# CLINICAL AND HEMATOLOGICAL CHANGES IN SHEEP INDUCED BY *Escherichia coli* LIPOPOLYSACCHARIDE

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**ABSTRACT:** Organic response to infection is characterized by a systemic reaction known as acute phase response (APR). In order to know the effect of the administration of *Escherichia coli* lipopolysaccharide (LPS) on physiological, hematological and biochemical variables, 10 sheep weighing 45 ± 5 kg were divided in two groups: Experimental group treated with 3 doses of 1 μg.kg$^{-1}$ LPS and control group treated with saline solution (SS) at the same frequency as experimental group. Body temperature (BT°), heart rate (HR) and respiratory rate (RR) were monitored. Blood samples for hemogram and enzyme activity for aspartate amino transferase (AST) and gamma glutamyl transferase (GGT) were collected between 1 and 24 h post-LPS. LPS-treated sheep presented mean values of BT (41.2 ± 0.4°C), HR (132 ± 12.3 beats/min) and RR (107.2 ± 25 cycles/min) higher than those observed in control sheep (39.8 ± 0.2°C, 88.8 ± 8.7 beats/min and 53.6 ± 17.1 cycles/min respectively). Between 4 and 8 hours post-injection (hpi) of LPS the leukocyte count was associated with lymphopenia, followed by leukocytosis at 24 hours. No changes were observed in the activity of AST and GGT enzymes. The results characterize APR induced by LPS in sheep, representing a useful model to study cardiovascular, hematological and biochemical responses to infection.
**KEYWORDS:** Endotoxin. *Escherichia coli.* Fever. Hematology. Inflammation.

## ALTERAÇÕES CLÍNICAS E HEMATOLÓGICAS INDUZIDAS PELA ADMINISTRAÇÃO DE LIPOPOLISSCARÍDEO DE *Escherichia coli* EM OVELHAS

**RESUMO:** A resposta orgânica à infecção é caracterizada por uma reação sistêmica conhecida como resposta de fase aguda (RFA). Para conhecer o efeito da administração de lipopolissacárideo (LPS) de *Escherichia coli* sobre as variáveis fisiológicas, hematológicas e bioquímicas, 10 carneiros pesando 45 ± 5 kg foram divididos em dois grupos: o grupo experimental tratado com três doses de 1 μg.kg -1 de LPS e grupo controle tratado com solução salina (SS) na mesma frequência que o grupo experimental. Temperatura corporal (T°C), frequência cardíaca (FC) e frequência respiratória (FR) foram monitoradas. As amostras de sangue foram tomadas para hemograma e atividade da enzima aspartato aminotransferase (AST) e gama-glutamiltransferase (GGT) entre uma e 24 h pós-LPS. Ovelhas tratadas com LPS apresentaram valores médios de T°C (41,2 ± 0,4°C), FC (132 ± 12,3 batimentos/min) e FR (107,2 ± 25 ciclos/min) acima dos observados em ovelhas do tratamento controle (39,8 ± 0,2°C, 88,8 ± 8,7 batimentos/min e 53,6 ± 17,1 ciclos/min, respectivamente). Entre 4 e 8 horas após a injeção de LPS, a contagem de leucocitos foi asociado com linfopenia, seguida de leucocitose as 24 horas. Nenhuma mudança na atividade das enzimas AST e GGT foi observada. Os resultados caracterizam uma resposta de fase aguda induzida por LPS em ovelhas, o que representa um modelo útil para estudar os sistemas cardiovascular, hematológico e bioquímico em resposta à infecção.
**PALAVRAS CHAVE:** Endotoxina. *Escherichia coli.* Febre. Hematologia. Inflamação.

