TECHNICAL NOTE

Effect of Oral Administration of a Biopreparation Containing *Lactobacillus plantarum* CAM-6 on the Relative Weight of Digestive, Internal, and Immune Organs of Growing Pigs

César Augusto Betancourt Hurtado [*], Román Rodríguez Bertot [**], Yordan Martínez Aguilar [***]

*Faculty of Veterinary Medicine and Zootechnia, University of Cordova. Department of Livestock Sciences. Montería, Colombia. Carrera 6 No 76-103, 230002, Montería, Colombia.
[**] Center for Animal Production Studies Faculty of Agricultural Sciences, Granma, Granma, Cuba.
[***]Department of Agricultural Production, Agricultural Pan-American School of Zamorano, Honduras
Correspondence:ymartinez@zamorano.edu

Received: May 2020; Accepted: June 2020; Published: June 2020.

INTRODUCTION

The prohibition of growth promoting antibiotics in many countries increased the interest of researchers to find strategies to keep pig health and productivity, in addition to meeting consumer demands of healthy and safe meat (Ayala *et al.*, 2018). These restrictions urged researchers to seek novel alternatives, like probiotics, to replace antibiotics.

Moreover, the transition into weaning is one of the most critical moments of swine breeding, and this process may contribute to the dysfunction of the immune and intestinal systems (Campbell, Crenshaw, and Polo, 2013). Behind the occurrence of this event are alterations of the intestinal structure, changes in the relative weight of entrails and digestive organs, atrophy of villi, and crypt hyperplasia, which diminish the secretion of digestive enzymes, nutrient uptake, and therefore, growth retardation, and immunosuppression in pigs (Tsukahara *et al.*, 2015).

Lactobacillus plantarum was certified as one of the most prominent food probiotics, with beneficial effects to gastrointestinal health, and growth of pigs at weaning (Hou *et al*., 2015). The aim of this work is to evaluate the effect of oral administration of a biopreparation of *Lactobacillus plantarum* CAM-6, on the relative weight of digestive, internal, and immune organs of growing pigs.

How to cite (APA)
Betancur Hurtado, C., Rodríguez Bertot, R., & Martínez Aguilar, Y. (2020). Effect of Oral Administration of a Biopreparation Containing Lactobacillus plantarum CAM-6 on the Relative Weight of Digestive, Internal, and Immune Organs of Growing Pigs Journal of Animal Production, 32(2) //revistas.reduc.edu.cu/index.php/rpa/article/view/e3478

DEVELOPMENT

The experiment was conducted in the experimental area of the University of Cordoba, Colombia, upon approval by the Scientific and Veterinary Council of the institution, according to the Official Colombian Standard NOC Nº 001/2016, on animal welfare (Resolution No. 001, 26 January, 2016).

A number of 36 castrated pigs were used in the experiment [(Landrace x Pietrain) x Duroc], at 49 days of age, in the first stage, with a start weight of 10.37 ± 0.5 kg. The animals were distributed in a completely randomized experimental design, with 12 repetitions per treatment, in which every animal was an experimental unit. During the experiment, the pigs were lodged in collective pens measuring 4 x 2 m, on concrete flooring. Each pen was provided with a linear, canoe-type feeder made of PVC drainpipe, placed alongside the pen, with a metallic trough with nipple. The water and food were supplied *ad libitum.* The food was made from corn and soybean, to meet the 5-20 kg nutritional requirements. The diets were formulated according to the NRC (2012 (Table 1).

Table 1. Composition and contribution of experimental diets to growing pigs

Ingredients%	Start (5-20 kg)	Growth (20-50 kg)
Cornmeal	58.40	68.60
Soybean meal	29.78	24.00
Wheat bran	3.52	4.00
Plant oil	3.00	0.00
Methionine	0.22	0.00
Growing feed [1]	0.00	2.00
Startup feed[2]	2.50	0.00
Monocalcium phosphate	0.90	0.70
Calcium carbonate	1.28	0.40
Salt	0.40	0.30
Calculated nutritional contribution, dry base %		
Crude protein	19.0	17.0
Lysine	1.30	1.16
Methionine + Lysine	0.72	0.69
Tryptophan	0.22	0.21
Calcium	0.76	0.75
Total phosphorous	0.62	0.56
Metabolizable energy (kcal.kg^{-1})	3 285	3 180

