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Lipopolysaccharide (LPS) from *E. coli* has detrimental effects on the intestinal morphology of weaned pigs¤

El lipopolisacárido (LPS) de E. coli deteriora los parámetros morfológicos intestinales de cerdos posdestete

O lipopolissacarídeo (LPS) de E. coli afeta negativamente os parâmetros intestinais dos suínos apos desmame

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(Recibido: 18 marzo, 2011; aceptado: 6 septiembre, 2011)

Summary

Early weaning predisposes the pig intestine to structural and functional alterations, due to the increase in E. coli populations. These bacteria use the lipopolysaccharide (LPS) derived from their cell wall as an important pathogenic factor. Little is known about the effects of LPS on the intestinal morphology. Such knowledge could be helpful in understanding the pathogenesis of post-weaning enteritis, which is needed to design therapeutic strategies. Objective: this study aimed to evaluate the effects of the oral intake of LPS on the morphology of intestinal villi and glands of weaned pigs. Methods: the study used 52 pigs weaned at 21 days. The animals were fed a basal diet added with four levels of LPS (0.0, 0.3, 0.5 and 1.0 µg/mg of food) for 10 days. Pigs were sequentially slaughtered on days 1, 5, 7 and 10 after weaning, and samples of small intestine were taken to evaluate morphological parameters by computerized image analysis. The statistical design used was randomized blocks in a 4x4 factorial arrangement. Results: results showed that LPS decreases the height and area of intestinal villi, and increases the width of the villi and the depth and width of the intestinal glands. These effects probably contribute to a decreased intestinal nutrient absorption and increase co-infection with other pathogens, thus leading to the post-weaning diarrhea

¤ To cite this article: Parra J, Agudelo J, Ortiz L, Ramírez MC, Rodríguez B, López A. Lipopolysaccharide (LPS) from *E. coli* has detrimental effects on the intestinal morphology of weaned pigs.Rev Colomb Cienc Pecu 2011; 24:598-608

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syndrome. Conclusions: this study stresses the usefulness of computerized morphometric analysis to evaluate the effect of LPS on intestinal morphology, so it may be used in future studies to investigate the pathophysiology of the causative agents of enteritis and to evaluate therapeutic strategies.

Key words*: histology, LPS lipopolysaccharide, morphometry, small intestine, weaned pigs.*

Resumen

El destete precoz de los cerdos predispone al desarrollo de alteraciones estructurales y funcionales en el intestino y a enteritis causadas por la bacteria Escherichia coli; la cual utiliza el LPS de su pared como uno de sus principales factores patogénicos. Debido a que se conoce poco sobre los efectos del LPS sobre los parámetros morfológicos intestinales, y a que ese conocimiento es necesario para comprender la patogenia de las enteritis postdestete y para diseñar estrategias terapéuticas. Objetivo: se realizó este estudio con el objetivo de evaluar el efecto de la administración de LPS de E. coli sobre la morfología de las vellosidades y las glándulas intestinales en cerdos recién destetados. Métodos: El estudio experimental se realizó con 52 cerdos destetados a los 21 días de edad. Los animales fueron alimentados con una dieta basal adicionada con cuatro niveles de LPS (0.0, 0.3, 0.5 y 1.0 µg/mg de alimento) durante 10 días. Los cerdos se sacrificaron escalonadamente los días 1, 5, 7 y 10 posdestete y se tomaron muestras de intestino delgado para determinar algunos parámetros morfológicos mediante análisis computarizado de imágenes. El diseño estadístico empleado fue bloques al azar en un arreglo factorial 4x4. Resultados: como resultados se obtuvo que el LPS disminuye la altura y el área de las vellosidades y aumenta su ancho, así como la profundidad y ancho de las glándulas intestinales. Estos efectos probablemente disminuyen la absorción intestinal de nutrientes, incrementan la co-infección con otros agentes patógenos y la presentación del síndrome de diarrea posdestete. Conclusiones: Este estudio muestra la utilidad del análisis morfométrico computarizado para evaluar el efecto del LPS sobre los parámetros morfológicos intestinales, por lo que podría utilizarse en futuros estudios para investigar la fisiopatología de los agentes causantes de enteritis y para evaluar estrategias terapéuticas.

