# Evaluation of clinical parameters and lesions in pig organs during the post-weaning period

## Valoración de parámetros clínicos y lesiones en órganos de cerdos durante el período posdestete

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## Abstract

The effect of early weaning on clinical parameters, development and occurrence of lesions in organs of systemic importance, and weight gain in pigs was evaluated. The experiment was conducted in the San Pablo Production Research Center of the Universidad Nacional de Colombia (Medellín). We used 16 weaned pigs at 21 days of age. The animals were fed for 10 days with a basal diet (milk). Four pigs were slaughtered on days 1, 5, 7 and 10 post-weaning and, samples of intestine, stomach, liver, pancreas, heart, lungs, kidneys and spleen were extracted. Congestion, edema, and hemorrhage were the lesions determined; a value according to the degree of presence was assigned: absent (0), mild (1), mild-moderate (2), moderate-severe (3), severe (4). The animals were weighed on weaning day, and the day of slaughter. Statistical difference ($P < 0.01$) was found in macroscopic appearance of lesions, organ weight, rectal temperature, and weight gain. On the first day of post-weaning the highest values were observed. On the other hand, the lowest values were observed in the day fifth. However by day 10 after weaning an increase of the injuries was observed. The variable occurrence of diarrhea showed an opposite performance ($P < 0.01$). Weaning is associated with multiple factors leading to the early inflammatory response and the high incidence of diarrhea during post-weaning period.

**Key words:** Diarrheas, fever, pigs, weaning.

## Resumen

El destete de cerdos está asociado con múltiples factores que generan respuestas inflamatorias tempranas en órganos internos y alta incidencia de diarreas. En el Centro de Investigación San Pablo de la Universidad Nacional de Colombia sede Medellín, se evaluaron los parámetros clínicos y las lesiones en órganos internos en 16 cerdos destetados a 21 días de edad, que fueron alimentados durante 10 días con una dieta a base de leche. Cada uno, cinco, siete y diez días posdestete, se sacrificaron cuatro cerdos y se tomaron para estudio muestras de intestino delgado, estómago, hígado, páncreas, corazón, pulmones, riñones y bazo. Las lesiones determinadas fueron congestión, edema, y hemorragia; se asignó un valor según el grado de presentación: ausente (0), leve(1), leve-moderada(2), moderada-severa(3), severa(4). Los animales fueron pesados al destete y en el momento de sacrificio. Se encontraron diferencias ($P < 0.01$) en la aparición macroscópica de lesiones, peso de órganos, temperatura rectal y ganancia de peso. Los mayores valores se encontraron en el día uno posdestete y los menores en el día cinco; no obstante, para el día 10 posdestete se observó una recuperación de las lesiones. La variable ocurrencia de diarreas presentó un comportamiento posdestete diferente y tendió a disminuir (P < 0.01).

**Palabras clave:** Cerdos, destete, diarreas, fiebre.

## Introduction

Apart from its digestive functions, the gut wall has an active role in body defense avoiding bacterial and endotoxins movement from the gastrointestinal tract to the systemic circulation (Pitman and Blumberg, 2000). However, gut contains large amounts of antigens (innocuous) from food and commensal bacteria and is subject to infections by pathogens. Due to that, gut epithelial cells can act as receptors for the immune system (Eckmann *et al.*, 1995) and as response for pathogen organism, but not to the innocuous antigens (Pluske *et al.*, 1997).

After weaning, especially if it is done untimely, stress can be manifested as a short fasting period, changes in the gut microbiome and activation of the adaptive immune response (Lallès *et al.*, 2004). The intake of a new solid portion after weaning results in the alteration of specific substrate availability for the microbes, and a gastro physiological change that benefits increase on pathogen flora in the entire intestinal tract. Therefore, weaning causes reduction in microbe population specially lactobacillus that are predominant in the stomach and intestine, and the increase on *Escherichia coli* population, a bacteria that releases proinflammatory products like lipopolysaccharide (LPS) (Amador *et al.*, 2007).

LPS is the causing agent of sepsis and is recognized as an important pathogen unit by any mammal host (Pitman and Blumberg, 2000; García-Herrera *et al.*, 2003). LPS can activate both, innate and acquired, immune responses through the production of small peptides called cytokines (Pié *et al.*, 2004). Cytokines play a dual pathophysiological role as chemical mediators of inflammation and as immune-regulators that are important in defense against bacterial infections and clinical manifestations of disease (García-Herrera *et al.*, 2004). Additionally, this cytokines generate important changes in gut structure and functional capacity.

