Histochemistry distribution of mucins and number of leucocytes in the lungs of postweaning pigs exposed to \textit{E. coli} lipopolysaccharide (LPS)

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Abstract

Introduction. Currently, little is known about the effect of lipopolysaccharides (LPS) derived from \textit{E. coli} on the number of goblet cells and the type of mucins secreted in the airways of piglets. Likewise, the differential distribution of these mucins and their relationship with the development of respiratory infections in the post-weaning period is not well understood. This information is useful for porcine production for the development of preventive and sanitary management strategies. Objective. This study aimed to evaluate the effect of the oral intake of \textit{E. coli} LPS on the mucins distribution and the number of leukocytes in the lungs of early weaned piglets. Methods. 52 piglets weaned at 21 days were used. Animals were fed a basal diet with four levels of LPS (0.0, 0.3, 0.5, and 1.0µg /mg feed) for 10 days. Pigs were sequentially slaughtered on days 1, 5, 7, and 10 after weaning. Lung samples were taken for histochemical stainings (H-E, Alcian blue pH 1.0, 2.5, and PAS) in order to determine the amount and type of leukocytes as well as the number of goblet cells and their type of mucins produced. Mucins were classified according to their dyeing characteristics: acidic sulfated, non-sulfated, and neutral, all by computerized image analysis. The statistical design used was a randomized blocks in a 4x4 factorial arrangement (four experimental diets and four days post-weaning). Results and discussion. Results showed a decrease in the number of goblet cells and neutral mucins in both bronchi and bronchioles in the control piglets, followed by a recovery in day 10 post-weaning. In groups with LPS such recovery was inhibited. In all study groups acidic mucins increased in bronchi on day five. There were no acidic mucins in their bronchioles. Moreover, the treatment with the higher concentration of LPS caused a decrease in the number of neutrophils on day 10 and had no effects on the amount of other leukocytes. Conclusions. LPS affects the recovery of goblet cells in the lung airway epithelium and decreases the number of neutrophils altering the...
innate immunity in early weaning. Consequently, it may facilitate the entry of pathogens and an allergen sensitization.

Key words: goblet cells; lungs; swine; weaning; neutrophils.

Distribución histoquímica de mucinas y número de leucocitos en pulmones de cerdos pos-destete expuesto a lipopolisácaridos (LPS) de E. coli

Resumen

Introducción. Actualmente, se sabe poco sobre el efecto de los lipopolisácaridos derivados de E. coli en el número de células de cáliz y el tipo de mucinas secretadas en las vías respiratorias de lechones. Igualmente, no ha sido bien entendida la distribución diferencial de estas mucinas y su relación con el desarrollo de infecciones respiratorias en el periodo pos-destete. Esta información es importante para el desarrollo de estrategias preventivas y de manejo sanitario de las explotaciones porcinas. Objetivo. Este trabajo buscó evaluar el efecto de la ingestión de LPS de E. coli en la distribución de mucina y el número de leucocitos en los pulmones de lechones destetados. Materiales y métodos. Fueron usados 52 lechones destetados a los 21 días de nacidos. Los animales fueron alimentados por 10 días con la dieta estándar con diferentes niveles de LPS (0.0, 0.3, 0.5 y 1.0 µg/mg). Los lechones fueron sacrificados secuencialmente los días 1, 5, 7 y 10. Se tomaron muestras de pulmón para tinción histoquímica (H-E, Alcianblue pH 1.0, 2.5 y PAS) y posterior evaluación de la cantidad y tipo de leucocitos, y número de células de cáliz y tipo de mucinas producidas. Las mucinas fueron clasificadas de acuerdo a sus características de tinción por análisis digital de la imagen:ácidas-sulfatadas, no sulfatadas o neutras. Resultados y discusión. Los resultados muestran una disminución en el número de células de cáliz y mucina neutra en bronquios y bronquiolos en los lechones control con recuperación a los 10 días. En los grupos con LPS la recuperación fue inhibida. En todos los grupos las mucinas ácidas aumentaron en los bronquios al día cinco, y no se detectaron en los bronquiolos. Adicionalmente, el tratamiento con mayor concentración de LPS causó disminución en el número de neutrófilos al día 10 y no tuvo efecto sobre el resto de leucocitos. Conclusiones. Los LPS afectan la recuperación de las células de cáliz en el epitelio de las vías respiratorias y disminuye el número de neutrófilos, alterando la inmunidad innata en el destete temprano. Consecuentemente, esto puede facilitar la entrada de patógenos y la sensibilización con alérgenos.