Palabras clave: *cerdos destetados, histología, intestino delgado, lipopolisacárido (LPS), morfometría.*

Resumo

O desmame precoce dos suínos predispõe o desenvolvimento de alterações estruturais e funcionais no intestino e à enterite causada pela bactéria Escherichia coli, que usa o LPS da parede como um dos principais fatores patogênicos. Devido a que pouco se sabe sobre os efeitos do LPS sobre os parâmetros morfológicos intestinais, e que esse conhecimento é necessário para compreender a patogênese da enterite pós-desmame e projetar estratégias terapéuticas. Objetivo: este estudo foi realizado para avaliar o efeito administração de LPS de E. coli sobre a morfologia das vilosidades e glândulas intestinais em suínos desmamados. Métodos: o estudo experimental foi realizado com 52 leitões desmamados aos 21 dias de idade. Os animais foram alimentados com uma dieta basal suplementada com quatro níveis de LPS (0.0, 0.3, 0.5 e 1.0 µg/mg de alimento) durante 10 dias. Os suínos foram abatidos em escalonadamente aos 1, 5, 7 e 10 dias pós-desmame e foram tomadas amostras do intestino delgado para determinar alguns parâmetros morfológicos através da análise computacional de imagens. O delineamento estatístico utilizado foi em blocos casualizados em um arranjo fatorial 4x4. Resultados: o resultado foi que LPS diminuiu a altura e a área das vilosidades e aumenta sua largura e profundidade e amplitude das glândulas intestinais. Estes efeitos podem diminuir a absorção intestinal de nutrientes, aumento de co-infecção com outros patógenos ea apresentação do pós-desmame síndrome diarréica. Conclusões: este estudo mostra a utilidade da análise morfométrica computadorizada para avaliar o efeito do LPS sobre parâmetros morfológicos intestinais, de modo que poderiam ser utilizados em futuros estudos para pesquisar a fisiopatologia da enterite agentes causadores e avaliar estratégias terapêuticas.

Palavras chave: *histologia, intestino delgado, morfometria, suínos desmamados.*

Introduction

The pig industry has advanced in the development of precocious genetic lines with better production traits and the weaning of piglets at younger ages (7 - 21 days) (Touchette *et al*., 2002; Gómez, 2006). As a result, piglets are lighter at weaning and have a less developed digestive system, which makes them more susceptible to digestive problems (Reis *et al*., 2007a).

Early weaning results in a short period of fasting right after weaning, and the disappearance of the lactobacilli population that was predominant in stomach and intestine. This creates an imbalance which favors the increase of the *E.coli* population, which cause the release of lipopolysaccharide (LPS) from the cell wall of these bacteria (Amador *et al*., 2007).

LPSs are pathogenic compounds that increase indiscriminate paracellular transport of molecules within the intestine, causing diarrhea, structural and functional alterations (Zhenfeng *et al*., 2008), and in consequence, deficient absorption and utilization of nutrients (Pitman and Blumberg, 2000; Fan, 2002; García-Herrera *et al*., 2003).

Previous studies have described pre and post weaning intestinal morphology in pigs (Pluske *et al*., 1991; Reis *et al*., 2007b; Gomez *et al*., 2008), but little information exists on the combined effect of weaning and LPS on the morphology of the intestine in this species (Albin *et al*., 2007).

The prevention of post-weaning diarrhea syndrome has traditionally been based on the dietary inclusion of antibiotics, copper, and zinc. However, increased bacterial resistance and environmental concerns have led to an international trend to ban the use of antibiotics in animal diets and to reduce mineral inclusion levels. Such policy changes require alternative methods that allow the control of post weaning disorders. Therefore, it is necessary to understand the detailed mechanisms causing structural and functional alterations in the gut during this period in order to develop comprehensive management and control alternatives.

This study aimed at evaluating intestine structural changes, specifically the effect of supplying LPS from *E. coli* on the morphology of the villi and glands of weaned pigs. The assessment of these changes is important to understand the influence of nutrition on intestinal development after weaning, to identify therapeutic targets and to test therapeutic strategies aimed to efficiently treat post-weaning diarrhea.