Since the knowledge about the relation between immune response and the different manifestations of stress in domesticated animals is scarce, it is necessary to develop an experimental model that allows the evaluation of the early weaning effects on clinical parameters, development and presence of lesion in organs of systemic importance and the live weight gain in pigs.

## Materials and methods

### Ethical considerations

All the experimental proceedings were done according to the guides proposed by The International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1985). This research was supported by the Ethics Commitee on Animal Experimentation of the Universidad Nacional de Colombia – Medellín (CEMED 001 January 26, 2009).

### Location

Field work was done in the Research Center San Pablo from the Universidad Nacional del Colombia – Medellín, located in El Tablacito in Rionegro, 2100 MASL, temperature between 12 and 18 °C, and a life zone of lower montane per-humid forest (bmh-MB) (Holdridge).

### Animals

16 pigs were used, they came from a Duroc x Landrace alternate cross, exactly weaned at 21 days of age with a weight of 6.5 ± 0.5 kg. These pigs were kept in groups of four animals in cages with canoe feeder and waterer pacifier, which were in rooms with controlled temperature 26 ± 3 °C. Animals had available water at their will during their experimental

time and had no solid food during the breast-feeding period.

## Diets

Basal diet feed to the piglets was composed of powder milk and some of its derivatives, it was enriched with vitamins, minerals and HCL lysine. Diet was balanced to fulfill with the minimum nutrients required and proposed by NRC (1998) (Table 1 and 2). The amount of food given per cage was 300g/day, however, when needed extra food was given. Experimental diet was given from day 1 till day 10 post-weaning.

**Table 1**. Basal diet ingredients.

| Ingredients | Percentage |
|---|---|
| Powder milk | 59.0 |
| Casein | 6.05 |
| Dairylac 80 (lactose)[A] | 15.0 |
| Proliant 1000 (serum)[B] | 8.00 |
| Hemoglobine | 2.50 |
| Corn starch | 4.32 |
| Palm oil | 2.37 |
| Sea salt | 0.20 |
| Monocalcium phosphate | 0.31 |
| Common salt | 0.40 |
| Lysine | 0.44 |
| Methionine | 0.32 |
| Treonine | 0.28 |
| Triptofane | 0.06 |
| Toxin absorbent[C] | 0.05 |
| Vitamines[D] | 0.36 |
| Minerals[E] | 0.12 |
| Flavorants[F] | 0.21 |

[A]Dairylac 80 (Pro-Ag Products Ltd, Winnipeg, Canada)
[B]Proliant 1000 (Alitecno S.A.C., Lima, Perú)
[C]Toxibond (Biomix, Medellín, Colombia)
[D]Composition per kg of food: vitamine A 1020 UI, vitamine D 198 UI, vitamine E 6 UI, vitamine K 1.20 mg, riboflavine 7.20 mg, vitamine $B_{12}$ 0.04 mg, coline 968.58 mg, niacine 36 mg, panthotenic acid 16.55 mg, thiamine 30 mg, pyridoxine 31 mg, biotine 0.08 mg, folic acid 0.75 mg.
[E]Composition per kg of food: cupper 14.40 mg, iron 120 mg, manganese 36 mg, selenium 0.30 mg, iodine 0.96 mg, zinc 144 mg.
[F]Sweet vanilla, fruit essence (Prodia, Medellín, Colombia).

**Table 2**. Proximal analysis of the basal diet.

| | |
|---|---|
| Raw protein (%) | 21.00 |
| Ethereal extract (%) | 8.35 |
| Ashes (%) | 5.42 |
| Humidity (%) | 7.22 |
| Gross energy (Kcal/kg) | 3708.0 |

