Artículo Original

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

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Abstract

Introduction. Currently, little is known about the effect of lipopolysaccharides (LPS) derived from *E. coli* on the number of goblet cells and the type of mucins secreted in the airways ofpiglets. 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 to porcine production for the development of preventive and sanitary management strategies. **Objective.** This study aimed to evaluate the effect of the oral intake of *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 characteristicsas: 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 inday 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

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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: acídicas-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 acídicas 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 y 1.0 μg/mg). Os leitões foram sacrificados sequencialmente os dias 1, 5, 7 y 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-sede acordo com as características de coloração por análise digital da imagem: acidas-sulfatadas, no sulfatadas o 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, pulomões, suínos, desmame, neutrófilos.

Introduction

The swine industry has advanced in thedevelopment of enhanced genetic lines with better production rates and has standardized management protocols in which early weaning can fall between seven and 21 days of age (Gomez et al., 2008). The pig immune response can be negatively compromised due to stressful practices including abrupt nutritional alterations, housing modifications, and animal regrouping (Dunlop and Malbert, 2007). Consequently, these changes are associated with predisposition to gastrointestinal and respiratory diseases, evidenced by a decrease in weight gain (Plonait and Bickhardt, 2001; Pluske et al., 2006).

Early weaning causes a short period of fasting anda diminution of the beneficial lactobacilli populations,allowing an increase in the pathogenic enterobacteria populations,specially *E. coli*, causing the release of lipopolysaccharide (LPS) fromtheir cell wall (Amador et al.,2007; Mejía-Medina et al., 2011; Ospina et al., 2012; Parra et al., 2011).

The LPS is a pathogenic compound that stimulates innate immunity and inflammatory response (Basit et al.,2006;Dauphinee et al, 2006.,Zhan et al., 2008; Dänicke et al., 2013). Some studies have shown that it can induce changes in the morphology of goblet cells and thus the expression of mucins in different epithelia (Smirnova et al., 2003; Hauber et al., 2007).

Mucins are highly glycosylated proteins, which forms a barrier that protects epithelial cells from noxious agents (Deplancke and Gaskins, 2001). Mucins are produced by goblet cells and are an important component of mucus (Farzana, 2011). According to their biochemical nature, mucins can be classified as neutral and acidic,and at the same time acidic mucins can be sulfated and non-sulfated.The physiological application of the

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 importantto clarify some aspects of the pathophysiology of respiratory diseases in the weaning period and also it can contributein knowing the effects of management practices onthese 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 001del 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 Ríonegro, 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 ± 0.5kgand 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 milkby-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 Pathologyat the

Universidadde Antioquia. Tissues were paraffin-embedded by standard techniques and sliced to 4μm thick cuts. Four glass microscope slidesof 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 pH1.0 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 mm^2 with the H-E stain, three assessments by slidewere carried with a 1000X magnification evaluating a total area of 90mm^2.For goblet cells and mucinsanalysis a contour was performed, using the program Zen® 2011, of three bronchial and three bronchioles epithelium per slide. Goblet cell counting (cells/mm^2) was performed on slides stained with H-E. Identification of mucins was done with the previously described histochemical staining procedure. A total area of 6$mm^2$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 increasesin rectal temperature (above 38 °C). However, animals didnot 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"abaseline 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)

Variable	Post-weaning period (cells/mm²)				
	Day 1	Day 5	Day 7	Day 10	SEM
Neu	0.345[a]	0.365[a]	0.425[a]	0.275[b]	0.016
Macro	1.725[a]	2.028[b]	1.633[a]	1.368[a]	0.066
Linf	0.175	0	0.158	0.425	0.135
Eos	0.008	0	0.008	0.018	0.005
Leucglob	0.255	0	0	0	0.063

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 showa 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 E.coliLPSon the number of leukocytes at different post-weaning periods.