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). This research was approved by the Animal Experimentation Ethics Committee of the Universidad Nacional de Colombia, Medellín (CEMED 001 from January 26, 2009).

Location

Fieldwork was conducted in the Centro San Pablo of the Universidad Nacional de Colombia, Medellín, located in the municipality of Río Negro, at an altitude of 2100 meters above the sea level, with average temperatures between 12 and 18ºC, 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 0.3 µg of LPS / mg of food.
3. Diet 2 (D2): BD plus 0.5 µg of LPS / mg of food.
4. Diet 3 (D3): BD plus 1.0 µg of LPS / mg of food.

Animals and diet

The experiment used 52 pigs obtained by alternate crossing of Duroc x Landrace. Piglets were weaned exactly on their twenty first day after birth, weighing 6.5 ± 0.5 kg. The weaned pigs were housed in groups of eight with *ad libitum* water, in a controlled-temperature room at 26 ± 3 °C. The basal diet consisted of milk and milk by-products enriched with vitamins, minerals, and lysine-HCL. Diets were formulated to meet the minimum nutritional requirements proposed by the NRC (1998) (Tables 1 and 2).

Table 1. Composition of the basal diet.

Ingredients	%
Powdered Milk	59.0
Casein	6.05
Dairylac 80 (lactose)[A]	15.0
Proliant 1000 (whey)[B]	8.0
Hemoglobin	2.5
Corn starch	4.32
Palm oil	2.363
Sea salt	0.203
Monodicálcic phosphate	0.314
Common salt	0.4
Lysine	0.439
Methionin	0.326
Threonine	0.279
Tryptophan	0.061
Toxins[C] adsorbent	0.05
Vitamins[D]	0.36
Minerals[E]	0.12
Flavors[F]	0.217

Table 2. Proximal analysis of the basal diet.

Crude Protein (%)	21.0
Ethereal Extract (%)	8.35
Ashes (%)	5.42
Humidity (%)	7.215
Gross energy (Kcal/kg)	3708.0

[A]Dairylac 80 (Pro-Ag Products Ltd, Winnipeg, Canadá)

[B]Proliant 1000 (Alitecno S.A.C., Lima, Peru)

[C]Toxibond (Biomix, Medellín, Colombia)

[c] Composition per kg of food: vitamin A 1020 UI, vitamin D 198 UI, vitamin E 6 UI, vitamin K 1.20 mg, riboflavin 7.20 mg, vitamin B_{12} 0.04 mg, coline 968.58 mg, niacin 36 mg, pantothenic acid 16.55 mg, thiamine 30 mg, pyridoxine 31 mg, biotin 0.08 mg, folic acid 0.75 mg.

[c] Composition per kg of food: copper 14.40 mg, iron 120 mg, manganese 36 mg, selenium 0.30 mg, iodine 0.96 mg, zinc 144 mg.

[Ff] Sweet Vanilla, fruit essence (Prodia, Medellín, Colombia)

The amount of food offered per cage was 300 g/day; however, additional food was supplied when required. The experimental diets were offered from days 1 to 10 post weaning. During lactation no solid food was offered to the piglets.

Throughout the experimental phase all 52 pigs were sequentially slaughtered, as follows: the first day (day of weaning; day 1), four pigs representing the reference group were slaughtered to check the overall health and to evaluate the macro and microscopic condition of the organs before the beginning of the experiment. On days 5, 7 and 10 post weaning four pigs were slaughtered in each treatment. Diets were provided from the time of weaning up to 2.5 hours before slaughter.

Sampling of the small intestine

The animals were sedated by inhalation of carbon dioxide for 3 minutes, and then slaughtered by exsanguination through a section on the jugular vein. After slaughter, the pigs were placed in supine position to remove the small intestine (from the pyloric junction to the ileocecal valve) through an abdominal incision. The intestine was aligned on a table, measured without any tension, divided into three sections (duodenum, jejunum, and ileum) of equal size, and 20 cm sections were taken from each segment's center. Once the portions were cut, the content from each one was removed by washing with cold saline infusion as previously described by Makkink *et al*. (1994), and Reis *et al*. (2005). Then, 1cm long sub-samples were obtained from each segment. Samples were preserved in 10% buffered formalin and subsequently stored until performing laboratory determinations.