## ALTERACIONES CLÍNICAS Y HEMATOLÓGICAS INDUCIDAS POR LA ADMINISTRACIÓN DE LIPOPOLISCÁRIDO DE *Escherichia coli* EN OVEJAS

**RESUMEN:** La respuesta orgánica a la infección se caracteriza por una reacción sistémica conocida como respuesta de fase aguda (RFA). Para conocer el efecto de la administración de lipopolisacárido (LPS) de *Escherichia coli* sobre las variables fisiológicas, hematológicas y bioquímicas, 10 ovejas con un peso de 45 ± 5 kg se dividieron en dos grupos: grupo experimental tratado con 3 dosis de 1 μg.kg-1de LPS y grupo control tratado con solución salina (SS) en la misma frecuencia que el grupo experimental. Temperatura corporal (T°C), frecuencia cardíaca (FC) y frecuencia respiratoria (FR) fueron monitoreadas. Se tomaron muestras de sangre para hemograma y actividad enzimática para el aspartato amino transferasa (AST) y gamma glutamil transferasa (GGT) entre 1 y 24 h post-LPS. Las ovejas tratadas con LPS presentaron valores medios de T°C (41.2 ± 0.4°C), FC (132 ± 12.3 latidos / min) y FR (107.2 ± 25 ciclos/min) por encima de los observados en ovinos controles (39.8 ± 0.2°C, 88.8 ± 8.7 latidos/min y 53.6 ± 17.1 ciclos/min respectivamente). Entre las 4 y 8 horas después de la inyección de

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LPS, el recuento de leucocitos se asoció a linfopenia, seguida de leucocitosis a las 24 horas. No se observaron cambios en la actividad de las enzimas AST y GGT. Los resultados caracterizan una respuesta de fase aguda inducida por LPS en ovinos, representando un modelo útil para estudiar los sistemas cardiovascular, hematológico y bioquímico en respuesta a la infección.
**PALABRAS CLAVE:** Endotoxina. *Escherichia coli.* Fiebre. Hematología. Inflamación.

## INTRODUCTION

Bacterial infections are one of the main factors that reduce performance of livestock production systems, increasing morbidity and mortality of animals (DUFF; GALYEAN, 2007). One of the main effects of these infections is sepsis, which arises as a complex inflammatory deregulation, when the body's immune response to a bacterial infection is unable to control it successfully (BURAS; HOLZMANN; SITKOVSKY, 2005). Physiological response to infection consists of local inflammation and the initiation of a series of events leading to a systemic response called acute phase response (APR). The multiplicity of effects that occur during APR include both metabolic and physiological reactions (PEATMAN et al., 2007), such as fever and leukopenia followed by leukocytosis (GRYDLEY; MILLER; PECAUT, 2007). These reactions occur as well as changes in the concentration of a group of plasma proteins known as acute phase proteins (APP), which are synthesized in the liver and are related to a wide range of activities related to immunity (PEATMAN et al., 2007). The APR starts at a local inflammatory site and involves basically three key processes: (1) hemodynamic changes, (2) altered vascular permeability, and (3) changes in leukocyte count. These processes are initiated by the activation of cells such as monocytes, mast cells and basophils that circulate in the bloodstream. As a result, several soluble, vasoactive and chemotactic substances that affect vascular permeability and stimulate pain receptors are released (BLATTEIS, 2006). There is evidence that bacterial endotoxins, such as lipopolysaccharides (LPSs), are an important factor in the pathophysiology of septic shock generated by Gram negative microorganisms (RAMACHANDRAN, 2014).

A LPS is a glycolipid molecule that is attached to the outer membrane of Gram-negative bacteria, and plays an important role in the activation of the immune system to constitute the most important surface antigen of this type of bacteria.It has a potent endotoxic action and is responsible for septic shock induced by these of pathogens (HURTADO; IREGUI, 2010). *Escherichia coli* LPS has been widely used as an alternative model for infections, because of its potent pyretic and immuno-stimulatory capacity in animals (MORGAN et al., 2008).In addition, it has been used to create reproducible systems for the study of the pathogenesis of sepsis (BURAS; HOLZMANN; SITKOVSKY, 2005), since this endotoxin generates characteristic clinical signs of this phenomenon such as fever, tachycardia and leukocyte activation (PLESSERS et al., 2015).

