Probiotic effect of *Lactobacillus salivarius* C 65 on productive and health indicators of lactating piglets

Ana J. Rondón[1], Yurién Ojito[1], Fátima G. Arteaga[2], Marta Laurencio[1], Grethel Milián[1] and Y. Pérez[1]

[1]*Centro de Estudios Biotecnológicos, Universidad de Matanzas "Camilo Cienfuegos". Autopista a Varadero, km 3 1/2. Matanzas. Cuba*

[2]*Escuela Superior Politécnica Agropecuaria de Manabí "Manuel Félix". Calceta, Manabí, Ecuador*

Email: ana.rondon@umcc.cu

For evaluating the probiotic effect of a biopreparation with *Lactobacillus salivarius* C65 on productive and health indicators in lactating piglets, an experiment was realized with a completely randomized design and two treatments: basic diet (control) and basic diet + C65 biopreparation. Ten piglet litters of the Yorkshire – Landrace commercial cross x L35 were used from the first day of birth until 35 d of age. As a result from the utilization of this biopreparation, live weight (9.46 kg) of the animals treated with the probiotic improved ($P \leq 0.05$) regarding the control group (8.02 kg) at five weeks. Also there was a better weight increase and daily live weight gain. There was also a decrease in diarrhea incidence. It is confirmed the probiotic potential that this biopreparation has for creating beneficial effects on lactating piglet yield.

Key words: *probiotic, Lactobacillus salivarius, lactating piglets, nutritional additives*

Farm animals are very susceptible to enteric bacterial unbalances in the digestive tract, which leads to an insufficient nutrient conversion and growth delay (Armstrong 1986). To counteract these difficulties, diets were supplemented for years with antibiotics which are effective for decreasing diarrheas and as animal growth promoters (Weber *et al*. 2001). However, its discriminate use brings about the development of resistant pathogen strains to these anti-microbial. For that reason there is great interest in replacing these additives by others more natural that provoke less negative effects, as the probiotics (Callaway *et al*. 2003 and Liu *et al*. 2008).

Fuller (1989), González *et al*. (2003) and Lata *et al*. (2006) considered that probiotics are nutritional complements constituted by microorganisms alive that when ingested in adequate amounts colonized and modified the microbiota of the digestive tract, provoking a positive effect on health and on host physiology. Among the microorganisms mostly used as probiotics are those of Lactobacillus genus. They actively participate in the fermentative processes, with inhibitory activity before pathogen microorganisms, neutralizing enterotoxins, synthesizing vitamins and stimulating immunological response besides improving mineral absorption (Jacela *et al*. 2010).

Pig rearing in our country constitutes one of the most important lines of the economy. This species, as no other one, has differentiating characteristics which makes it preferential for many producers. It outstands by the heterogeneity of its diet, good conversion, adaptability and high proliferation, as well as high carcass yield, formed by representative levels of proteins and lipids (Fernández 2000).

The most critical stage in intensive pig rearing is lactation, due to unbalances produced in the gastrointestinal microbiota which bring about impairments in the productive yield and with that, the reduction of the expression of its genetic potential and the quality of the final product. These damages have been tried to be surpassed with the use of antibiotics. However, this practice can propitiate problems of microbial resistance and increase of the production costs. The objective of this study was to assess the probiotic activity of a biopreparation with *Lactobacillus salivarius* C65 on productive and health indicators of lactating piglets.

Materials and Methods

Production of the bacterial biopreparation. From *Lactobacillus salivarius* C65, 1 L of the biopreparation was produced according to the methodology described by Rondón (2009). Later, the viable counting was made through the method of serial dilutions, in an inoculation relationship of 1:10 (v/v) in peptone water (OXOID), from 10^{-1} to 10^{-12}. The three last dilutions were individually inoculated (1 mL) deeply in plates with MRS agar (De Mann *et al*. 1960). This procedure was replicated three times and plates were incubated at 37 ºC under anaerobiosis conditions for 48 h. The number of colony forming units (cfu) was determined with a magnifying glass, by visual counting of colonies. This biopreparation was preserved at 5º C until its utilization.