Histotechnical procedures

Samples from three regions of the small intestine were processed and analyzed in the Laboratory of Animal Pathology at the University of Antioquia. The tissues were sliced in 4 µm thick cuts, and stained with hematoxylin-eosin according to the methodology reported by Nabuurs *et al.* (1993). Three transverse cuts were mounted per slide.

Microscopic evaluation and morphometric analysis of images

The histological sections were analyzed quantitatively by computerized digital image processing, as follows: An optical microscope (Leica DMLB, Meyer Instruments, Houston, TX, USA) was used to identify tissue areas; then, the corresponding images were captured with a three-megapixel 200X zoom camera for instant digital microscopy (Moticam 2300, Motic, Hong Kong, China).The images were analyzed with Motic® Images Plus 2.0 image treatment software (Motic, Hong Kong, China).

The morphometric variables measured in each tissue section were: villus height, width and area. Intestinal glands' depth and width were also determined, as previously described by Nabuurs *et al.* (1993) and Marion *et al*. (2002). The average value for each variable was calculated after performing measurements in eight villi and their corresponding intestinal glands. Due to the fact that villus height may vary in each intestinal fold, being shorter at the apex, it was required that each region was equally represented in the assessment. In consequence, a circular fold of the mucosa was chosen, measuring two villous from the bottom, two on the right, two from the left side and two from the vertex. This procedure was repeated in each section of the small intestine (duodenum, jejunum and ileum) allowing to verify the effect of different diets on the villi according to their location. As far as we are aware, this analysis has not been performed previously.

Statistical analysis

The experiment was conducted as a randomized block design (two blocks) in a 4x4 factorial arrangement (four experimental diets and four periods after weaning) (Steel and Torrie, 1985). The animals were blocked by initial weight. Each animal was assigned one of 16 treatments, and each treatment had four repetitions. Statistical data analysis was conducted using the General Linear Models procedure (GLM) of SAS program (2006). A Duncan test was used to compare treatment means ($p<0.05$).

Results

Pigs fed the basal diet showed good health condition and behaved normally, whereas those receiving LPS showed increases in rectal temperature (above 38ºC) throughout the experiment. However, they didn´t show any signs of illness that would force their retirement or immediate slaughter. No food leftovers were observed during the experiment.

This study compared the data obtained with the BD in each of the post weaning periods and intestinal sections, in order to determine the effect of weaning on intestinal glands and villi morphology (Tables 3 and 4). Villi's height and area decreased from day one after weaning ($p<0.01$; Table 3). Animals with at least five days post weaning had the lowest values for height and area (324.7 µm and 35042 µm^2, respectively), while villi's width had the highest value (112.2 m). For gland's width and depth (Table 3) significant differences were observed ($p<0.01$) from day one, reaching their peak on day five after weaning (43.14 µm^2 and 207.1 µm^2, respectively). In each of the variables under study a partial recovery is shown as time goes by, specifically on day 10 after weaning. However, there were no statistically significant differences between days one and 10 post weaning ($p<0.01$).

Table 3. Changes in the villi and glands (µm)of the small intestine of pigs fed the basal diet(DB) during the post weaning period (effect of weaningz).

Variables	Post weaning Períod				EEM
	1	5	7	10	
Villi					
Height	369.8^a	324.7^b	332.4^c	361.6^a	3.06
Witdth	102.5^a	112.2^b	109.2^b	101.8^a	4.86
Area (µm^2)	37304^a	35042^b	36069^c	36511^a	340
Glands					
Depth	99.2^a	125.5^b	113.8^c	106.3^{ca}	5.78
Witdth	96.4^a	121.5^b	114.4^{bc}	101.2^{ca}	6.11
Villi:Gláná[1] Rate	3.72^a	2.58^b	2.92^b	3.40^a	1.62

[1]Villi: Gland: Height of the villi (µm)/depth of the gland (µm).

^{abc} Means with a different super index within the same row are statistically significant differences ($p<0.01$).