The complexity of sepsis makes its clinical study difficult; therefore, animal models have been developed as a way to create reproducible systems to study their pathogenesis (BURAS; HOLZMANN; SITKOVSKY, 2005). Within the literature, little data that describe the magnitude and connection between inflammatory parameters after exposure to low doses of *Escherichia coli* LPS in sheep, this is associated with the great variability of the reference values for the haematology and clinical biochemistry of these animals in different regions of the world (BRAUN; TRUMEL; BÉZILLE, 2010).

The main objective of the present study was to investigate the clinical and haematological response of sheep subjected to intravenous exposure of low doses of LPS,to develop a reproducible model that is useful to study the effect of infection and inflammation on the pharmacokinetic characteristics of veterinary drugs.

## MATERIALS AND METHODS

**Location and year of study.** This study was carried out during the winter season of the year 2015 in the Department of Clinical Sciences of the Faculty of Veterinary Sciences of the University of Concepción, Chillán Campus in Chile (36° 36'S, 72° 7'O).

**Experimental animals.** A group of 10 clinically healthy *Suffolk Down* sheep of 12 months of age and 40-50 kg body weight (bw) were selected for the study. Sheep were housed in collective pens, which met individual requirements of space, dry bedding, ventilation and free access to water. Sheep were fed daily with alfalfa hay, supplemented with grain oats (300 g/sheep).All procedures were approved by the Bioethics Committee of the Faculty of Veterinary Sciences, University of Concepción, Chile.

**Reagents.** Purified LPS from *Escherichia coli* O128-B12 was purchased from Sigma-Aldrich (Santiago, Chile). An endotoxin stock solution was prepared by diluting 2 mg of LPS in 10 mL of sterile pyrogen-free saline solution (SS). Then, 1 mL of the solution was diluted to a final concentration of 20 $\mu g\ mL^{-1}$, used to apply a dose of 1 mL 20 $kg^{-1}$ bw.

**Experimental design.** A 2x2 factorial design was used, which consisted of the use of animal pairs (blocks) similar in body weight and corresponding to the same sex. Animals were divided into two groups. Experimental group was treated with three intravenous doses of 1 $\mu g\ kg^{-1}$ bw of LPS through the right jugular vein at 0, 8 and 24 hours (h) after initiating the study to induce an APR.Control group was treated with the same volume of SS at intervals similar to those of LPS group.

**Record of Physiological Constants.** During the test period (0 to 36 hours), heart rate (beats/min), respiratory rate (cycles/min) and rectal temperature (°C) were recorded every 15 minutes using a multi-parameter monitor (Mek´s, MP 800, Kangwon-do, New Global Leader, Korea). An increase of 0.8 °C above the basal rectal temperature of the sheep was considered as an indicator of fever. In addition, the ambient temperature was measured by a maximum and minimum temperature thermometer.

**Processing of blood samples.** Blood samples (3 mL) were obtained by jugular puncture vein before, during 1, 4, 8, 12 and 24 h and after the first administration of LPS or SS. Blood was collected in a tube with ethylene diamine tetraacetic acid (EDTA) for the blood count analysis and in a tube without anticoagulant for the biochemical analysis. Ani-

mals were visually inspected for any adverse effects during each sampling time over a 36 h period.

**Analysis of blood samples.** Globular volume (%) of blood was determined by the microhematocrit method (FELDMAN et al., 2000). Total leukocyte count was determined using the Hayem B technique (FELDMAN et al., 2000). In addition, the differential count of leukocytes and platelet count were determined by means of optical microscopy using Romanowsky staining. Activity of the aspartate amino transferase (AST) and gamma glutamyltranspeptidase (GGT) enzymes was determined using a microplate in which the commercial reagent from the Wiener® laboratory (Buenos Aires, Argentina) corresponding to the evaluated analyte was added to each well. The concentrations of each analyte are proportional to the enzymatic-colorimetric reaction, which were measured at a wavelength of 540 nm by spectrophotometry (Multiskan Go, Thermo Scientific®, Vantaa, Finland).