Treatments and experimental conditions. The research was developed at the "Gelpis" Integral Pig Center, belonging to the "Matanzas" farm from the Agricultural Enterprise of FAR. A completely randomized design was applied with the inclusion of two treatments: control in which only feed was supplied and that corresponding to the application of the biopreparation at a dosage of

10^9 cfu/kg. The experiment began when the piglets were eight days old, when they started the intake of small portions of the concentrate.

For the design of the experiment ten litters of the Yorkshire – Landrace commercial cross x L35 were used from the first day of birth until 35 d of age, offspring from second farrowing sows. Litters were adjusted to a same size (10 piglets), with two treatments and five litters (50 piglets or replications) each one: the control and that treated with *Lactobacillus salivarius*. The dosage of the probiotic biopreparation was of 5 mL (10^9 cfu/mL) per kilogram of feed, which was supplied mixed with the concentrate until the end of the experiment. The probiotic had an initial concentration of 10^{11} cfu/mL, though it was diluted in water (1:3, v/v of biopreparation and water) before using it for increasing the volume, attaining greater homogenization and facilitating its application in the concentrate. The litters of each treatment were placed distant one from the others for avoiding the self-inoculation. Five samplings were carried out (7, 14, 21, 28 and 35 d of birth).

Diet. From the eighth day, besides the colostrum and maternal milk, a starter diet was supplied to all piglets until the end of the experiment (160 g/animal/d). The concentrate was distributed in each occasion through the biopreparation. Table 1 shows the raw material and the percentage of inclusion utilized for the formulation of the concentrate and its bromatological composition.

Determination of the effect of the biopreparation on productive and health indicators. The productive indicators as final weight were registered weekly. Weight increase and average daily gain of the piglets were calculated for the lactation stage, according to the specifications of the technical instructions (Anon 1998). Mortality and diarrhea incidence were the health indicators evaluated from daily observation of the animals.

Statistical analysis. Results were processed with the INFOSTAT system, version 1, (Balzarini *et al*. 2001) through an analysis of variance of simple classification. In the necessary cases Duncan's (1955) test was used for mean comparisons.

Table 1. Formulation of the concentrate

Raw material	Inclusión, %
Wheat meal	70.36
Soybean meal	27.00
NaCl	0.50
Calcium carbonate	0.50
Vitamin and mineral premix[1]	1.00
Choline	0.14
Dicalcium phosphate	1.00
Calculated analysis	
DM (%)	90.81
CP (%)	19.00
ME (MJ/kg)	18.97
Ca (%)	0.61
P (%)	0.49
Ash (%)	4.55

[1]Vitamin and mineral premix per kg concentrate: vitamin A 12,000 IU, vitamin D 2,600, vitamin E 30 IU, vitamin B12 12 µg, vitamin K 3 mg, D-calcium pantothenate 15 mg, nicotinic acid 40 mg, choline, 400 mg, Mn 40 mg, Zn 100 mg, Fe 90 mg, Cu 8.8 mg, I, 0.35 mg, Se 0.3 mg

Results and Discussion

The biopreparation was produced and in the viable counting it was found a concentration of 11 Log cfu/mL. These results coincide with the values attained by Rondón (2009) for obtaining this biopreparation which demonstrates that the additive shows repeatability under the same conditions.

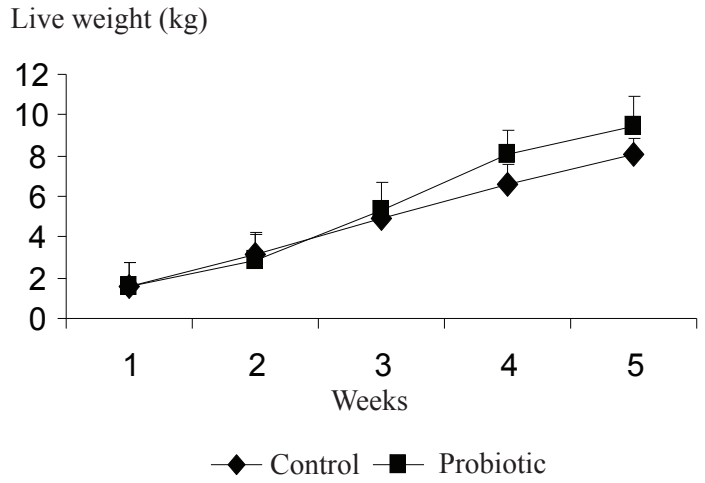
In figure 1 is shown that with the use of *Lactobacillus salivarius* C65, live weight of the animals treated with the biopreparation increased regarding the control group. From 21 d, weight of piglets receiving the biopreparation increased ($P < 0.05$) respecting the control group. At 28 and 35 d the differences ($P < 0.05$) were more evident. It was even more evident at 35 d, when weaning took place.