SEM: Standard Error of the Means.

Table 4. Comparison between villi and glands (µm) in different section of the small intestine of pigs not exposed to LPS from *E. coli* (DB) until day 10 post weaning (effect of weaning).

	Intestinal Section			
Variables	**Duodenum**	**Jejunum**	**Ilion**	**EEM**
Villi				
Height	354.4[a]	314.6[b]	307.8[b]	3.06
Witdth	100.5[a]	112.3[b]	109.2[b]	5.86
Area (µm²)	34789[a]	33847[b]	33121[b]	340
Glands				
Depth	112.4[a]	138.5[b]	128.2[b]	5.78
Witdth	89.9[a]	114.5[b]	108.3[b]	7.11
Villi:Gland[1] Rate	3.15[a]	2.27[b]	2.40[b]	1.62

[1]Villi: Gland: Height of the villi (µm)/depth of the gland (µm).

[ab] Means with a different super index within the same row are statistically significant differences ($p < 0.01$).

SEM: Standard Error of the Means.

Significant differences were found among sections of intestine ($p<0.01$) for the variables studied, where the proximal section (duodenum) showed the highest values for villi height and area (354.4 and 34789 µm², respectively) and the lowest values for gland depth and width (112.4 and 89.9 µm, respectively) (Table 4). Jejunum had the highest values for villi and glands width, (112.3 and 114.5 µm, respectively). Nevertheless, there were no statistically significant differences between the middle section (jejunum) and distal (ileum) for the studied variables ($p>0.01$).

The average values of the intestinal variables are presented in Tables 5, 6, and 7. There was no statistical interaction between the factors involved (LPS concentrations and post weaning slaughtering periods) for any of the variables studied. Significant decreases were observed ($p<0.01$) among diets regarding villi's height (Figure 1) and area (Table 5). Animals on D3 had the lowest values for these traits (256.4 µm, and 29,309 µm² respectively) compared to samples from those on BD (347.1 µm and 36232 µm², respectively). Conversely, villi's width for animals on D3 (128.4 µm) showed a significant increase ($p<0.01$) in comparison with those on BD (106.4 µm). For depth and width of the glands, animals on D3 showed ($p<0.01$) higher values (246.1 µm and 121.5 µm, respectively) compared to samples from animals on DB (111.2 and 108.4 µm, respectively).