**Statistical analysis.** Results were expressed as means and standard error of the mean (SEM), and were compared by analysis of variance (ANOVA) for repeated measurements associated with a Tukey multiple comparison test. A value of $P \leq 0.05$ was considered to establish statistically significant differences.

## RESULTS AND DISCUSSION

Inoculation of animals with pure or mixed bacterial agents has been a common tool for the study of the mechanisms of animal sepsis, and is used to develop a reproducible and rapid disease model compared to human sepsis models (BURAS; HOLZMANN; SITKOVSKY, 2005). The utility of LPS as an exposure model is that it is relatively cheap and easy to reproduce, since it is not always possible to develop reliable disease models to carry out pharmacokinetic studies in food-producing animals (POST et al., 2003). The model used in the present study allowed the generation of an APR with three repeated doses of LPS to simulate an inflammatory response to infection. This was done in order to characterize the changes in the physiological, hematological and biochemical variables in healthy sheep.

Mean body temperatures (BT°s) observed in both study groups of sheep are shown in Figure 1a. Experimental group and control group had a baseline BT° of 39.6 ± 0.12 and 39.7 ± 0.1 °C, respectively. In control group after administration of saline solution, BT° remained within normal ranges throughout the study period. In contrast the sheep of experimental group presented a febrile response as the main clinical sign of infection.

The increase in temperature started 1 h after LPS administration and was maintained until 5.5 hpi, reaching a maximal difference of 1.7 °C at 3.5 h after the first LPS injection, and 1.3 °C at 1.25 h after the second injection. These differences were significant with respect to baseline ($P < 0.05$) and values observed in the control group ($P < 0.05$). The return to baseline BT° occurred at 6 h after each LPS application. Similar results were obtained in sheep by Jones et al. (2000). Studies carried out on rabbits by Marca et al. (2009) and Huang et al. (2008) describe similar febrile responses.

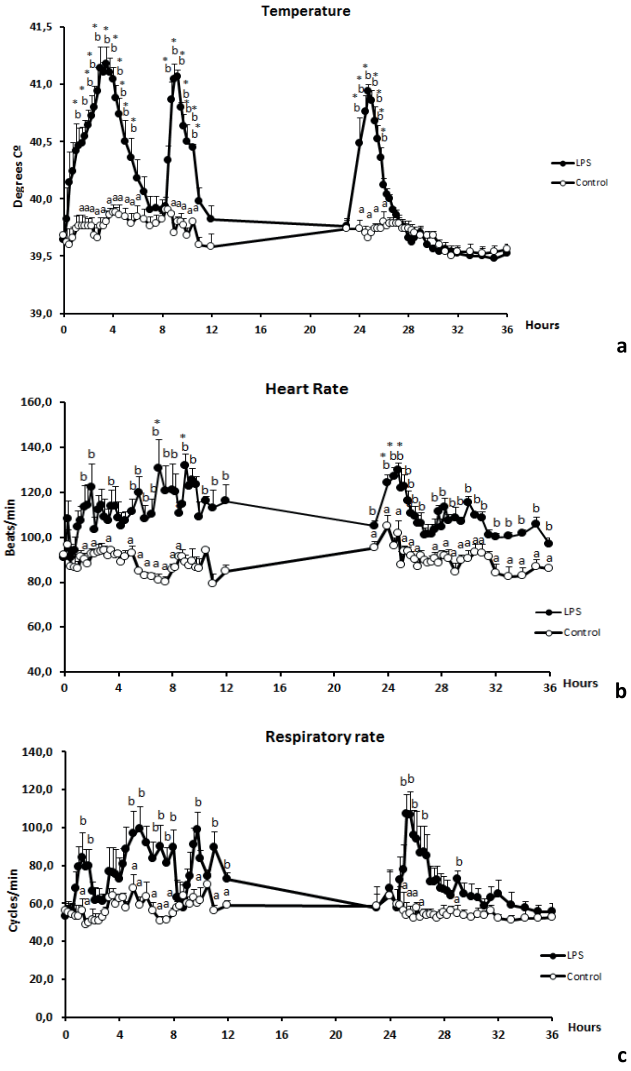
The initial increase in temperature is due to the participation of pyrogenic cytokines derived from reticuloendothelial cells and phagocytes. These cytokines (IL-1, IL-2, IL-6, interferons and tumor necrosis factor) are transcribed, translated and subsequently reach the central nervous system (CNS) by crossing areas with leakage in the blood-brain barrier through the organum vasculosum terminalis, where they act on certain structures to trigger fever, either by their direct action on the receptors or by the biosynthesis of other cytokines (DALAL; ZHUKOVSKY, 2006). The delay in reaching the temperature peaks is explained by the need for synthesis and/or presence of prostaglandin $E_2$ ($PGE_2$) in the ventromedial nucleus of the anterior hypothalamic pre-optic area (thermoregulatory center of the organism), whose synthesis from the arachidonic acid is selectively regulated by pyrogenic cytokines (BLATTEIS, 2003; DALAL; ZHUKOVSKY, 2006). The shorter time to reach the second temperature rise is because cytokines are already present in the area, and therefore their synthesis and transfer to the hypothalamus is not necessary.