With these results it is demonstrated that *L. salivarius* biopreparation influenced on the live weight increase of the piglets. It is known that the microorganisms residing in the gastrointestinal tract interact with the host animal. This microbiota varies with the animal species, the site of digestive system where it resides, the age, the diet consumed and the environment. Healthy animals maintain a balanced microbial population, which is in agreement with the eubiosic state of the gastrointestinal ecosystem. This condition is closely related to the productivity and health of the animals (Yeo and Kim 1997 and Patterson and Burkholder 2003).

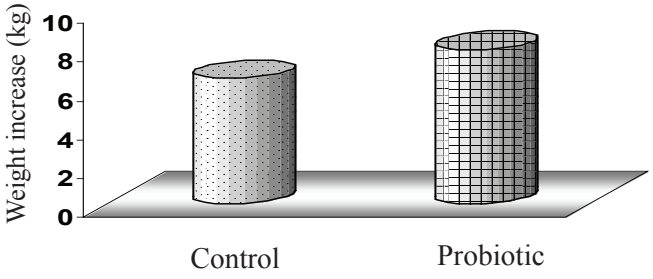
In this study the *L. salivarius* C65 strain, selected by Rondón *et al*. (2008) was utilized since it showed *in vitro* probiotic potential and provoking *in vivo* improvements in the productive and health indicators of broilers (Rondón 2009). Nonetheless, throughout the development of this experiment it was demonstrated that it causes positive effects on lactating piglets. In the literature it is reported that *Lactobacillus salivarius* are normally found in the microbial population residing in the digestive tract of all animals of agricultural interest, as pigs (Nemcova *et al*. 1997 and Korhonen *et al*. 2007), calves (Schneider *et al*. 2000) and ducks (Ehrmann *et al*. 2002).

In figure 2 are shown the results from the weight increase of piglets during the lactation stage. With the application of the biopreparation, there was higher weight increase ($P < 0.05$) in the group of animals treated with *Lactobacillus salivarius* C65 regarding the control.

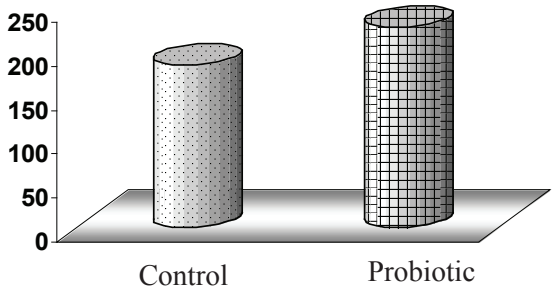
The assessment of average daily gain demonstrated the probiotic effect of the biopreparation (figure 3), since



Points in the line differ at $P < 0.05$ (SE ± 0.77) (Duncan 1955)
Figure 1. Effect of the probiotic activity of *Lactobacillus salivarius* C65 on the live weight performance of lactating piglets during the first five weeks of life



Columns differ at $P < 0.05$ (SE± 0.67) (Duncan 1955)
Figure 2. Weight increase of piglets during the lactation stage



Columns with different values at $P < 0.05$ (SE± 0.007) (Duncan 1955)
Figure 3. Average daily gain of piglets

there were differences ($P < 0.05$) between the control group and that treated with probiotics. In this latter an increase of the results was found.