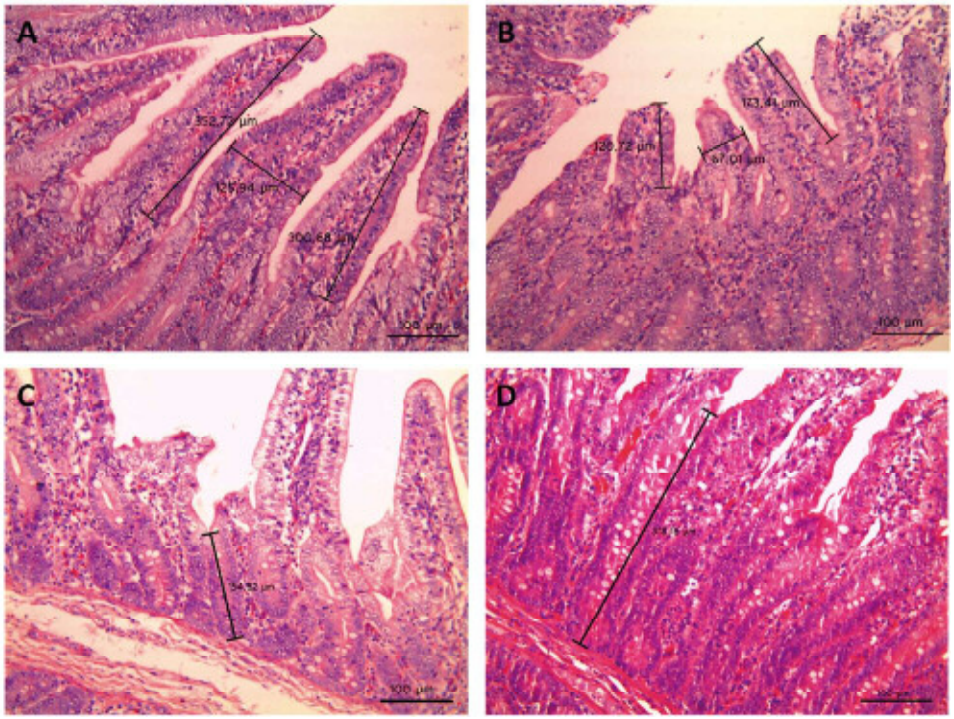

Figure 1. Jejunum. Measurement of morphometric parameters in the mucosa. (A) Basal diet(control), with longer hairs. (B) diet 3, villous atrophy. (C) Basal diet, intestinal glands with less depth. (D) Diet 3 intestinal glands further. Hematoxylin-eosin staining.

Table 5. Changes in villi and glands (µm) of the intestinal mucosa of pigs that consumed the experimental diets until day 10 after weaning.

Variable	Diets				EEM
	DB	D1	D2	D3	
Villi					
Height	347.1[a]	309.3[b]	273.2[b]	256.4[c]	9.42
Witdth	106.4[a]	115.1[b]	122.1[c]	128.4[d]	1.31
Area (µm^2)	36232[a]	34584[b]	32197[b]	29309[c]	743
Glands					
Depth	111.2[a]	182.1[b]	207.5[c]	246.1[d]	11.01
Witdth	108.4[a]	112.9[b]	119.9[c]	121.5[d]	2.29
Villi:Gland[1] Rate	3.15[a]	1.69[b]	1.31[c]	1.04[d]	0.06

DB: Basal diet, without adding LPS from *E. coli*; **D1**: DB plus 0.3 µg of LPS from *E. coli* /mg of food; **D2**: DB plus 0.5 µg de LPS de *E. coli* /mg of food; **D3**: DB plus 1.0 µg de LPS from *E. coli* /mg of food.

[1]Villi: Gland: Height of the villi (µm)/depth of the gland (µm).

[abcd] Means with a different super index within the same row are statistically significant differences (p<0.01).

SEM: Standard Error of the Means.

Table 6. Changes in villi and glands (µm) in the intestinal mucosa of pigs exposed to different concentrations of LPS from *E. coli* at various times post weaning.

Variables	Post weaning period				EEM
	1	5	7	10	
Villi					
Height	369.8[a]	316.1[b]	279.1[b]	259.1[c]	9.42
Witdth	102.5[a]	112.2[b]	123.3[c]	130.9[d]	1.31
Area (µm^2)	37304[a]	34593[b]	32123[c]	30579[d]	743
Glands					
Depth	99.2[a]	143.9[b]	171.7[c]	197.1[d]	11.01
Witdth	96.4[a]	115.9[b]	131.9[c]	132.4[c]	2.29
Villi:Gland[1] Rate	3.72[a]	1.16[b]	0.92[c]	0.70[d]	0.06

[1]Villi: Gland: Height of the villi (µm)/depth of the gland (µm).

[abcd] Means with a different super index within the same row are statistically significant differences (p<0.01).

SEM: Standard Error of the Means.

Table 7. Comparison between villi and glands (µm) in different section of the small intestine of pigs exposed to LPS from *E. coli* (DB) until day 10 post weaning (effect of weaning).

Variable	Intestinal Section			EEM
	Duodenum	Jejunum	Ilion	
Villi				
Height	337.7[a]	306.2[b]	298.3[b]	9.42
Witdth	112.1[a]	126.5[b]	122.3[b]	1.31
Area (µm^2)	39084[a]	32643[b]	31109[b]	743
Glands				
Depth	180.3[a]	238.5[b]	249.3[b]	11.01
Witdth	88.7[a]	123.9[b]	119.2[b]	2.29
Villi:Gland[1] Rate	1.87[a]	1.28[b]	1.19[b]	0.06

[1]Villi: Gland: Height of the villi (µm)/depth of the gland (µm).

[ab] Means with a different super index within the same row are statistically significant differences (p<0.01).

SEM: Standard Error of the Means.