The return of BT° to baseline values occurred at 6 h after each LPS application. The rapid recovery of the basal temperature could be explained by a dose-dependent effect of LPS, since at low doses the fever presents a monophasic temperature pattern with a rapid recovery after the temperature peak is reached, in contrast to what happens with moderate to high doses of LPS, where biphasic or triphasic patterns have been observed with a slower recovery of basal temperature (RUDAYA et al., 2005). This has been demonstrated by Jacobsen, Toelboell and Andersen (2005), who administered increasing doses of LPS of 10-100-1000 μg $kg^{-1}$ to cows, and found that these responded with a mono, bi and three-phase BT° pattern respectively, correlating the doses with basal BT° recovery. These BT° patterns are explained by different "waves" of $PGE_2$ synthesis (BLATTEIS et al., 2005).

The mean heart rate (HR) values corresponding to both groups are presented in Figure 1b. In the control group, HR remained within normal physiological ranges throughout the study, presenting no significant differences ($P > 0.05$) with respect to the baseline value (92 ± 3.2 beats per minute). Significant increases ($P < 0.05$) in HR were observed in the experimental group compared to the baseline (90.8 ± 3.18 beats per minute), at 7 - 9.5 - 24.5 - 24.75 h after initiation of the study. The statistical comparison of the averages observed between the two groups showed that there were significant differences in heart rate during a large part of the study, with the greatest difference occurring 1 h after injection of the second dose of LPS ($P < 0.05$).

The sustained increase in heart rate is correlated with the first phase of sepsis, known as the hyper dynamic phase (BURAS; HOLZMANN; SITKOVSKY, 2005) which is characterized by a decrease in systemic vascular resistance (SVR) and an increase in cardiac output mediated initially by the release of bradykinin and histamine (BERMEJO; DUARTE, 2003). Tissue perfusion decreases and therefore oxygen consumption also decreases. Moreover, the activation of white blood cells releases vasoactive agents so that in some areas of the vascular bed there is dilatation, and in others constriction (BERMEJO; DUARTE, 2003). The tachycardia present in these animals was similar to that described by Yates et al. (2011), who found increases in heart rate in sheep treated with 1.5 μg $kg^{-1}$ of LPS. Plessers et al. (2015) had similar results in calves treated with doses of 0.5 μg $kg^{-1}$ LPS.

Figure 1. Mean (± SE) of rectal temperature (a), heart rate (b) and respiratory rate (c) in control group sheep and those treated with *E. coli* LPS (n = 5).




*Significant differences (P <0.05) with respect to baseline. Different lowercase letters vertically (a, b) indicate significant differences between groups (P <0.05).

