at the entrance and the end of the feedlot in order to identify the type and prevalence of parasites present in these animals at each moment of the feedlot period. Rectal faecal samples were preserved in the refrigerator until they could be analysed (approximately two hours). Flotation with saturated NaCl solution and sedimentation technique were used as qualitative detection methods. As a quantitative method, the McMaster technique was used.

2.4 Data analysis

Descriptive statistics were calculated for the pre- and post-scores of the haematological, biochemical and hormonal parameters. Specifically, mean, standard deviation (SD), percentile 2.5 and percentile 97.5. T-tests for paired samples were applied. T-tests for paired samples were applied. The t-test for dependent samples compares the difference in the mean values between pre- and post-scores of the haematological,

biochemical and hormonal parameters. In order to apply this test, the difference between pre- and post-scores must be normally distributed and/or the sample size must be large. This test can be applied to all the pairs of pre- and post-scores in this study, due to the large sample size (410 paired scores for each parameter). The results were considered as significant when P-values were < 0.05 . The statistical software SPSS[®] version 19 was used (IBM. New York).

3. RESULTS

Mean \pm SD for the haematological, hormonal and biochemical parameters at the two previously defined time points are shown in Table 1. Descriptive statistics of the blood parameters at the beginning and at the end of the feedlot periods are listed in Table 1. Mean, SD, 2.5th percentile, 97.5th percentile and percentage for each of the sampled parameters are provided.

Most values ($>75\%$) for RBC, Haemoglobin, WBC, Neutrophils, Eosinophils, Basophils, Lymphocytes, Urea, Creatinine, CPK and Total Protein are within the reference range for clinically healthy animals described by other authors for this breed (Miranda-de la Lama, 2009b; Lepherd et al., 2009). On the other hand, over 77% of samples showed elevations of Haematocrit, MCHC, and Cortisol values beyond the reference range for healthy animals. Whilst for other parameters (MCV, platelets and AST), only half of the sampled animals were within previously stated reference ranges for lambs. Moreover, statistically significant differences were found for Haematocrit, MCV, MCHC, Eosinophils, Cortisol, CPK and Total Proteins mean values between the beginning and the end of the feedlot periods.

Haematocrit presented higher values than normal for this breed at both time points and MCHC presented lower values than normal at the two time points; furthermore, at the beginning of the feedlot period, lambs showed higher mean levels of Haematocrit (%), MCV and Eosinophils, and lower mean levels of MCHC than at the end of the feedlot period. These changes could be explained by the relationship between MCHC, Haemoglobin and Haematocrit expressed in the following equation: $MCHC = (Haemoglobin/Haematocrit) \times 100$ (Guerrero-Mayares, et al., 2004). These results are presented in Table 1. It must be mentioned that during the experimental

period, mortality rate and disease incidence was zero.

Cortisol values were out of range, being higher than normal values (Table 1), according to those described by Rivero (2007) in non-transported lambs of the same type and breed (7.44 ± 3.0 ng/ml). Statistically significant differences were found between cortisol mean levels at the end compared to the beginning of the feedlot period ($P < 0.001$), with higher mean values at the end.

All mean \pm SD of biochemical parameters are within the normal range for this breed (Table 1). Statistically significant differences were observed for the mean of some variables at the two time points (beginning and end of the feedlot period). Lambs showed higher mean levels of CPK at the beginning compared to the end. On the other hand, Urea and Total Proteins showed higher mean levels at the end. The most remarkable elevation of Urea levels was in August with a clear difference of 31.55 mg/dl. In the rest of the studied months, only minor variations were found (see Figure 1).

Statistically significant differences were not observed between the presence of *Eimeria* ($p = 0.465$) or *Strongylida* ($p = 0.056$) and the feedlot period, but we observed higher values of these parasites at the beginning (13322.49 ± 72233.72 and 40.61 ± 266.67 of *Eimeria* and *Strongylida*, respectively) than at the end of the feedlot (10566.95 ± 20651.74 and 15.25 ± 63.94 of *Eimeria* and *Strongylida*, respectively). This could be due to the application of Decoquinate, which is a coccidiostat and one of the most commonly used antiparasitic in lamb production.