During the post-weaning period (Table 6), villi's height and area decreased (p<0.01) from day one, with animals slaughtered 10 days after weaning presenting the lowest values (259.1 µm and 30579 µm^2, respectively), while villi's width had the highest values (130.9 µm). For depth and breadth of glands (Table 6), significant increases were observed (p<0.01) since day one, reaching the highest level on day 10 post weaning (197.1 and 132.4 µm).

Significant changes (p<0.01) were observed among intestinal portions for the variables under study (Table 7). The proximal portion (duodenum) had the highest values for villi's height and area (337.7 µm and 39084 µm^2, respectively) and the lowest values for glands depth and width (180.3 and 88.7 µm, respectively). For width, both in villus and glands, jejunum had the highest values (126.5 and 123.9 µm, respectively). However, there were no statistically significant differences between the small intestine's mid (jejunum) and distal (ileum) sections for the variables studied (p>0.01)

Discussion

At weaning, the observed values for villi's height and width, as well as those for depth and width of glands, were similar to the ones reported by other authors (Nabuurs *et al*., 1993; Marion *et al*., 2002; Reis *et al*., 2005). It was further verified that weaning decreases villi's height and area, and induces an increase in villi´s width. Weaning also increases intestinal glands' depth and breadth, which is in agreement with previous reports (Hedemann *et al*., 2003). It was also confirmed that recovery of these parameters occurs around day five, which agrees with previous work (Hedemann *et al*. 2003; Vente-Spreeuwenberg *et al*. 2004ab) reporting that villi's growth recovery occurs between 5-8 days post weaning, returning to normal levels between 9-14 days after weaning, under normal conditions (Nabuurs *et al*.1993; Hedemann *et al*. 2003; Vente-Spreeuwenberg *et al*., 2004a).

The ratio between villi's height and glands' depth is of great importance and should be at a maximum, due to the fact that minimum values

are associated with a decrease in digestion and absorption during the first week post weaning. After weaning, the ratio between villi and glands is affected by a change in the microbial population, the intake of solid food, and allergic reactions (Rodrigues *et al*., 2007).

Although the mechanisms underlying the changes observed in this study are not fully understood at present, some researchers postulate that such changes are due to the fact that piglets are subjected to environmental, social and nutritional changes at weaning, which favor the appearance of various stress symptoms (Lallès *et al*., 2004a). Due to this, the post weaning period is characterized by an immediate but transient reduction in food intake, leading to a state of malnutrition and stunted growth. This affects several aspects of the small intestine's architecture and functions, ultimately causing diarrhea. Among others, the following changes have been reported: villi atrophy (Van Beers-Schreurs *et al*., 1998), deepening of intestinal glands, and decreased digestive enzyme activity (Vente-Spreeuwemberg *et al*., 2001; Hedemann *et al*., 2006).

Some research has shown early signs of inflammation after weaning, including leukocyte infiltration, increased expression of several proinflammatory cytokines, enhanced cytoprotection (by over-expression of heat shock proteins), tissue alterations caused by proteases, and epithelial function disorders related to absorption, mineral secretion and intestinal permeability. Nonetheless, after these disorders, there is an intestinal regeneration phase, probably stimulated by the return of feed consumption, which leads to a restored normality in these parameters (Lallès *et al*., 2004a).

Supplying LPS from *E. coli* had an additive effect on these variables, altered as a result of weaning. Such effect was dose-dependent, since the greatest changes occurred with the highest dose (D3). The observed shortening of villus' height is probably due to a decrease in villus cells numbers, from the adverse effects of LPS, and alteration in cell turnover rate, which were significantly lower in the animals on DB. Similar effects have been observed after experimental infection of gnotobiotic pigs with *E. coli* (Willing *et al*., 2007).

Changes in villi and glands' morphology' (McCracken *et al*., 1999; Li *et al*., 2001) represent a balance between gland cells production (Nabuurs *et al*., 1993; Jin *et al*., 1994) and loss of villus cells. The decrease in villus height reduces the area for digestion and absorption of nutrients during this period (Rodrigues *et al*., 2007). The food that is not digested and absorbed in the small intestine ends up in the cecum and colon, generating intense activity and proliferation of microbial population, mainly enteropathogenic, which triggers diarrhea processes that can cause death (Lallès *et al*., 2004b).