Variable	Weaning period(cells/mm²)				
	Day 1	Day 5	Day 7	Day 10	SEM
Neu	0.335[a]	0.365[a]	0.440[a]	0.288[b]	0.0419
Macro	1.715[a]	2.023[b]	1.900[b]	1.536[a]	0.03860
Linf	0.043	0	0.056	0.087	0.0077
Eos	0.002	0.002	0.010	0.008	0.0052
Leucglob	0.002	0. 066	0.188	0.333	0.0642

Neu: neutrophils; Macro: macrophages; Linf: lymphocytes; Eos: eosinophils; leucglob: globular leukocytes.Each value corresponds to the average of cells for the three doses of LPS in each day of study.
[abc] Means with a different super index within the same row are statistically significant differences (p>0.05).
SEM: Standard Error of the Means.

Table 3. Effect of E. coli LPS dose on the number of leukocytesat different post-weaning periods.

Variable	Diets with LPS(cells/mm²)				
	BD	D1	D2	D3	SEM
Neu	0.335[a]	0.360[a]	0.414[a]	0,128[b]	0.0149
Macro	1.710	1.900	1.836	1.8367	0.0385
Linf	0.043	0.075	0.043	0.0250	0.0078
Eos	0.002	-	0.003	0.0217	0.0024
Leucglob	0.002	0.182	0.167	0.2167	0.0642

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 decreas(P< 0.05).e in the number of goblet cells in both bronchus and bronchioles (Figure 3).

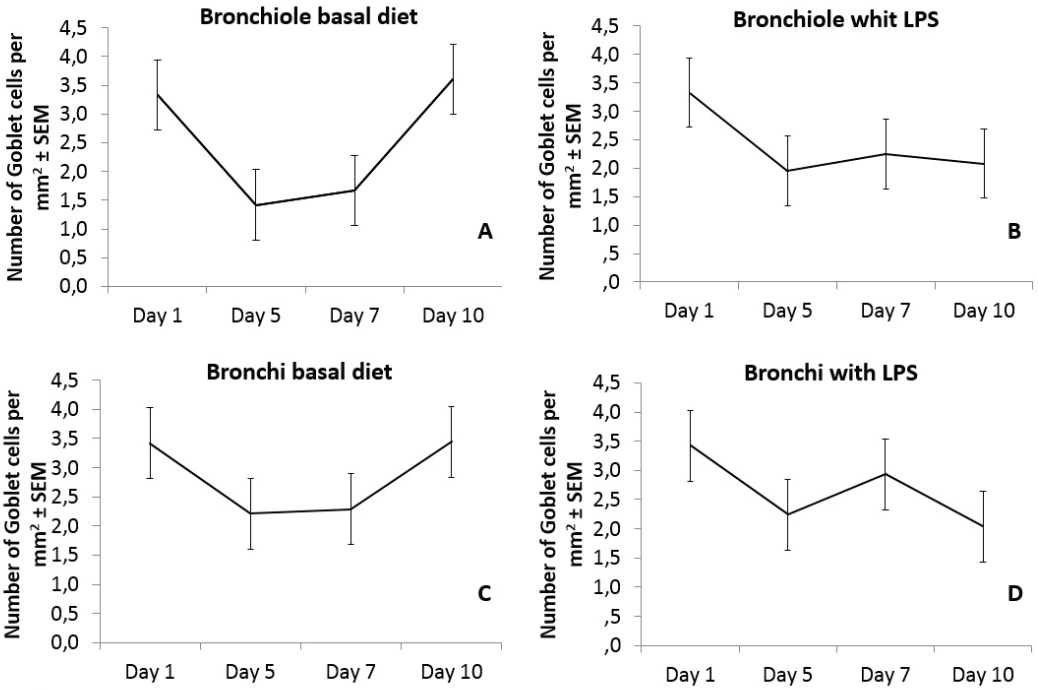
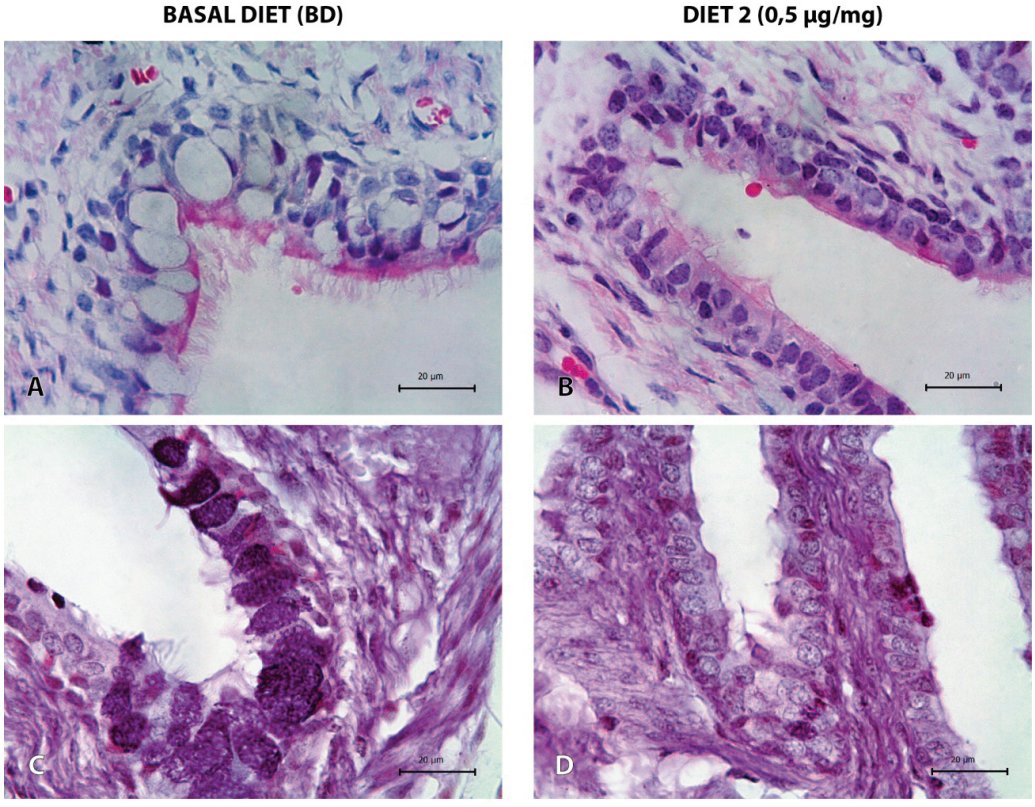