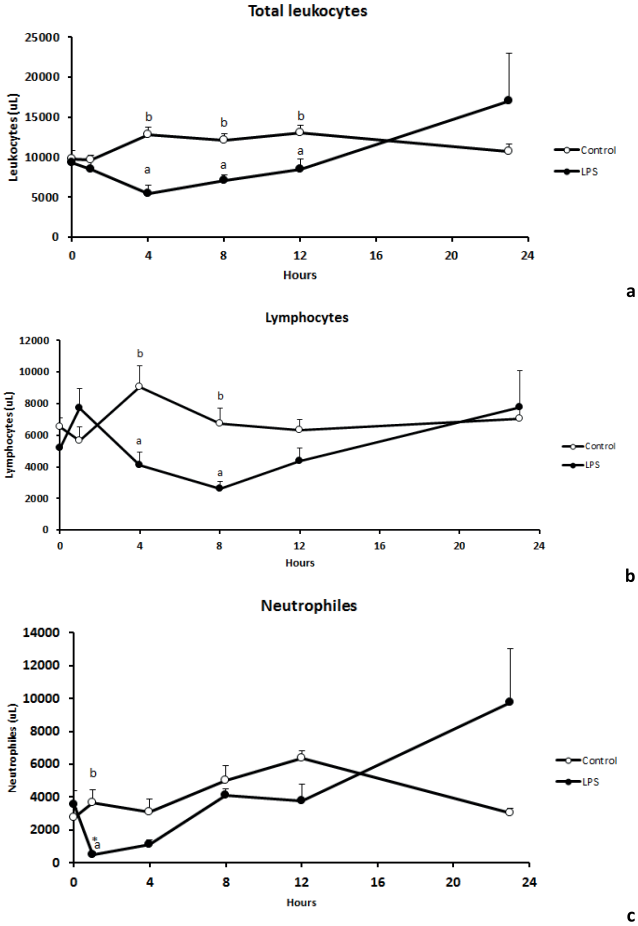
Figure 1c shows the averages of respiratory rate (RR) for both experimental groups. In the control group, RR remained within normal physiological ranges throughout the study, with no significant differences (P> 0.05) with respect to baseline (56 ± 3.7 cycles per minute). In the experimental group, there were statistically significant differences (P <0.05) with respect to baseline (53.2 ± 3.7 cycles/min), at 25.5 and 25.75 hpi. Respiratory rate showed a similar tendency to heart rate and was higher in LPS-treated animals. Comparison of mean values showed that there were significant differences in respiratory rate between the two groups over all part of recording. Some treated animals had mild coughing periods.

The increase in RR represents the response of the organism to an increase in BT° as a way to increase heat dissipation through panting and tachypnea. Meanwhile periods of coughing should be due to injury and/or irritation of the respiratory tree syndrome caused by the endotoxin. At 8 h after the first LPS injection the RR was normalized, which coincides with the decrease in BT°. The anti-inflammatory action of activated protein C (APC) would aid in the cessation of respiratory symptomatology (BURAS; HOLZMANN; SITKOVSKY, 2005). APC is an important route in the regulation of coagulation and inflammation during septicemia, as it has anti-inflammatory action and also improves tissue perfusion (BURAS; HOLZMANN; SITKOVSKY, 2005). Intravenous LPS administration models have been made in rats to determine the effect of APC on lung injury. These studies demonstrated that exogenous APC reduces LPS-induced lung injury through inhibition of cytokine production and activation of leukocytes (BURAS; HOLZMANN; SITKOVSKY, 2005).

Average total leukocyte counts per group are presented in Figure 2a. The control group had a baseline total white blood cell count of 9760 ± 1116.7 $\mu L^{-1}$ cells, remaining at similar values throughout the sampling period. Meanwhile, the experimental group had a baseline count of 9280 ± 723 leukocytes $\mu L^{-1}$, with no difference between baseline values in both groups. However, at 4hpi, a decrease in white blood cell count was observed in LPS's treated sheep, resulting in a slight leukopenia. Notwithstanding, this difference was not statistically significant with respect to the baseline value, but it was statistically significant with respect to the values of control group (P <0.05). This early cellular response is mainly attributed to the infiltration and storage of white line cells in organs such as the liver and spleen, generating in the latter the splenomegaly characteristic of Gram negative sepsis (GRIDLEY et al., 2007).