The increased villi´s width observed during the post weaning period agrees with previous descriptions (Cranwell, 1995; Yen, 2002). Such change suggest a compensatory response to decreased height (villous atrophy), which occurs in the days after weaning, as described above (Pluske *et a*l.1997; Vente-Spreeuwenberg *et al*., 2001).

The observed changes in the glands' width and height in jejunum and ileum have been reported by some authors as a normal process related to adaptation, which takes place in the post weaning phase, indicating an accelerated mitotic activity (Pluske *et al*. 1997; Vente-Spreeuwenberg *et al*., 2004a). Hedemann *et al*. (2003) reported that the jejunum's villi are between 350 to 450 µm long at weaning and reduce their size from then, returning to their normal size 14 days after weaning. In consequence, villous atrophy and cell renewal, which determines their recovery and growth, vary according to the intestinal section (Reis *et al*., 2005). In this study we observed that these changes were increased in jejunum and ileum due to LPS intake, so these sections are probably more susceptible to the effect of this bacterial component, probably due to an increased specificity for its receptors. This hypothesis must be tested in future research.

In the present study, the effect of *E. coli*'s LPS on intestinal morphology could be largely due to the inflammatory response induced by this component. LPS is known to have the ability to activate a

variety of signaling pathways (Amador *et al*., 2007; Foot *et al*., 2004) through proinflammatory cytokines such as IL-8, IL-18 and TNF-α (Johnson *et al*., 2005). These cytokines affect normal cell growth and induce changes in the intestine's structure and functional capacity (Garcia-Herrera *et al*., 2008; Yoo *et al*., 2000). In general, this may be attributed to two factors: 1) inflammation causes changes in the intestinal cells architecture and normal functions, promoting the release of inflammatory mediators (Johnson *et al*., 2005); 2) enterocytes' normal physiology can be profoundly affected by factors derived from coagulation and blood complement system (Yuji *et al*., 2003).

In addition to the above, some research has shown that Fas receptors co-stimulation with TNFα or INFγ on human enterocytes can induce apoptosis (Ruemmele, 1999). Studies performed on gnotobiotic piglets indicate that conventional *E. coli* bacteria, contrary to the *L. fermentum* bacteria, increases general cell turnover by stimulating apoptosis through the expression of FasL, TNFα and increased cell proliferation. However, in pigs, it has not yet been established whether a similar mechanism causes the decrease in intestinal villi cells, and consequently their atrophy after LPS action.

Another mechanism that could be involved in intestinal disorders arising from LPS-induced inflammation is the disruption of intestinal permeability caused by TNF-α. This cytokine alters intestinal permeability through its effect on tight junctions' structure between epithelial cells (Fengjun *et al*., 2005), mainly in jejunum (Foot *et al*., 2004; Lallès *et al*.2004a). The above mentioned, combined with *E. coli*'s LPS effect, which causes the shortening of actin filaments within the enterocyte, could permeate tight junctions structure to infection-promoting bacteria and compounds, leading to inflammation (Berkes *et al*., 2003).

From this study we conclude that *E. coli*'s LPS has an effect on intestinal morphological parameters, specifically decreasing villi's height and area, and increasing glands' depth and width. Such effect probably contributes to lowering intestinal nutrient absorption, co-infection with other pathogens, and onset of the post-weaning diarrhea syndrome.

This study shows the usefulness of computerized morphometric analysis to reliably, objectively and reproductively evaluate LPS effects on intestinal morphological parameters. In consequence it could be used in future research to investigate the physiopathology of this and other enteritis-causing agents, and for evaluating therapeutic strategies.

From these findings it is suggested the need for more research on the digestive physiology associated with pathology, microbiology and immunology, in order to improve the understanding of the mechanisms responsible for digestive problems during the critical post-weaning period.

Acknowledgements

The authors thank the Research Committee (CODI) of the University of Antioquia for co-funding this research (code 20096000), and the Medellin Research Direction (DIME) of the National University of Colombia for financing it (project codes QUIPU 20301007862 and 20101007950), which made this research possible.

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