4. DISCUSSION

In sheep, as in other species, blood parameters are indicative of animal health (Knowles and Warris, 2007; Bórnez, 2009). However, these parameters can undergo variations depending on different factors such as species and breed. Data regarding reference range for haematological, hormonal and biochemical parameters from clinically health animals are limited. Therefore, it is not clear if it is appropriate to use these reference intervals for lambs of different breeds that are managed under a variety of management systems.

In lambs, changes in blood parameters have been attributed to stress and other damage (Knowles and Warris, 2007), which could lead to direct and indirect losses (i.e., loss of weight, poor conversion rates and

Table 1. Statistical results for haematological, hormonal and biochemical parameters at different blood sampling times.

Analyte	Entry to feedlot (2 days after transport)				Exit from feedlot (slaughter)				P-value
Analyte	Mean and Standard deviation	P _{2.5} *	P _{97.5} *	Percentage in reference interval	Mean and Standard deviation	P _{2.5} *	P _{97.5} *	Percentage in reference interval	P-value
RBC* (x 10 ⁶ cells/μl)	1.30 x 10 ⁷ ± 2.38 x 10 ⁶	8.70 x 10 ⁶	1.78 x 10 ⁷	76.34 %	1.30 x 10 ⁷ ± 1.82 x 10 ⁶	9.91 x 10 ⁶	1.68 x 10 ⁷	85.36 %	0.925
Haemoglobin (g/dl)	11.88 ± 2.35	9.60	14.40	98.78 %	12.14 ± 4.28	9.60	14.97	97.07 %	0.261
Haematocrit (%)	52.41 ± 8.83	38.00	70.48	21.21 %	51.23 ± 8.48	35.22	68.81	15.36 %	0.035
MCV* (fl)	40.45 ± 2.96	35.13	46.95	45.61 %	39.92 ± 2.90	33.70	45.70	54.15 %	0.001
MCHC* (g/dl)	21.77 ± 3.02	15.80	28.72	0.24 %	22.65 ± 2.61	17.40	29.04	0 %	0.000
WBC* (x 10 ³ cells/μl)	7781.88 ± 2760.64	3955	14190	90.73 %	7686.40 ± 3002.77	3265	14862.50	90.97 %	0.593
Neutrophils (10 ⁶ cells/μl)	2918.56 ± 1889.24	229.95	7129	85.85 %	2738.61 ± 1789.63	390	7810.45	87.80 %	0.144
Eosinophils (10 ⁶ cells/μl)	115.72 ± 270.48	0	849.45	98.29 %	68.91 ± 157.258	0	569.68	99.76 %	0.001
Basophils (10 ⁶ cells/μl)	3.15 ± 24.01	0	1.45	99.76 %	12.24 ± 147.56	0	0	99.27 %	0.218
Lymphocytes (10 ⁶ cells/μl)	4402.05 ± 2311.91	994.95	9635.25	84.88 %	4668.19 ± 2431.50	1037.45	9770.45	88.78 %	0.070
Monocytes (10 ⁶ cells/μl)	279.98 ± 504.47	0	1608.30	91.22 %	212.65 ± 545.50	0	1647.78	93.66 %	0.061
Platelets (x 10 ³ cells/μl)	7.74 x 10 ⁵ ± 2.87 x 10 ⁵	320650	1512025	56.59 %	7.42 x 10 ⁵ ± 3.14 x 10 ⁵	299000	1550000	55.61 %	0.120
Cortisol (ng/dl)	42.97 ± 30.30	0	118.71	22.93 %	51.43 ± 32.45	1.27	129.72	17.80 %	0.000
Urea (mg/dl)	41.71 ± 15.65	16.93	80.77	87.32 %	46.42 ± 16.13	19.00	82.43	80.49 %	0.000
Creatinine (mg/dl)	0.97 ± 0.24	0.6	1.50	95.61 %	1 ± 0.26	0.60	1.50	96.34 %	0.069
AST* (U/l)	74.39 ± 27.38	20	140.18	60.24 %	76.71 ± 31.16	36.28	152.73	59.51 %	0.198
CPK* (U/l)	374.12 ± 496.42	61.20	2035.38	75.36 %	269.50 ± 310.73	25.20	1024.43	83.41 %	0.000
T. Proteins* (g/dl)	6.35 ± 0.77	4.41	7.87	76.58 %	6.69 ± 0.57	5.70	7.90	93.66 %	0.000

* RBC (Red Blood Cell), MCV (Mean Corpuscular Volume), MCHC (Mean Cell Haemoglobin Concentration), WBC (White blood Cell Distribution), AST (Aspartate Aminotransferase), CPK (Creatine Phosphokinase), T. Proteins (Total Proteins). P_{2.5} and P_{97.5} represent the 2.5 percentile and the 97.5 percentile, respectively. A percentile is a statistical measure indicating the value below which a given percentage of observations in a group of observations falls.