[1]The pre-mix containing vitamins and minerals supplied per kilogram of food: 15,000 UI vitamin A; 3,750 UI vitamin D3; 28 UI vitamin E; 18 mg vitamin K; 2.5 mg thiamine; 5.5 mg riboflavin; 5.5 mg pyridoxine; 0.03 mg vitamin B12; 28 mg niacin; 23 mg Ca-panthotenate; 400 mg folic acid; 0.03 mg biotin; 22 mg Cu (as copper sulfate); 20 mg Zn (as zinc oxide); 90 mg Mn (as manganese oxide); 0.4 mg I (as potassium iodine); 0.4 mg Co (as $Co2O_3$-$7H_2O$); 0,12 mg Se (as Na_2SeO_3-$5H_2O$). [2]The premix of vitamins and minerals supplied per kilogram of food: 20,000 UI vitamin A; 4,000 UI vitamin D3; 80 UI vitamin E; 16 mg vitamin K; 4 mg thiamine; 20 mg riboflavin; 6 mg pyridoxine; 0.08 mg vitamin B12; 120 mg niacin; 50 mg Ca-panthotenate; 2

mg folic acid; 0.08 mg biotin; 15 mg Cu (as copper sulfate); 56 mg Zn (as zinc oxide); 73 mg Mn (as manganese oxide); 0.3 mg I (as potassium iodine); 0.5 mg Co (as $Co2O_3$-$7H_2O$); 0,4 mg Se (as Na_2SeO_3-$5H_2O$).

The probiotic biopreparation was produced in the biotechnology laboratory at the University of Cordoba. The treatments consisted in: control group with a diet based on the commercial feed without antibiotics or additives (T0); group treated with ciprofloxacin (250 mg/kg) in the feedstuff (T1); and group treated with 5 mL of the microbial biopreparation (10^9 UFC.mL^{-1} *L. plantarum* CAM-6), per animal (T2). The suspension was applied orally using a syringe, one hour prior to the administration of the commercial feedstuff without antibiotics, for 90 days throughout the experiment.

At the end of the experimental stage (139 days of age), four pigs were randomly chosen per treatment (each animal is an experimental unit), and fasted for 12 h, only consuming water *ad libitum*. Finally, they were weighed. The animals were sedated with tranquilizer Xylazina (Rompum®), with a 5 mL/50 kg live weight, intramuscularly. Then they were sacrificed by bleeding through the jugular vein, at the experimental slaughterhouse of the Faculty of Veterinary Medicine, University of Cordoba, Berastegui venue.

Following sacrifice, the organs were removed and placed on sterile trays, and the entrails were weighed (stomach, liver, gall bladder, heart, and kidneys), and the spleen, as immune organ. The removal of intestines was done by cutting the mesenteron first. Then the ases were stretched, and the cecum was separated. Later, the small intestine (SI), and the large intestine (LI) were separated as well. The length of the SI was determined with a measure tape, and theweight of SI, LI, and empty cecum were weighed on a digital scale (OSBORNE®, model 37473®, with ± 0.1 g precision). To calculate the relative weight of organs, organ weight was divided by the final weight of each animal at sacrifice.

The probiotic biopreparation significantly increased ($p<0.05$) the relative weights of the cecum, liver, and spleen, as well as the length of the cecum. Additionally, the antibiotic reduced the relative weight of the stomach, liver, and spleen ($p<0.05$). Other measurements showed no significant differences between the treatments ($P>0.05$) (Table 2).

Table 2. Effect of a biopreparation of *Lactobacillus plantarum* CAM-6 on the relative weight of digestive organs and morphometry of SI of growing pigs.

Indicators	T0	T1	T2	SE±	W
Stomach %	1.32[a]	1.07[b]	1.26[a]	0.08	0.045
Gall bladder %	0.89	0.91	0.91	0.01	0.921
Small intestine, %	2.51	2.80	2.55	0.19	0.181
Large intestine, %	3.82	4.10	3.95	0.11	0.247
Cecum, %	0.28[b]	0.27[b]	0.35[a]	0.01	0.026
Length of SI, cm	16.51[b]	17.58[a]	17.24[a]	0.26	0.045
Kidney, %	0.185	0.198	0.185	0.011	0.330

Heart, %	0.361	0.350	0.366	0.018	0.245
Liver, %	1.85[ab]	1.75[b]	1.99[a]	0.094	0.012
Pancreas, %	0.297	0.326	0.320	0.028	0.527
Spleen, %	0.174[b]	0.143[c]	0.197[a]	0.005	0.027

[a,b] Means with unequal letters on the same row differ for P<0.05 (Duncan, 1955) SI: small intestine.