Palabras clave: células de cáliz, pulmones, suinos, destete, neutrófilos.

Distribuição histoquímica de mucinas e número de leucócitos em pulmões de porcos desmamados expostos a lipopolissacarídeos (LPS) de E. coli

Resumo

Introdução. Atualmente, se sabe pouco do efeito dos lipopolissacarídeos derivados da E. coli no número de células do cálice e o tipo de mucinas secretadas nas vias respiratórias dos leitões. Igualmente, não tem sido bem entendida
a distribuição diferencial das mucinas e sua relação com o desenvolvimento de infecções respiratórias no período de desmame. Esta informação é importante para o desenvolvimento de estratégias preventivas e manejo sanitário das fazendas de porcos. **Objetivo.** Este trabalho avaliou o efeito da ingestão de LPS de *E. coli* na distribuição de mucina e no número de leucócitos nos pulmões de leitões desmamados. **Materiais e métodos.** Foram usados 52 leitões desmamados aos 21 dias do nascimento. Os animais foram alimentados por 10 dias com uma dieta padrão com diferentes níveis de LPS (0.0, 0.3, 0.5 e 1.0 µg/mg). Os leitões foram sacrificados sequencialmente os dias 1, 5, 7 e 10. Tomaram-se amostras de pulmão para coloração histoquímica (H-E, Alcian blue pH 1.0, 2.5 y PAS) com avaliação posterior da quantidade e tipo de leucócitos, e o número de células do cálice e tipo de mucinas produzidas. As mucinas classificaram-se de acordo com as características de coloração por análise digital da imagem: acidas-sulfatadas, no sulfatadas e neutras. **Resultados y discussão.** Os resultados evidenciam uma diminuição no número de células do cálice e mucina neutra em brônquios e brônquicos em leitões controle com recuperação aos 10 dias. Nos grupos com LPS a recuperação foi inibida. Em todos os grupos as mucinas acidas aumentaram nos brônquios ao dia cinco, e não se detectaram nos brônquicos. Adicionalmente, o tratamento com maior concentração de LPS causou diminuição no número de neutrófilos ao dia 10 e não teve efeito sobre os outros leucócitos. **Conclusões.** Os LPS afetam a recuperação das células do cálice no epitélio nas vias respiratórias e diminui o número de neutrófilos, alterando a imunidade inata no desmame inicial. Consequentemente, pode-se favorecer o ingresso de patógenos e sensibilização com alérgenos.

**Palavras chave:** células do cálice, pulmões, suínos, desmame, neutrófilos.
different subtypes of mucins is not well understood, but it is considered that acidic mucins, mainly sulfated, protects against bacterial translocation since they are less degradable by glycosylases and bacterial proteases (Deplancke and Gaskins, 2001; Machado-Neto et al., 2013).

The effects of E. coli LPS on the normal distribution of the different types of mucins in the lung of the post-weaned pig is currently unknown. Understanding the effect of LPS on mucins distribution is important to clarify some aspects of the pathophysiology of respiratory diseases in the weaning period and also it can contribute in knowing the effects of management practices on these diseases.

The following study aims to evaluate the effect of weaning and E. coli LPS on mucins distribution in the lungs of weaning piglets and aims to provide a methodology for the study of cell populations in the lung.

### Materials and Methods

#### Ethical considerations

All experimental procedures were conducted according to guidelines suggested by “The International Guiding Principles for Biomedical Research Involving Animals” (CIOMS, 1985). The experimental procedures were approved by the Experimentation Ethics Committee for Animals of the Universidad Nacional de Colombia, Medellín (CEMED 001 del January 26, 2009).

#### Location

Fieldwork was conducted in the San Pablo Experimental Unit, of the Universidad Nacional de Colombia, Medellín, located in the municipality of Rionegro, at an altitude of 2100 meters above the sea level, with average temperatures between 12 and 18°C, corresponding to an area of very humid low montane forest (bmh-MB).

#### Type of study

Four experimental diets were evaluated: a control diet (basal diet), and three others containing LPS from E. coli, serotype 0111: B4 (Sigma- Aldrich, Sigma-Aldrich, St Louis, MO, USA), as follows:

1. Basal Diet (BD): without LPS.
2. Diet 1 (D1): BD plus 0.3µg of LPS/mg of feed.
3. Diet 2 (D2): BD plus 0.5µg of LPS/mg of feed.
4. Diet 3 (D3): BD plus 1.0µg of LPS/mg of feed.