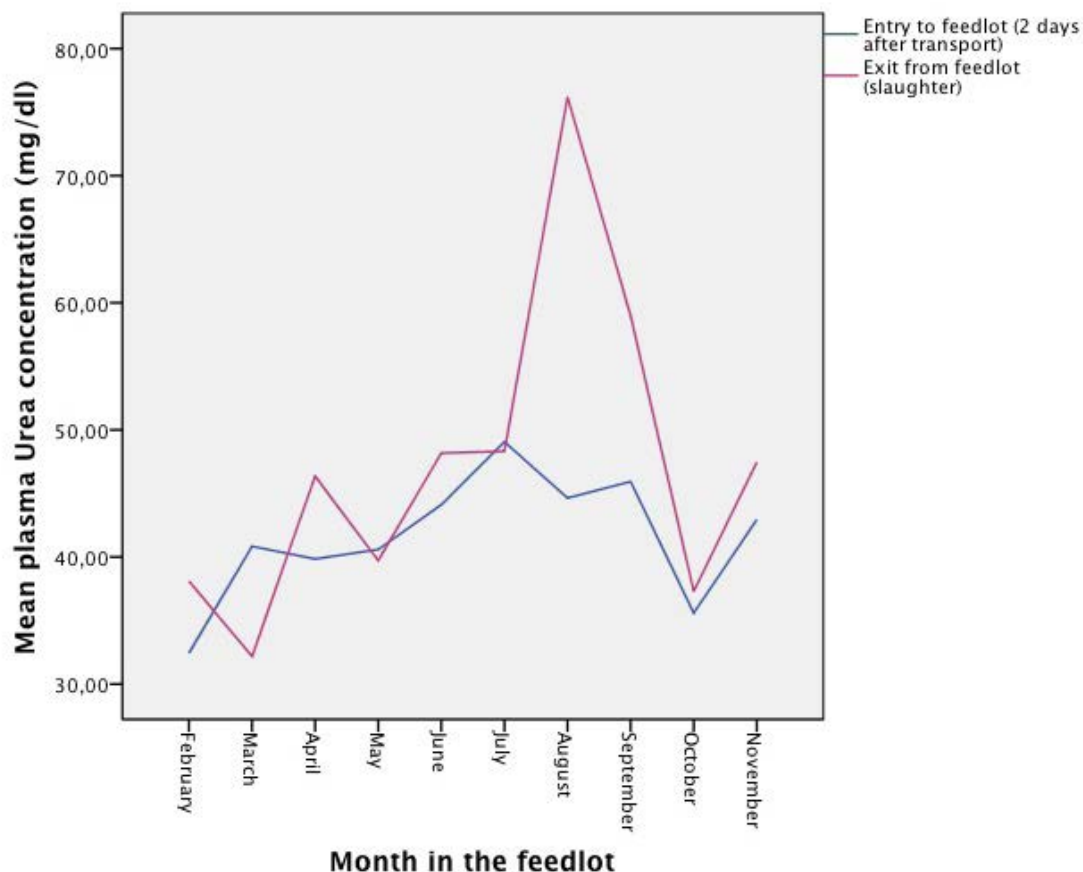


Figure 1. Time plot for urea concentration at the beginning and at the end of the feedlot period.

increasing numbers of lesions in the transport and feedlot periods). In our study, some parameters showed values outside of the reference range, including Haematocrit, MCHC and Cortisol levels. These values could indicate stress factors in handling and transport as has been discussed in previous studies (Weeks 2008, Manteca 2008).

In general, the statistically significant differences found in the mean haematological and biochemical parameters between the beginning and end of the feedlot period were not very noticeable. One highlight was the higher values of eosinophils at the beginning of the feedlot period. This could be due to the higher count and prevalence of parasites (*Eimeria* spp. and *Strongylida* spp.) at the beginning of feedlot period. Eosinophils had lower values at the end of the feedlot period due to coccidial treatment (Adamu et al., 2013).