## Evaluation of the clinical manifestations

In order to avoid the inclusion of animals that were previously sick or with diarrhea, a clinical and paraclinical monitoring of the animals was done before starting the experiment. That monitoring was done daily (three times per day) during all the experiment. During the experimental time all the alterations presented by the animals were registered. Rectal temperature was measured daily early in the morning (08:00 h) with a rectal thermometer of mercury which was introduced for 60 seconds with a reference temperature of 38 °C. Feces consistency was measured daily by observing the animals while the temperature was taken and using a scale (0-3), O for normal feces (absence of diarrhea); 1= light, thick diarrhea; 2 = moderate, semi-liquid diarrhea; 3 = severe, very liquid diarrhea. Daily qualifications were summed during the experimental time to calculate the index of diarrhea severity (Reis de Souza *et al.,* 2010), according to the following equation

$$ISD = \sum CFd/Pe$$

where, $ISD$ = Index of diarrhea severity, $CFd$ = Qualification of the daily fecal consistency, and $Pe$ = Experimental period (days).

## Organs extraction

Each 4 days for a total of 16 pigs were slaughtered. Day 1 or initial day of weaning, four pigs were slaughtered representing the reference group; their general health was checked and they were used for the macroscopic evaluation of the organs state before administer the experimental diet. Evaluated organs were stomach, small and large intestines, liver, pancreas, heart, lungs, kidneys and spleen. Days 5, 7 and 10 post-weaning four pigs were slaughtered per day. All the pigs were slaughtered 2.5 hour after their last meal. Animals were sedated by carbon dioxide inhalation for 3 minutes and were slaughtered by exsanguination cutting the jugular vein.

After slaughter, pigs were put in a supine position. For the cavity aperture, an incision till the thorax entrance was done exposing all the rib cage. Following the cut, the abdominal cavity was opened till the pubis. Next, a ligature in the cardias was done and liver, stomach and gut were extracted. Afterwards, kidneys were extracted along with the urinary bladder and genitalia. For the thoracic organs study, heart and lung were extracted together (Segalés and Domingo, 2003). Finally, the extracted organs were washed with a cold saline solution (Reis de Souza *et al.*, 2005).

### Histotechnical procedure

Samples obtained from the different organs were processed and analyzed in the Animal Pathology Lab in Universidad de Antioquía. Preserved samples were included in paraffin, cut at 4 μm thickness and colored with Hematoxylin-Eosin according to the method described by Nabuurs *et al.* (1993). Each slide had three transversal cuts.

### Microscopic evaluation of organ lesions

The lesions identified in each histological cut were congestion, edema and hemorrhage; and a value was assigned depending on the level of presence, as follows: absent (0), mild (1), mild to moderate (2), moderate to severe (3), severe (4). Afterwards, the presence percentage of lesions in each organ was calculated.

### Weight gain estimation

All the animals used in the experiment (16 pigs) were weighted the day of weaning (day 1) and the final day of the experiment (slaughter day), and weight variation was expressed as percentage of the initial weight.

### Experimental design

The experiment was done in a complete randomized design, with a total of four replicates per treatment (post-weaning age). Statistical analysis was done using a lineal general modeling procedure with SAS (2006). For mean comparison between treatments Duncan´s test ($P < 0.05$) was performed.

## Results

Piglets presented a good health at the slaughter moment, although some presented a rise in rectal temperature over 38 °C during all the experimental period, they did not show any disease symptoms that cause their immediate removal and/or slaughter. The amount of food was enough, there were not rejection or excess of it.

The general appearance of lesions in the animals feed with the basal diet can be observed in Figure 1. The obtained data show that between day 1 and 5 post-weaning there is an increase in macroscopic lesions in the different organs studied. With exception of the digestive system organs (stomach and small