In the present study, the initial leukopenia is due to a decrease in the lymphocyte count whose values were lower than 50% of the basal value, as observed in Figure 2b. Similar results were found by Peñailillo et al. (2016) in rabbits treated with LPS, where a decrease in leukocytes was observed at 4 hpi and then showed an increase between 12 and 24 hpi. In the LPS treated group of sheep, the initial leukopenia was followed by an increase in leukocytes at 8 and 24 h, with statistically significant differences to the control group (P <0.05) at 8 and 12 hpi. These results are consistent with those reported by Rose and Semrad (1992), Bieniek et al. (1998), Bannerman et al. (2003) and Jacobsen, Toelboell and Andersen (2005) in cattle. Peñailillo et al. (2016) observed an increase in the neutrophil counts from 12 h post treatment in rabbits. In the literature, it has been speculated that this phenomenon could be attributed to a synergistic effect between LPS and the release of corticosteroids in the body. The latter condition leads the animal to produce a higher level of chemo attractant cytokines such as interleukin 1 (IL-1) and necrosis factor alpha (FNTa), which stimulate the release of immature cells from the bone marrow into the circulation (ALTENBURG et al., 2002).

Figure 2. Average total white blood cell (WBC. / µL$^{-1}$) (a), lymphocyte count (Nº of lymphocyte µL$^{-1}$) (b), neutrophil count (Nº of neutrophils µL$^{-1}$) (c) in control sheep and those treated with *E. coli* LPS (n = 5).




*P<0.05 vs baseline. Different lowercase letters vertically (a, b) indicate significant differences between groups (P <0.05).

Neutrophil count averages are presented in Figure 2c. The control group had a baseline count of 2763.4 ± 538.8 µL$^{-1}$ neutrophils, remaining at similar averages throughout the sampling period. The experimental group had a baseline count of 3526.8 ± 844.5 µL-1 neutrophils, with no statistically significant difference (P> 0.05) found between baseline values in both groups.

In sheep treated with LPS, a decrease in neutrophil counts was observed after 1hpi, resulting in mild neutropenia. This difference was statistically significant with respect to baseline and control group (P <0.05). Then, from 4 hpi an increase was observed in the number of neutrophils arriving at their peak at 24 hpi, with statistically significant differences with respect to the basal value and to the control group (P <0.05).

Table 1 shows the averages of the globular volume (GV%), hemoglobin concentration, differential monocyte count, platelet count, enzymatic activity of GGT and AST obtained in both groups. Statistical analysis did not show significant variations in mean globular volume (GV), hemoglobin, and platelet count in LPS-treated sheep. These results are similar to those reported by Bieniek et al. (1998) in calves, and by Peñailillo et al. (2016) in rabbits treated with LPS. However, Yates et al. (2011) describe an increase in GV in sheep treated with LPS, an effect attributed to the splenic contraction that results in the release of erythrocytes into the bloodstream.

Activity of the AST and GGT enzymes did not show significant variations with respect to their baseline value after administration of LPS. These results agree with those obtained by Ramírez (2011) in sheep treated with similar doses of LPS. Therefore, it is inferred that the dose of LPS was not sufficient to cause alterations in liver functionality. However, significant increases have been observed in the mean values of these enzymes in rabbits treated with high doses (100 µg kg$^{-1}$) of LPS (ELMAS et al., 2008).