The elongation of the SI in treatments T1 and T2 may have been associated to a larger absorption surface of nutrient uptake (Ly *et al*., 2014), thus the reduction of metabolites or toxic substances that modify the intestinal morphology can improve the proliferation of epithelial cells (Ayala *et al*. (2018). Also, Hou *et al*. (2015) reported that *L. plantarum* raises intestinal activity and morphometry through growth inhibition of opportunistic pathogens, and an increase in the height of villi, enzymatic activity, transport, and nutrient uptake in the intestinal epithelium, which benefits the usage pattern of the diet.

Furthermore, the size of cecum is associated to the diet and cellular and microbial metabolic processes that take place there. Hence, probiotics, especially those based on *Lactobacillus* spp., which are stimulated by high lactose levels, can colonize the cecal epithelium, and use their peculiar metabolism to produce short-chain fatty acids (SCFA), which stimulate the proliferation of the intestinal epithelium, and influence the elongation of that organ.

The the relative weight of the spleen demonstrates that probiotics can increase the immunologic activity of pigs. According to Ayala *et al*. (2008), a greater activity of this hematopoietic organ influences on IgM immunoglobulin production due to the input of antigens filtrated from the blood torrent and the production of opsonins, which are important in bacterial phagocytosis. These authors reported similar results when a probiotic mix (*L. acidophilus* and *L. rhamnosus*) was compared to a growth promoting antibiotic. It proves that probiotics can increase pig immunity, contrary to growth promoting antibiotics (GPA), which reduced the relative weight of the spleen.

CONCLUSIONS

The oral administration of a biopreparation containing *Lactobacillus plantarum* CAM-6 increased the relative weight of cecum and spleen, as well as the length of the SI, without changing the relative weight of other immune organs and entrails of growing pigs.

REFERENCES

Ayala, D.I., Chen, J.C., Bugarel, M., Loneragan, G.H., den Bakker, H.C., Kottapalli, K. R., & Nightingale, K.K. (2018). Molecular detection and quantification of viable probiotic strains in animal feedstuffs using the commercial direct fed microbial *Lactobacillusanimalis* NP51

as a model. *Journal of Microbiological Methods*, *149*, 36-43. DOI: https://10.1016/j.mimet.2018.04.012

Campbell, J. M., Crenshaw, J. D., & Polo, J. (2013). The biological stress of early weaned piglets. *Journal of Animal Science and Biotechnology*, *4*, 19. DOI: https://10.1186/2049-1891-4-19

Hou, Ch., Zeng, X., Yang, F., Liu, H, & Hou, Sh.Q. (2015) Study and use of the probiotic *Lactobacillusreuteri* in pigs: a review. *Journal of Animal Science and Biotechnology*, *14*, 15-14. DOI: https://10.1186/s40104-015-0014-3

Ly, J., Almaguel, R., Ayala, L., Lezcano, P., Romero, A. & Delgado, E. 2014. Digestibilidad rectal y ambiente gastrointestinal de cerdos jóvenes alimentados con dietas de levadura torula. Influencia de la fuente de carbohidratos. *Revista Computadorizada de Producción Porcina*, *21*(3), 134-139. http://www.iip.co.cu/rcpp/213/213_artJLy.pdf

Tsukahara, T., Inoue, R., Nakatani, M., Fukuta, K., Kishino, E., Ito, T. (2015). Influence of weaning age on the villous height and disaccharidase activities in the porcine small intestine. *Animal Science Journal*, *87*(1). DOI: https://10.1111/asj.12399

AUTHOR CONTRIBUTION

Conception and design of research: CABH, RRB, YMA; data analysis and interpretation: CABH, RRB, YMA; redaction of the manuscript: CABH, RRB, YMA.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.