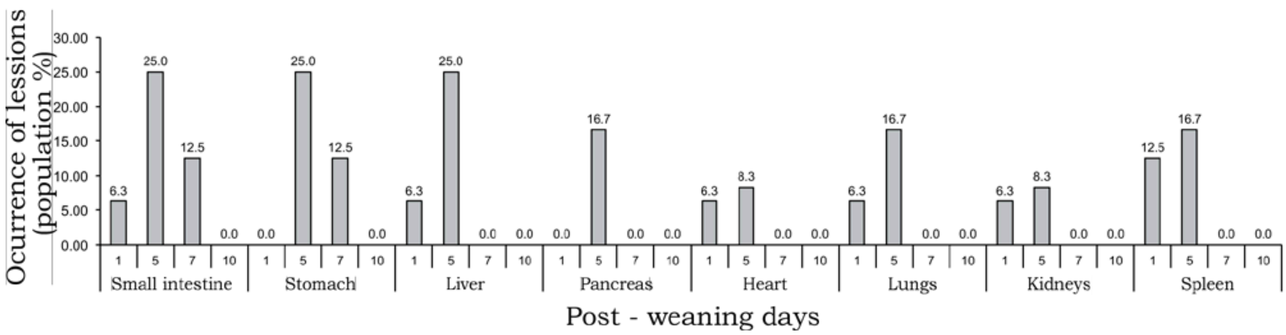


**Figure 1**. Percentage of lesions in different organs of pigs during post-weaning.

intestine) in the other organs there was a reduction on those lesions after the day 5 post-weaning, with a minimum value in day 10. Most common lesion in both organs was hyperkeratosis, and for the other organs were congestions and hemorrhages.

In Figure 2 is observed that from day 1 till day 5 post-weaning, there is a considerable increase in diarrhea occurrence; however, from day 5 the problem decreases and it is minimum in day 10 post-weaning.

In this study was evaluated, as well, the weaning effect on organ weight variation in the different experimental periods (Table 3). For all the organs there was a significant live weight reduction (P < 0.01) in each period, the highest on day 1 and lowest on day 5 post-weaning. However, there were no differences (P > 0.01) in this variable in day 1 compared to day 10, indicating that the organ weight at day 10 post-weaning reaches the one of day 1post-weaning.

In Table 4 is shown that the body temperature changed significantly (P < 0.01) between the different post-weaning periods, being the lowest (38.3 °C) on day 10, which

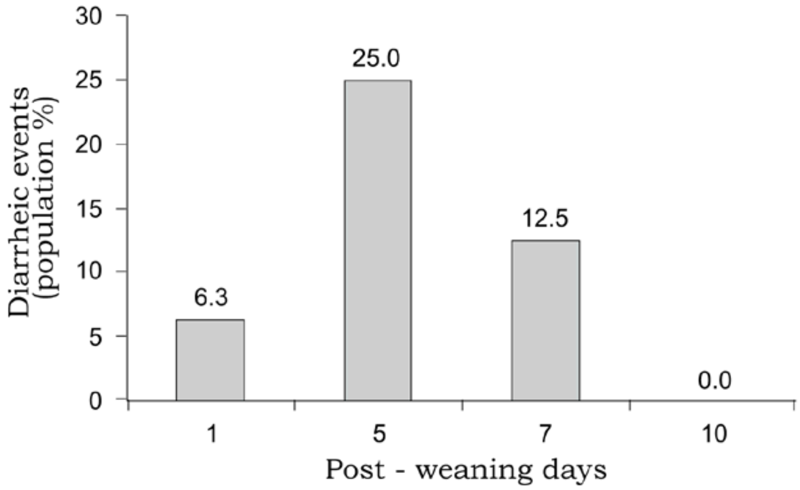


**Figure 2.** Percentage of diarrheic events in pigs during the post-weaning period.

**Table 3.** Organ weight (%PV) of pigs feed with basal diet during different post-weaning periods (weaning effect).

| **Organ** | **Post-weaning period (day)** | | | | **SEM** |
|---|---|---|---|---|---|
| | **1** | **5** | **7** | **10** | |
| Small intestine | 12.34a | 10.82b | 11.48bc | 12.08ca | 0.24 |
| Stomach | 3.04a | 2.92b | 2.95b | 3.03a | 0.02 |
| Liver | 1.32a | 1.23b | 1.26b | 1.33a | 0.02 |
| Pancreas | 0.92a | 0.75b | 0.81bc | 0.85ca | 0.03 |
| Heart | 0.99a | 0.88b | 0.91bc | 0.96ca | 0.02 |
| Lungs | 3.17a | 2.78b | 2.92b | 3.14a | 0.06 |
| Kidneys | 0.67a | 0.52b | 0.58bc | 0.63ca | 0.02 |
| Spleen | 0.39a | 0.32b | 0.37a | 0.41a | 0.01 |

* In the same row, means with different letter are statistically different (P < 0.01). SEM: Standard error of the mean. %PV: percentage of live weight.

indicates that in the weaning day there is a rise in body temperature that keeps rising till day 5, and decreases on day 10.