According to Maede et al. (1990), the significantly higher mean of MCV value found at the beginning of the feedlot period were indicators of the stressful

conditions during transport, capture and placement of the animals in the feedlot. Bórnez et al. (2009) did not find statistically significant differences in MCV, between suckling and light lambs. The difference between our results and those of previous reports could be due to the stress during different handling procedures (Bórnez et al., 2009).

Haematocrit data were outside of normal range at the two time points, presenting higher values at the beginning of the feedlot period. The effects of transport, classification and regrouping that the lambs experienced could explain this (Ferguson and Warner, 2008), possible dehydration during long trips and the first hours of the feedlot period (Broom et al., 1996; Chacon et al., 2005), or excessive handling (Marco et al., 1999).

On the other hand, mean MCHC values were lower than the reference range at both time points. The lower numbers of MCHC values were noted at the beginning of the feedlot period coinciding with high parasite counts. This observation is in agreement with Patra et al. (2010) and Adamu et al. (2013), who demonstrated

pathological changes, such as anaemia, in animals with an elevated presence of parasites.

In regards to biochemical parameters, statistically significant differences were found in Urea concentrations, which were higher at the end compared to the beginning to the feedlot period. Although both values are biologically normal, the increase could be due to warmer months coinciding with a change to a diet richer in protein leading to increased blood urea concentrations; moreover, other authors have shown that exposure to cold temperatures significantly decreased plasma urea nitrogen (Horton, 1981); higher blood urea nitrogen values in lambs indicated that their protein allowance was more than the required (Kaushish et al., 2000).

CPK is released into the blood in response to muscle damage, or to vigorous exercise. Hence, CPK has been used as an indicator of trauma, high levels of physical activity or other damage during handling and transport in farm animals (Knowles and Warris, 2007). In our study, CPK also presented statistically significant differences, showing higher mean levels at the beginning of the feedlot period. Individual examination of this parameter allowed us to affirm that this increase occurred in all but one of the studied feedlots. This could be due to the effect of transportation from the farm to the feedlot. In the feedlot that experienced lower levels, the most distant farms were 100 kilometres away. This is unlike the others feedlots where the farms were as far as 180 kilometres away, increasing muscular damage from traumas and exposure to a larger quantity of stressors during transport (Tadich et al., 2009).

Bórnez et al. (2009) noted that the usefulness of physiological indicators in animal welfare might vary depending on blood sampling time or the age of the animals. Contrary to the results presented here, Bórnez et al. (2009) did not find significant differences in CPK values between blood samples taken on the farm and after transport, while our results suggest that both transportation and handling produce muscle damage in lambs, increasing the CPK parameter. This difference is explained by good handling of lambs and no trauma during loading, travel or unloading in their study (Bórnez et al., 2009).

The last biochemical parameter with significant differences was total proteins value. Although it is within the normal range (Kaneko, 1980), higher mean

values at the end of the feedlot period were found. This may have been due to protein supplementation, which was provided due to increasing requirements for growing lambs (Beatty et al., 1994), feed intake and digestibility (Köster et al., 1996).

Cortisol levels are a good indirect indicator of acute stress in animals (Baldock and Sibly, 1990). This parameter was higher in the two studied time points than normal range values for this breed (Rivero, 2007). This suggests that both transport and handling produce higher values of cortisol. Moreover, other details such as dietary monotony or even nutritionally balanced diets could influence this parameter. Catanese et al. (2013) observed that restricting lambs' dietary choice produced changes in blood and behavioural parameters indicative of stress in sheep.

5. CONCLUSIONS

The results of the current study indicate that both transportation and handling practices cause changes outside of normal Haematocrit, MCHC and cortisol values. Moreover, both practices can produce statistically significant differences in the mean of several haematological (Haematocrit, MCV, MCHC and Eosinophils), biochemical (Urea, CPK and Total Protein) and hormonal parameters (Cortisol level), throughout the study period. These parameters are good indirect indicators of the stress experienced by Merino lambs during transport or handling.

6. CONFLICT OF INTEREST STATEMENT

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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