Table 1. Mean ± SEM of globular volume, hemoglobin, platelet count, monocyte count, AST and GGT enzyme activity in control group sheep and those treated with *Escherichia coli* LPS.

| | Control group (n = 5) | | | | | | Treated group (n = 5) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time in hours | | | | | | Time in hours | | | | | |
| Variables | 0 | 1 | 4 | 8 | 12 | 24 | 0 | 1 | 4 | 8 | 12 | 24 |
| GV (%). | 33 ± 1,7 | 30,8 ± 1,1 | 31,6 ± 0,7 | 30,2 ± 0,6 | 32,2 ± 1,4 | 33,6 ± 1,2 | 32,6 ± 0,4 | 31,6 ± 0,9 | 33 ± 0,3 | 30 ± 0,5 | 32,2 ± 0,7 | 32,3 ± 1,3 |
| Hemoglobin (g/dL) | 10,9 ± 0,55 | 10,3 ± 0,39 | 10,5 ± 0,2 | 10,1 ± 0,21 | 10,7 ± 0,46 | 11,2 ± 0,38 | 10,9 ± 0,11 | 10,5 ± 0,31 | 11 ± 0,08 | 9,9 ± 0,16 | 10,7 ± 0,23 | 10,7 ± 0,43 |
| Monocytes (uL) | 361 ± 101 | 277 ± 54 | 614 ± 111 | 389 ± 151 | 332 ± 77 | 435 ± 78 | 298 ± 47 | 245 ± 56 | 257 ± 90 | 270 ± 40 | 248 ± 67 | 555 ± 214 |
| Platelets (uL) | 285,2 ± 45 | 282,4 ± 56 | 333 ± 26 | 311 ± 60 | 290,2 ± 69 | 286,8 ± 49 | 313,2 ± 54 | 321,4 ± 51 | 325 ± 32 | 220 ± 27 | 145,2 ± 26 | 244,8 ± 41 |
| AST (U/L) | 166,2 ± 57,7 | 121,2 ± 15,8 | 127 ± 18,2 | 129 ± 15,7 | 129,6 ± 12,3 | 127,2 ± 13,3 | 86,2 ± 7,9 | 103,8 ± 8,4 | 117 ± 6,9 | 123 ± 9,6 | 126,3 ± 13,7 | 119 ± 10,6 |
| GGT (U/L) | 86,8 ± 21,2 | 79,4 ± 10,8 | 80 ± 11,5 | 82 ± 11,3 | 80,6 ± 11,7 | 79,6 ± 12,7 | 73 ± 6,9 | 74 ± 4,8 | 79,2 ± 4,9 | 75,6 ± 2,9 | 77,2 ± 3,97 | 80,6 ± 1,77 |

It is known that LPS is attached to the outer membrane of Gram-negative bacteria (HURTADO; IREGUI, 2010). Within these *E. coli* LPS is one of the most widely used endotoxins as an alternative model to infections (MORGAN et al., 2008), although this model is not a generic substitute for bacterial infections and acute inflammatory responses (POST et al., 2003), it is easy to use and relatively similar to these (ELMAS et al., 2008).

There is evidence indicating that the pharmacokinetics of drugs vary during the occurrence of a pathological state or an infection that involves an inflammatory component, therefore, the ability to handle these drugs is diminished (RENTON, 2005), these effects result from the altered expression of certain enzymes of the Cytochrome P450 family (CYP 450) and of drug transport proteins, which are regulated downward (down regulation) during the generation of host defense mechanisms (MORGAN et al., 2008) . This has been demonstrated by different studies where significant modifications in the pharmacokinetics of drugs have been observed between animals treated LPS and control animals (GORALSKI et al., 2003; ELMAS et al., 2006; PÉREZ et al., 2015; PÉREZ et al., 2016).

## CONCLUSIONS

Repeated dose administration of 1μg $kg^{-1}$ LPS of *Escherichia coli* induced an APR in sheep and produced significant changes in physiological variables, resulting in increases in BT°, HR, RR and fever in treated animals. Also, the total and differential leukocyte counts decreased in the first 8 h of LPS injection, followed by leukocytosis.

The observed changes allow the characterization of the acute response to infection by Gram negative microorganisms, mainly *Escherichia coli.*

We conclude that this is an animal model that generates minimal damages to the study subjects, which is easily reproducible, and that is useful to study the effects of APR on the physiological and immunological responses of the organism, and for potential future studies over the effects of infection on the pharmacokinetics of drugs used in veterinary medicine.

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