#### Animals and diet

A total of 52 crossbred pigs (Duroc x Landrance) with an initial body weight of 6.5 ± 0.5kg and an age of 21 days were used. Animals were randomly allotted in groups of eight with water ad libitum and with a controlled temperature of 26 ± 3 °C. The basal diet consisted of milk and milky-products enriched with vitamins, minerals, and lysine HCL. Pigs were fed based on the NRC (2012) nutritional requirements. The experimental diets were fed during the first 10 days of weaning at a rate of 300g/day. During lactation no solid food was offered to the piglets.

#### Sampling

Throughout the experiment, animals were slaughtered sequentially, as follows: the first day (day of weaning, day 1), four piglets representing the reference or control group for all diets studied were sacrificed. On days 5, 7 and 10 post weaning four pigs from each diet were sacrificed, so a total of 52 pigs were used during the experimental phase. Sedation was performed by inhalation of carbon dioxide for three minutes with subsequent exsanguination. Pigs were placed in supine position, the chest cavity was exposed, and lung tissue was extracted by taking fragments of 1cm long and 0.5cm thick. The airways were clearly visible in the extracted tissue. Samples were preserved in 10% buffered formalin.

#### Histotechnical procedures

Samples were processed and analyzed in the Laboratory of Animal Pathology at the
Universidad de Antioquia. Tissues were paraffin-embedded by standard techniques and sliced to 4µm thick cuts. Four glass microscope slides of each sample were mounted for histochemical staining with Hematoxylin-eosin (H-E) for goblet and white blood cell count (neutrophils, macrophages, lymphocytes, eosinophils, plasma cells, and globular leukocytes); Alcian Blue pH 2.5 was used for identification of acid mucins; Alcian blue pH 1.0 for the identification of sulfated acidic mucins; finally, PAS staining was used to identify neutral mucins; according to the method by Armed Forces Institute of Pathology (AFIP, 1994). Positive lung structures for the different colorations were used as positive internal control, these structures were the cartilage, basement membranes, and mucous glands in each sample. Pig brain was used as negative control.

Microscopic evaluation and morphometric analysis of images
For quantitative evaluation of histological sections, an optical microscope Leica DMLB (Meyer Instruments, Houston, TX, USA) was used, then the corresponding images were captured using a digital camera for instant Leica EC3 microscopy (Leica Microsystems, Heerbrugg, Switzerland). Images were analyzed with the image processing software ZEN (blue edition, Carl Zeiss, 2011). The procedure was performed as follows: For leukocytes identification and counting per mm² with the H-E stain, three assessments by slide were carried with a 1000X magnification evaluating a total area of 90mm². For goblet cells and mucins analysis, a contour was performed, using the program Zen® 2011, of three bronchial and three bronchioles epithelium per slide. Goblet cell counting (cells/mm²) was performed on slides stained with H-E. Identification of mucins was done with the previously described histochemical staining procedure. A total area of 6mm² for bronchial and bronchiole epithelium was analyzed. Margins were determined by the apical and basal limits of these structures.

Statistical analysis
The experiment was conducted as a randomized block design in a 4x4 factorial arrangement (four experimental diets and four periods after weaning) (Steel and Torrie, 1985). Each animal was assigned one of 16 treatments, and each treatment had four replicates. Experimental data was analyzed by a multivariate general linear model with the statistical program SPSS® (version 19, 2010). Duncan test was used to compare treatment means (p<0.05).

Results
Pigs fed the basal diet showed good health, whereas those receiving LPS showed sporadic increases in rectal temperature (above 38 °C). However, animals did not show severe clinical manifestations that would force their exclusion from the study (data not shown). No diet rejection was observed during the experiment. In order to determine the effect of early weaning on each of the variables, and to define a baseline to compare with data obtained from animals with LPS, a comparison between the different days of animals consuming the basal diet (BD) was conducted. In day “zero” a baseline leukocyte population was found. The number of neutrophils increased to day seven and decreased (p<0.05) at day 10 post-weaning. Macrophages significantly increased (P<0.05) at day five compared to the weaning day. There was absence of plasma cells in all evaluated slides. Also lymphocytes, eosinophils, and globular leukocytes counts were not significantly different (p>0.05) between the evaluated groups (Table 1).
Table 1. Number of leukocytes in the post-weaning period in pigs fed the basal diet (weaning effect)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Post-weaning period (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Neu</td>
<td>0.345a</td>
</tr>
<tr>
<td>Macro</td>
<td>1.725a</td>
</tr>
<tr>
<td>Linf</td>
<td>0.175</td>
</tr>
<tr>
<td>Eos</td>
<td>0.008</td>
</tr>
<tr>
<td>Leucglob</td>
<td>0.255</td>
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</table>

Neu: neutrophils; Macro: macrophages; Linf: lymphocytes; Eos: eosinophils; Leucglob: globular leukocytes.
abc Means with a different super index within the same row are statistically significant differences (p<0.05).
SEM: Standard Error of the Means.