These results demonstrate the additive effect in the digestive physiology of the animals, since it is evident a better use of the nutritive ingredients of the diet. Giang *et al*. (2011) stated that on supplying a probiotic complex (*E. faecium*, 3 x 10^{11} cfu/kg, *L. acidophilus*, 4 x 10^{9} cfu/kg and *L. plantarum*, 2 x 10^{9} cfu/kg) in the concentrates to piglets obtained better results in daily weight gain and better conversion during the first and second week after weaning.

In Cuba, Brizuela (2003) and Rondón (2009) formulated biopreparations with Lactobacillus achieving positive effects on the productive indicators of pigs and poultry, respectively. It is known that lactobacilli released enzymes that improve the digestive capacity of the animals, inactivate efficiently the toxic metabolites of the harmful biota and increase the absorption process due to a better hairiness cell status. Also, they provoke greater vitamin synthesis and inhibit enteropathogens due to the increase of secretion of bacteriostatic and bactericide substances as the bacteriocins (Segura y De Bloss 2000). Results obtained with the application of *L. salivarius* confirm the effect that this microorganism has on the intestinal microbiota of these animals.

More and more support is obtaining the use of probiotics for intensive pig rearing in view of their large diversity of advantages (Guarner 2007). In studies of Nazef *et al*. (2008) it was demonstrated that *Lactobacillus salivarius* can eliminate harmful bacteria, due to its effect on the gastrointestinal tract, as the pH decrease, formation of aggregates with other bacteria that are eliminated, competition for proteins and other feeds and bacteriocin production.

The positive effect of probiotics on the gastrointestinal tract makes to decrease the development of pathogen bacteria, which contributes to the intestinal microorganism balance. This fact improves the digestive processes of the host, which is evidenced in the increase of live weight increase and the decrease of feed conversion of animals consuming these formulations (Simon *et al*. 2003 and Nazef *et al*. 2008).

These results coincide with those reported by Marín *et al*. (2007), who conducted two experiments for studying the possible probiotic effect of a cream of protein biomass obtained by simplified way with a mixed culture of yeasts and lactic bacteria.

The biopreparation with *Lactobacillus acidophilus* was supplied orally or in the feed to lactating piglet litters, improving weight increase and daily mean weight. Also diarrhea incidence diminished and there were no deaths due to digestive upsets.

These results can be attributed to the function played by lactic acid bacteria in the gastrointestinal tract of young pigs, since the lactic acid derived from lactobacilli supply the necessary acidity for the digestive processes. It is known that until the third or fourth week of life hydrochloric acid secretion is not intensified. Stomach acidity in piglets contributes to pathogen germ control and to the optimum acidity for pepsine activity that must be between pH 2 and 4 (Mejía *et al*. 2007). Lactobacilli, also, increase amino acid availability and improve the efficiency in energy utilization and other diet components, as the fiber (Mroz *et al*. 2000). Another activity that must have exerted its influence in the gastrointestinal tract of piglets is the Lactobacillus adherence to the intestinal mucosa, since it supplies an eubiotic environment. Iñiguez *et al*. (2011) confirmed that *Lactobacillus salivarius* strains are adhered to the carbohydrates of the intestinal mucosa through proteins called lectines, present at the bacteria surface. This characteristic contributes to mucosa protection and to the stimulation of immunological activity (Guerin *et al*. 2001 and Zhang *et al*. 2011).

Gamuza (2012) stated that the presence of *L. salivarius* in the small intestine could assure protein absorption, what makes more efficient the digestive capacity. This effect propitiates the increase of protein availability, bringing to the organism the necessary elements for producing hormones and enzymes, maintaining the nutritive integrity and, in turn, improves the productive pig yield.

There are evidences that on using probiotics, mainly Lactobacillus strains, as monocultures or mixtures, retention of nutrients included in the diet are increased, especially by N, P and Ca assimilation (Nahashon *et al*. 1994, Schneitz *et al*. 1998 and Ángel *et al*. 2005).