Figure 1. Goblet cells on days five, seven and 10.



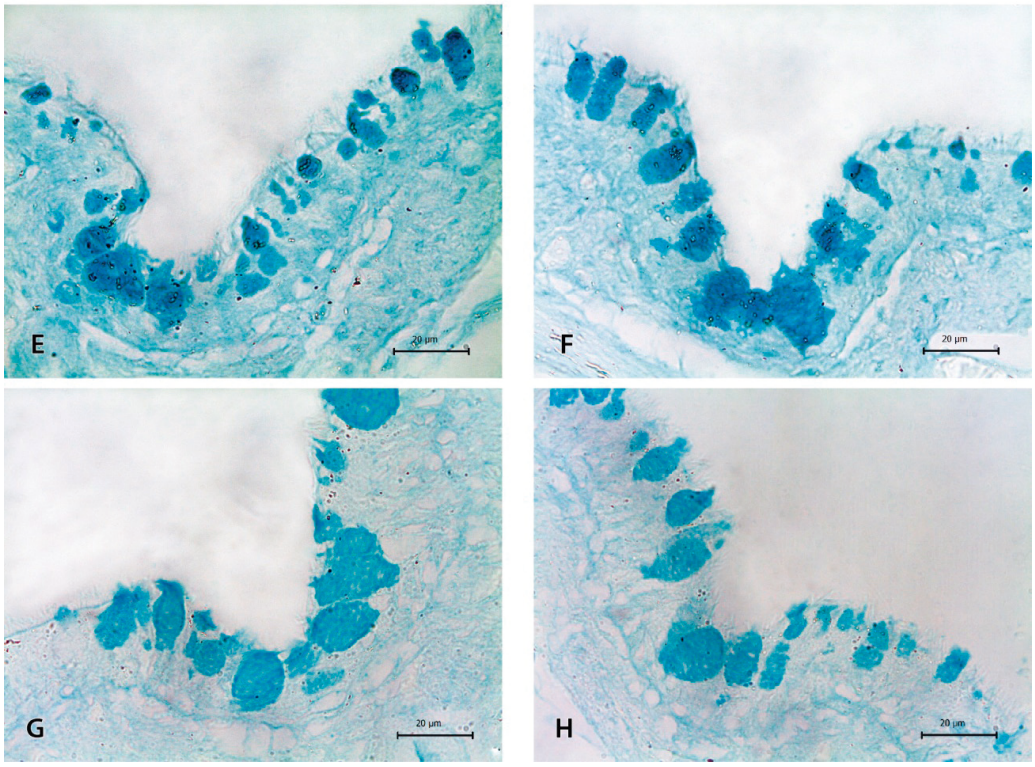


Figure 2. Goblet cells with the different LPS treatments, stained with both AA 2.5 and AA 1.0. Left column, basal diet. Right column, diet.

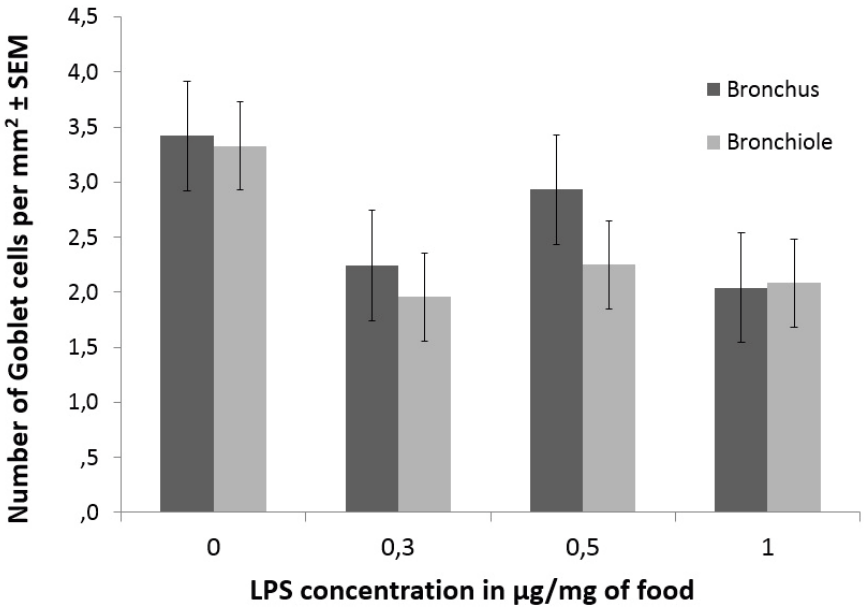


Figure 3. Goblet cells in both bronchus and bronchioles at all doses of LPS

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 BDhad 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/mm^2).

Variable	Post-weaning period (basal diet)				
	Day 1	Day 5	Day 7	Day 10	SEM
NM BI	5.52^a	5.49^a	4.37^a	7.55^b	0.35
NM Br	4.75^a	4.61^a	3.87^a	6.33^b	0.26
AM	2.58^a	6.0^b	4.33^c	4.44^c	0.30
ASM	2.66^a	4.89^b	3.54^a	3.55^a	0.21
Variable	Dietswith LPS				
	Day 1	Day 5	Day 7	Day 10	SEM
MN BI	5.52^a	5.40^a	4.46^b	5.44^a	0.162
MN Br	4.75^a	5.06^a	3.51^b	3,14^b	0.185
AM	2.58^a	4.58^b	2.95^a	3.86^{ab}	0.171
ASM	2.66	3.51	2.75	3.34	0.214

NM BI: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 daysseven and 10 compared to day one (Table 4).

With the different LPS treatments, goblet cells stained with both AA 2.5and AA 1.0, had a similar behaviorcompare 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 intravascular space of the lung increased. This is due to LPS altershomingreceptors 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 capillaryof the pigs' lung due to itslittle deformability (Doerschuk, 2001).