In animals treated with LPS, the number of neutrophils showed a similar behavior to the basal diet but with a greater decrease (P<0.05) at day 10. Other leukocytes did not show a significant difference (P>0.05) compared to the basal diet (Table 2). Animals on D3 showed the major decrease in the number of neutrophils (P<0.05) (Table 3). No statistical interaction was observed between the LPS concentrations and postweaning periods for any of the studied variables. Therefore, it was not necessary to analyze or break down said factors independently.

Table 2. Effect of E. coli LPS on the number of leukocytes at different post-weaning periods.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Weaning period (cells/mm²)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Neu</td>
<td>0.335a</td>
</tr>
<tr>
<td>Macro</td>
<td>1.710</td>
</tr>
<tr>
<td>Linf</td>
<td>0.043</td>
</tr>
<tr>
<td>Eos</td>
<td>0.002</td>
</tr>
<tr>
<td>Leucglob</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Neu: neutrophils; Macro: macrophages; Linf: lymphocytes; Eos: eosinophils; Leucglob: globular leukocytes.
abc Means with a different super index within the same row are statistically significant differences (p<0.05).
SEM: Standard Error of the Means.

In animals fed only the BD, a significant decrease (P<0.05) was observed in the number of goblet cells on days five and seven, while on day 10 reached similar values to the baseline levels (Figure 1). With the different doses of LPS, the behavior was similar to the basal diet until day seven, however by day 10 values decreased significantly compared to day one (P<0.05). All doses of LPS, but diet 2 (0.5 µg/mg of food), caused a significant decrease (P<0.05) in the number of goblet cells in both bronchus and bronchioles (Figure 3).
Figure 1. Goblet cells on days five, seven and 10.
**Figure 2.** Goblet cells with the different LPS treatments, stained with both AA 2.5 and AA 1.0. Left column, basal diet. Right column, diet.

**Figure 3.** Goblet cells in both bronchus and bronchioles at all doses of LPS.
In the bronchioles of all study units no goblet cells were observed expressing acidic mucins, this means that were not positive for Alcian Blue staining. Bronchioles were only positive for PAS staining (neutral mucins). Neutral mucins count in the BD had no significant differences until day seven, but increased by day 10 (p<0.05). In contrast, the different doses of LPS showed no increase at day 10 and no differences were found between any of the remaining days (Table 4). When comparing between LPS treatments, no statistically differences (p>0.05) were found.

The bronchi were positive for all types of evaluated mucins. Thenumber of neutral mucins (positive for PAS) in the BD showed a similar behavior to the bronchiole, in contrast, diets with LPS showed a significant decrease (p<0.05) on day 10 compared to day one (Table 4).

**Table 4.** Effect of LPS on the expression of different types of mucins in post-weaning piglets (cells/mm²).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Post-weaning period (basal diet)</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 5</td>
<td>Day 7</td>
<td>Day 10</td>
<td>SEM</td>
</tr>
<tr>
<td>NM Bl</td>
<td>5.52a</td>
<td>5.49a</td>
<td>4.37a</td>
<td>7.55b</td>
<td>0.35</td>
</tr>
<tr>
<td>NM Br</td>
<td>4.75a</td>
<td>4.61a</td>
<td>3.87a</td>
<td>6.33b</td>
<td>0.26</td>
</tr>
<tr>
<td>AM</td>
<td>2.58a</td>
<td>6.0b</td>
<td>4.33c</td>
<td>4.44c</td>
<td>0.30</td>
</tr>
<tr>
<td>ASM</td>
<td>2.66a</td>
<td>4.89b</td>
<td>3.54a</td>
<td>3.55a</td>
<td>0.21</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diets with LPS</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 5</td>
<td>Day 7</td>
<td>Day 10</td>
<td>SEM</td>
</tr>
<tr>
<td>MN Bl</td>
<td>5.52a</td>
<td>5.40a</td>
<td>4.46b</td>
<td>5.44a</td>
<td>0.162</td>
</tr>
<tr>
<td>MN Br</td>
<td>4.75a</td>
<td>5.06a</td>
<td>3.51b</td>
<td>3.14b</td>
<td>0.185</td>
</tr>
<tr>
<td>AM</td>
<td>2.58a</td>
<td>4.58b</td>
<td>2.95a</td>
<td>3.86ab</td>
<td>0.171</td>
</tr>
<tr>
<td>ASM</td>
<td>2.66</td>
<td>3.51</td>
<td>2.75</td>
<td>3.34</td>
<td>0.214</td>
</tr>
</tbody>
</table>