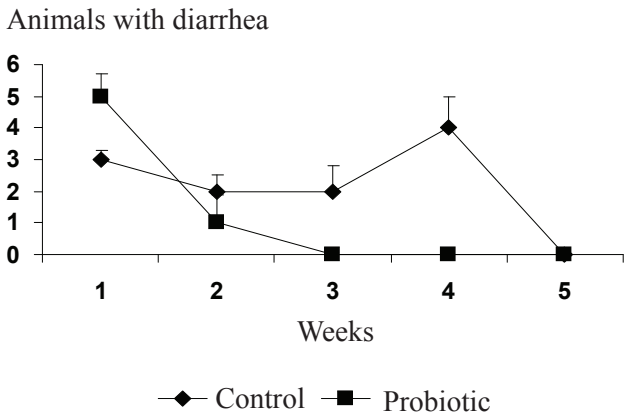
In figure 4 are shown the results of the probiotic activity of *L. salivarius* on the health indicators of lactating piglets. During the experiment diverse observations were carried out, among them the amount of animals with diarrhea presence. Favorable results ($P < 0.05$) were obtained from the first until the fifth week in the treated group with *L. salivarius*. This presented sick piglets, mainly during the first seven days, when animals have not still started consumption of the biopreparation, while in the control group there were a higher number of piglets with diarrhea, in a repetitive way, during the first 28 d. Regarding mortality there were no deaths in any of the treatments.

Results obtained agree with Cajarville *et al*. (2011) who indicated that the application of probiotics in pig exploitations contributes to a considerable reduction of gastrointestinal disorders, lower medicament expenses, especially antibiotics, decrease of mortality due to diarrhea, better feed conversion and reduction of the fattening period.

These results indicate that the supplemented lactobacilli could contribute to the decrease of pathogen microorganisms. It is known that these produce antimicrobial substances, as organic acids, hydrogen peroxide and bacteriocins (Ma *et al*. 2004). Riboulet-Bisson *et al*. (2012) studied the effect of the bacteriocin Abp produced by *Lactobacillus salivarius* on the intestinal microbiota of mice and pigs. These authors confirmed that this bacteriocin inhibits the development of different Gram-negative bacteria as *E. coli*. Segura and De Bloss (2000) found that lactic acid bacteria (LAB) fermented the carbohydrates from the diet and produced high levels of lactic and acetic acids that inhibited the growth of *E. coli*, *Salmonella thyphimurium* and *Clostridium perfringens*. This brought about benefits in weight increase, on decreasing the incidence of diarrheic diseases.

Piper *et al*. (2006) reported that the species *Lactobacillus salivarius*, *L. fermentum* and *L. acidophilus* are the most abundant lactobacilli of the microbial community of the pigs' ileum during the weaning period. Fuller (1989) stated that if animals are supplied with autochthonous strains of the gastrointestinal tract through the use of probiotics from the first birth hours, these bacteria will colonize the intestinal mucosa and will protect in a natural way against the growth of other microorganisms, especially those which are harmful or undesirable.

When probiotic microorganisms as lactobacilli, proliferate in the gastrointestinal tract, the production of organic acids becomes accentuated, followed by pH decrease. These conditions provoke the increase of the



Points in the line differ at $P < 0.05$ (SE± 0.88) (Duncan 1955)

Figure 4. Performance of the incidence of animals with diarrhea throughout the experiment.

enzymatic and absorptive activity by the host, as well as the potential control of enteropathogens. The lumen acidification also propitiates the dissipating effect of minerals, with its consequent bioavailability and higher nutritional contribution (Nomoto 2005).

Tsai *et al*. (2005) and Pérez *et al*. (2011) confirmed that LAP 5 and LF33 strains, isolated from pigs and chickens, respectively, were capable of inhibiting *in vitro Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus cereus*, mainly by the production of lactic acid. Higgins *et al*. (2007) and Nazef *et al*. (2008) indicated that lactic acid bacteria are high producers of organic acids that decrease pH of the intestine and prevent the colonization of undesirable bacteria that do not proliferate before this effect and cause the stimulation of the immunological system.

Casey *et al*. (2007) stated that the supply of a mixture of *Lactobacillus murinus*, *Lactobacillus salivarius*, *Lactobacillus pentosus* and *Pediococcus pentosaceous* strains to weaned piglets brought about lower incidence, severity and duration of diarrheas, when a *Salmonella typhimurium* strain was supplied orally.