Moreover, it has been proposed that a sustained exposure to LPS can decrease the expression of some cytokines such as Il 1B and Il 8 (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 behavesimilar to thegoblet 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 diminutionon 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 alsothat 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 formucus 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 mucins.Similarly, there are reports suggesting that the action of bacteria, both gram positive and gram negative,up-regulate mucins 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 mucins in patients with chronic bronchitis and bacterial infections were acidic sulfates.However,these studies analyzed the material exuded from the patient and were nota histopathological study. These findings suggested that after bacterial infection sulfated acidic-mucins are stimulated;this is consistent with reports founds 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 mucins. 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 mucins 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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References

AFIP, Armed Forces Institute of pathology. «Laboratory Methods in histotechnology.» American Registry of Pathology, 1994: p279.

Amador, P., García-Herrera, J., Marca, M.C., Osada, de la J., Acín, S., Navarro, M.A. «Intestinal d-galactose transport in an endotoxemia model in the rabbit.» J Membr Biol., 2007: 125-133.

Ansari, N., Khodagholi, F., Amini, M. and Shaerzadeh, F. "Attenuation of LPS-induced apoptosis in NGF-differentiated PC12 cells via NF-kB pathway and regulation of cellular redox status by an oxazine derivative." Biochimie, 2011: 899-908.

Basit, A., Reutershan, J, Morris, M.A, Solga, M, Rose, C.E and Ley, K. «ICAM-1 and LFA-1 play critical roles in LPS-induced neutrophil recruitment into the alveolar space. .» Am. J. Physiol. Lung Cell. Mol. Physiol., 2006: 291, L200–L207.

Blecha, F., Pollmann, D.S., Nichols D. A. «Weaning Pigs at an Early Age Decreases cellular immunity.» J. Anim. Sci., 1983: 395-393

Brown, D.C., Maxwell, C.V., Erf, G. F., Davis, M.E., Singh, S., Johnson, Z.B. «The influence of different management systems and age on intestinal morphology, immune cell numbers and mucin production from goblet cells in post-weaning pigs.» Veterinary Immunol. Immunopathol., 2006: 187–198.

Cagiola, M., Severi, G., Menichelli, M., Forti, K., Petrucci, P., Agostino, M., Pasquali, P., «In vitro down regulation of proinflammatory cytokines induced by LPS tolerance in pig CD14+ cells.» Vet. Immunol. Immunopathol, 2006: 316–320.

Cavaillon, J.M., Adrie, C., Fitting, C,.Adib-Conquy, M.A.«Endotoxin tolerance: is there a clinical relevance?» J. Endotox. Res., 2003: 101–107.

Choi, J, Klinkspoor, J.H., Yoshida, T., Lee, S.P. «Lipopolysaccharide from Escherichia coli stimulates mucin secretion by cultured dog gallbladder epithelial cells.» Hepatology, 1999: 29: 1352–1357.

Chung Hyum Kim, Yeonsu Oh, Kiwon Han, Hwi Won Seo, Duyeol Kim, Ikjae Kang, Changhoon Park, Ki Young Jang, Sung-Hoon Kim, Chanhee Chae«Expression of secreted mucins (MUC2, MUC5AC, MUC5B, and MUC6) and membrane-bound mucin (MUC4) in the lungs of pigs experimentally infected with Actinobacillus pleuropneumoniae.» Research in Veterinary Science., 2012: 486–491.

Dänicke, S., Brosig, B., Kersten, S., Kluess, J., Kahlert, S., Panther, P., Diesing, A.K., Rothkötter H.J. «The Fusarium toxin deoxynivalenol (DON) modulates the LPS induced acute phase reaction in pigs.» Toxicol Lett., 2013: 172– 180.

Dauphinee, M.S., Karsan, A. «Lipopolysaccharide signaling in endothelial.Cells.» Lab. Invest., 2006: 86: 9–22.

Degroote, S., Maes,E., Pascale, H.,Delmotte, P.,Lamblin, G., Roussel,P. «Sulfated oligosaccharides isolated from the respiratory mucins of a secretor patient suffering from chronic bronchitis.» Biochimie, 2003: 369-379.

Deplancke, B., Gaskins,H.R. «Microbial modulation of innate defense: goblet cells and the intestinal mucus layer.» Am. J. Clin. Nutr., 2001: 1131-1141.

Dholakiya, S., Benzeroual,K. "Protective effect of diosmin on LPS-induced apoptosis in PC12 cells and inhibition of TNF-a expression." Toxicol.In Vitro, 2011: 1039–1044.