NM Bl: Bronchiole neutral mucins; NM Br: bronchial neutral mucins; AM: Acidic Mucins; ASM: acidic-sulfated mucins.

abc Means with a different super index within the same row are statistically significant differences (p>0.05).

SEM: Standard Error of the Means.

In the basal diet (weaning effect), acidic mucins stained with A.A 2.5 showed a significant increase at day five (p<0.05), with a subsequent decrease for days seven and 10 post-weaning (p<0.05). Acidic-sulfated mucins (positive for A.A 1.0) showed a similar behavior to the acidic mucins (positive for A.A 2.5); however, no significant differences (p>0.05) were found for days seven and 10 compared to day one (Table 4).

With the different LPS treatments, goblet cells stained with both AA 2.5 and AA 1.0, had a similar behavior compared to the basal diet (figure 2), with an overall decrease in cells number, but without significant differences (p>0.05).

**Discussion**

This study suggests that E. coli LPS, after early weaning, affects the number of goblet cells in airway epithelium and the number of neutrophils in the interstitial tissues of the lung.

In terms of leukocyte response, it was observed that during weaning there is a resident population of leukocytes in the lung interstitium (granulocytes), lymphocytes appeared and increased over time, whereas plasma cells were not observed in the evaluated period. Time of exposure of LPS caused a gradual decrease in the number of neutrophils in the lung interstitium. These findings are similar to those found by Kandasamy et al. (2012), who demonstrated that after intravenously and aerogenous administration of LPS in pigs, the sequestration of neutrophils in the extravascular space of the lung increased. This is due to LPS altershoming receptors expression and vascular addressins such as P-selectin and the ICAM 1 (Basit et al., 2006; Kathirvel et al., 2012).

Additionally, other reports suggest that leukocyte retention is greater in the interior of the capillary of the pigs’ lung due to its little deformability (Doerschuk, 2001).
Moreover, it has been proposed that a sustained exposure to LPS can decrease the expression of some cytokines such as IL1β and IL8 (Yi-Hong et al., 2012), thereby inhibiting chemotactic stimuli and depressing the antimicrobial effect of neutrophils and macrophages. This phenomenon and the effect of pre-exposure to LPS, which reduces sensitivity to subsequent challenges, have been termed LPS tolerance (Cagiola et al., 2006). Some human and murine studies have associated LPS tolerance with a marked imbalance in the production of inflammatory mediators derived from leukocytes. It has been reported that deregulation of TNF and a reduced expression of the Toll Like Receptor 4 (TLR4) in the cell membrane are the characteristics of the LPS tolerance (Cavaillon et al., 2003; Cagiola et al., 2006).

The gradually appearance of lymphocytes and the absence of plasma cells during the study suggests that an adaptive immune response has not been established in tissue. It also suggests that the effects of LPS in the post-weaning period are given by stimulation and alteration of the innate immune responses.

Furthermore, early weaning also caused a decrease on the number of goblet cells in the airways of control animals, in which a recovery in the last period was seen. Goblet cells stained with H-E behave similar to the goblet cells expressing neutral mucins (PAS). In both cases the LPS showed an inhibitory effect on the recovery of the number of goblet cells producing neutral mucins. LPS caused no variations on the acidic mucins in control animals.

Some authors have reported that both LPS and early weaning have negative effects on intestinal epithelial cells, causing damage to the intercellular junctions and allowing the passage of harmful agents through the epithelial barrier (Parra et al., 2011). Moreover, this effect is enhanced when the two factors (LPS and weaning) act in combination (Hoang et al., 2010; Dänicke et al., 2013). Even though the effect of LPS on non-digestive organs epithelium cells is not completely determined, the present study showed a marked decrease in the number of goblet cells in the respiratory epithelium after early weaning. The pathogenic mechanisms by which occurs this diminution on goblet cells are not well understood, but it is suggested that the early weaned animals can suffer the effects from the LPS that enters via systemic and airborne ways (Dänicke et al., 2013) and also that LPS may induce apoptotic activation pathways in some cells (Ansari et al., 2011; Dholakiya and Benzeroual, 2011); however, the specific effects on the goblet cells are to be determined.