The daily weight gain (Table 4) was different (P < 0.01) between the post-weaning days, it was lower on the period five (-7.2), meaning that piglets were losing weight during the experimentation in comparison to the weight at the beginning of the experiment. Between the post-weaning days 1 and 10 there were differences (P < 0.01) in this variable, on day 10 there is a total recovery, surpassing the weight of day 1 post-weaning.

## Discussion

In this work was proven, again, that early piglet weaning reduces weight on the studied organs and, generates diarrhea events. Shan *et al.* (2007) demonstrate that pigs weaned later have a more developed and complex immune system, and a digestive system characterized by heavier and more functional organs. This gastrointestinal development reduces diarrheic events during the post-weaning period, because it favors the early food consumption by the pigs (Vente-Spreeuwenberg *et al.*, 2004), improving their growth and animal performance (Main *et al.*, 2004).

Diarrheic events during the post-weaning period are due, possibly, to the appearance of diverse stress manifestations (Lallès *et al.*, 2004) characterized by changes in the intestinal microbial population, the presence of acute inflammation signs and allergic reactions (Rodrígues *et al.*, 2007). Intestinal inflammation associated with weaning happens in diverse animals of productive type (Manzano *et al.*, 2002) and is represented by severe atrophy, immune system disequilibrium and release of inflammatory agents such as TNF-α (Jiang *et al.*, 2009; Wang *et al.*, 2008). Increments in TNF-α expression during the inflammatory response cause chlorine stimulation ($Cl^-$) in the ileum crypts (Burrell, 1994). Increases in $Cl^-$ secretion and reduction in sodium ($Na^+$) absorption in the villi are highly associated with diarrhea occurrence (Berkes *et al.*, 2003).

Additionally, TNF-α alters paracellular transport of toxic compounds to the systemic circulation and the process of cellular turnover (Manzano *et al.*, 2002), which leads to a not-regulated systemic response that can progress into a multiple organic failure (FOM). FOM is related to high mortality and is characterized by lung, cardiovascular, renal and gastrointestinal dysfunction (Bertelsen *et al.*, 2004). Due to the aforementioned, lesion occurrence and weight reduction in the different organs of study, could be due to the release of the inflammatory mediator TNF-α during the inflammatory response in the intestine. TNF-α activates a wide variety of signaling pathways (Pié *et al.*, 2004) that affect cellular turnover and growth because of the stimulation of apoptosis (Yu and Perdue, 2000).

The reduction in growth rate after weaning in the animals feed with the basal diet, can be associated with inflammatory and immune responses caused by weaning stress, like: abrupt separation from the mother, relocation of new social groups and change to solid food (Kojima *et al.,* 2007). During this phase, some nutrients intended for growth and development are used by cells involved in those responses (Rodrígues *et al.*, 2007). Moreover, physiological responses to stress require complex responses from the central nervous, endocrine and immune systems that have an effect on animal health and well-being, res-

**Table 4.** Body temperature (°C) and weight gain (%PV)in weaning pigs without exposition to *E. coli* LPS for different post-weaning periods (weaning effect).

| Variables | Post-weaning period (day) | | | | SEM |
|---|---|---|---|---|---|
| | 1 | 5 | 7 | 10 | |
| Temperature | 38.6a | 38.9b | 38.7ab | 38.3c | 0.03 |
| Weight gain | 0.0a | -7.2b | -4.07c | 5.12d | 0.25 |

* In the same row, means with different letter are statistically different (P < 0.01). SEM*:* Standard error of the mean. %PV: percentage of live weight.

ponding to the environmental conditions and management (Davis *et al.*, 2006).

Immune system activation and posterior response to the different types of stress can affect some productive functions of the animal, like growth and development, muscular protein deposition and, nutrient metabolism (Williams *et al.*, 1997). Those changes in nutritional metabolism can create an important competence for nutrient utilization by different cell types, especially for amino acids (Le Floc'h *et al.*, 2009).

Fever occurrence in the animals of this study could be due to the effect of proinflammatory cytokines production, specifically TNF-α and the immune factors involved. These factors could be part of process that develops fever and sepsis (Liu *et al.*, 2008).

## Conclusion

Pig weaning is associated with multiple factors that generate stress in animals and favors early inflammatory responses. This causes inhibition in organ growth, reduction in productive efficiency (represented as weight gain) and high incidence of diarrhea during the post-weaning period.

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