Dunlop, R., Malbert C.H.. Fisiopatología Veterinaria. Minessota: Acribia Ed, 2007.

Farzana, M. «Recent advances in mucin immunohistochemistry in salivary gland tumors and head and neck squamous cell carcinoma.» Oral Oncology, 2011: 797–803.

Gomez, A.S., Vergara, D., Argote, F. «Effect of diet and age of weaning on piglet digestive physiology. .» Revista Biotecnología en el Sector Agropecuario y Agronindustrial., 2008: 6:32-41.

Hauber, H.P., Goldmann, T., Vollmer, E., Wollenberg, B., Hung. H.L., Levitt, R.C., Zabel, P.«LPS-induced mucin expression in human sinus mucosa can be attenuated by hCLCA inhibitors.» J. Endotoxin. Res., 2007: 2 109-116.

Hoang, V.C., Williams, M.A., Simpson H.V. "Effects of weaning and infection with Teladorsagia circumcincta on mucin carbohydrate profiles of early weaned lambs." Vet. Parasitol., 2010: 354–360.

Kandasamy, K., Sahu, G., Parthasarathi, K. «Real-time imaging reveals endothelium-mediated leukocyte retention in LPS-treated lung microvessels.» Microvasc. Res., 2012: 323–331.

Machado-Neto, R., Pontin M.C.F., Nordi, W.M., Lima, A.L., Moretti, D.B. «Goblet cell mucin distribution in the small intestine of new born goat kids fed lyophilized bovine colostrum.» LivestockScience157, 2013: 125–131.

McNamara, N., Basbaum, C.«Signaling networks controlling mucin production in response to Gram-positive and Gram-negative bacteria.» Glycoconj J. 2001 Sep;18(9):715-22.

Mejía-Medina, J., Rincón-Ruiz, J., Gutiérrez-Vergara, C., Correa-Londoño, G., «Valoración de parámetros clínicos y lesiones en órganos de cerdos durante el período posdestete.» Acta Agronómica, 2011: 61-68.

Olof, S., y Arnebrant T. «Mucin layers and multilayers — Physicochemical properties and applications.» Current Opinion in Colloid & Interface Science, 2010: 395–405.

Ospina, D., Rodríguez, B., López, A., Parra, J. «Efecto de la inflamación causada por el Lipopolisacarido (LPS) de E. coli sobre la morfología y actividad enzimática de las vellosidades en lechones recién destetados.» PANVET, 2012.

Parra, J., Agudelo, J., Ramírez, M.C., Ortiz, L., Rodríguez, B., López, A. «Lipopolysaccharide (LPS) from E. coli has detrimental effects on the intestinal morphology of weaned pigs€.» Rev. Col. Cienc. Pec., 2011: 598-608.

Pierre-Regis, B., Nadel, J.A. «Mucus and Mucin-Secreting.» En Asthma and Chronic Obstructive Pulmonary Disease, de Barnes, P., Drazen, J.M., Rennard, S.I, Neil C. Thomson, N.C., 153-187. Omaha, USA: Alpha, 2008.

Plonait, Hans, y Klaus Bickhardt. Manual de las Enfermedades del Cerdo. Segunda Edición. Zaragoza: Acribia, 2001.

Pluske, J. R., Le Dividich, J., Verstegen, M. El destete en el ganado porcino. Zaragosa: España, 2006.

Smirnova, M.G., Guo, L., Birchall, J.P., Pearson, J.P.«LPS up-regulates mucin and cytokine mRNA expression and stimulates mucin and cytokine secretion in goblet cells.» Cell Immunol. 2003 Jan;221(1):42-9.

Zapata D.J., Rodríguez B.J., Ramírez M.C., López, A., Parra J.E. Escherichia coli lipopolysaccharide affects intestinal mucin secretion in weaned pigs. Rev col cienc pec 2015 Vol 28, No 3.

Zhan, Z., Ou, D., Piao, X., Kim, S.W., Liu, Y., Wang, J. «Dietary Arginine Affects Microvascular Development in the Small Intestine of Early-Weaned.» J Nutr. 2008 Jul;138(7):1304-9.

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

LPSthroughout 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).