Goblet cells have a fundamental role in innate defense, they are responsible for mucus and mucins production which is a protection against biological, enzymatic, chemical, and mechanical hazards of the respiratory tract (Smirnova et al., 2003; Pierre-Regis and Nadel, 2008). Also, goblet cells produce proteins of the trefoil factor family that promotes epithelialization and epithelial renewal after erosion (Brown et al., 2006). Therefore, a decrease of goblet cells in the post-weaning period in conjunction with the negative effects of LPS in the recovery of cell number, could indirectly affect epithelial renewal against damage.

A profound description of the genes that are expressed in the respiratory mucosa and are involved in mucins production has been made (Degroote et al., 2003; Pierre-Regis and Nadel, 2008). But a description of the distribution according to their biochemical nature has not been yet well defined, considering that it varies according to posttranslational changes (Olof and Thomas, 2010). However, some reports suggest that LPS up-regulates the expression of genes associated with mucins in goblet cells (Smirnova et al., 2003). In the present study it was observed that LPS had an effect on the
diminution in the number of goblet cells and the expression of neutral mucus. Similarly, there are reports suggesting that the action of bacteria, both gram positive and gram negative, up-regulate mucus production in the human respiratory tract (McNamara and Basbaum, 2001), but there is not clear information on its biochemical nature. Studies by Lo-Giudice et al. (1997) and Degroote et al. (2003) demonstrated that the predominant mucus in patients with chronic bronchitis and bacterial infections were acidic sulfates. However, these studies analyzed the material exuded from the patient and were not a histopathological study. These findings suggested that after bacterial infection sulfated acidic-mucins are stimulated; this is consistent with reports found in the gastrointestinal tract (Deplancke and Gaskins, 2001).

In a study conducted by Zapata et al. (2015), it was evaluated the effect of weaning and different diets containing E. coli LPS on pig’s intestines. They observed that there was a decrease in acidic mucin-producing cells during the weaning period and an increase, in similar proportions, of neutral mucus. Early weaning (21 d) and LPS addition to the diet affect mucin secretion and intestinal epithelium integrity by modifying goblet cell populations and their balance between acidic and neutral mucin secretion (Zapata et al., 2015). Deplancke and Gaskins (2001), suggested that the radius of neutral to acidic mucins in the intestine, generally increases between birth and weaning and decreases thereafter. Our findings show that after early weaning there is a decreasing tendency of goblet cells expressing neutral mucins, but in day 10 there is a significant increase, which could explain that neutral-mucins may have a different behavior in the respiratory tract compared to the intestinal tract during weaning period. However, it is necessary to perform additional studies to describe the normal distribution of the different types of mucus in the respiratory tract of the pigs.

The results of this study and the mechanisms mentioned in this discussion suggest that E. coli LPS reduces the innate immune response in the airways and may favor pathogen infection and allergen sensitization. Additionally, it is suggested that early weaning itself can generate this effect and that the presence of LPS have synergistic effects on the response.

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Evaluación de factores que afectan la comercialización de agro-productos de pequeños ymedianos productores del Oriente Antioqueño


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Figure 1. Effect of diets on the average number of goblet cells (GC). (A) Basal diet on bronchiole: GC average decreased on days five and seven, followed by an increase in day 10. (B) Diet with LPS in bronchiole: GC average did not increased in day 10. (C) Basal diet on bronchus: showed a similar behavior as BD on bronchiole. (D) Diet with LPS in bronchus: GC average did not increased in day 10 (SEM: standar error of the means).

Figure 2. Histochemical stainings in bronchi of control pigs (Basal Diet) and treated with D2 (0.5µg LPS/mg). A decrease in the number of GC with H-E (B) and PAS (D) staining’s at day 10 was observed in LPS-treated animals compared to control animals (A and C). There was no difference between the number of goblet cells expressing acidic mucins (A.A 2.5 and A.A 1.0) between the control animals (E and G) and LPS-treated animals (F and H).

Figure 3. comparison of the total number of goblet cells with different concentrations of
LPS throughout the study. In the bronchiole significant decrease was observed in all doses compared with the basal diet. In the bronchus there was no significant reduction with dose 2 (0.5µg/mg of food).