The end products of fermentation, as lactic acid and volatile fatty acids (VFA), mainly the acetic, propionic and butyric, provoke intestinal pH decrease, and by this mechanism the growth of pathogen bacteria is inhibited (McDonald *et al*. 2006 and Seifert and Watzl 2007). In the literature is reported that with the utilization of probiotics the production of VFA, especially butyrate is increased. This acid constitutes the main energy source for the colonocites. Roberfroid *et al.* (2007) indicated that its increase is the key of the positive effects on the intestinal functioning and health. According to the studies carried out, it was confirmed that *Lactobacillus salivarius* C65 contributed to the improvement of the productive and health indicators in the group of piglets treated with the biopreparation. These results corroborate the probiotic potential of this additive, on provoking beneficial effects on lactating pig yield.

References

Ángel, R., Dalloul, R.A. & Doerr, J. 2005. Metabolism and nutrition: Performance of broiler chickens fed diets supplemented with a direct-fed microbial. Poult. Sci. 84:1222

Anon 1998. Producción Avícola. Pollos de engorde. Tecnología de crianza y regulaciones sanitarias generales. Intructivo Técnico. Subdirección técnica, UECAN-IJA, La Habana

Armstrong, D.G. 1986. Gut active growth promoters. Control and manipulation of animal growth. Butterworths, London. p. 2-37

Balzarini, M.G., Casanoves, F., Di Rienzo, J.A., González, L.A. & Robledo, C.W. 2001. INFOSTAT, Versión 1. Córdoba, Argentina

Brizuela, M.A. 2003. Selección de cepas de bacterias ácido lácticas para la obtención de un preparado con propiedades probióticas y su evaluación en cerdos. PhD Thesis. Instituto de Ciencia Animal. La Habana, Cuba.

Cajarville, C., Brambillasca, S. & Zunino, P. 2011. Utilización de probióticos en monogástricos: aspectos fisiológicos y productivos relacionados al uso de sub-productos de agroindustrias y de pasturas en lechones. Rev. Porcicultura Iberoamericana 1: 2

Callaway, T.R., Anderson, R.C., Edrington, T.S., Elder, R.O., Genovese, K.J., Bischoff, K.M., Poole, T.L., Jung, Y.S., Harvey, R.B. & Nisbet, D.J. 2003. Preslaughter intervention strategies to reduce food-borne pathogens in food animals. J. Anim. Sci. 81:17

Casey, P., Gardiner, G., Casey, G., Bradshaw, B., Lawlor, P., Lynch, P., Leonard, F., Stanton, C., Ross, R., Fitzgerald, G. & Hill, C. 2007. A fivestrain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs cha lenged with *Salmonella enterica* serovar Typhimurium. Appl. Environ. Microbiol. 73: 1858

De Mann, J.C., Rogosa, M. & Sharpe, M.E. 1960. A medium for the cultivation of lactobacilli. J. Bacteriol. 23:130

Duncan, B. 1955. Multiple ranges and multiple F. Test Biometrics 11:1

Ehrmann, M.A., Kurzak, P., Bauer, J. & Vogel, R.F. 2002. Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. J. Appl. Microbiol. 92:966

Fernández, R. 2000. Necesidades nutricionales del lechón.

Porcino Aula Veterinaria 55:39

Fuller, R. 1989. Probiotics in man and animal. J. Appl. Bacteriol. 66:365

Ganuza, P. 2012. Suplementos Probioticos del R – jardín. Available:< http://www.wholeearthhealth.com/enzymes.> [Consulted: 10/04/12]

Giang, H., Vietl, T., Ogle, B. & Lindberg, J. 2011. Effects of Supplementation of Probiotics on the Performance, Nutrient Digestibility and Faecal Microflora in Growing-finishing Pigs. Asian-Aust. J. Anim. Sci. 24: 655

González, B., Gómez, M. & Jiménez, Z. 2003. Bacteriocinas de probióticos. Rev. Salud Pública y Nutrición. 4:1

Guarner, F. 2007. Papel de la flora intestinal en la salud y en la enfermedad. Nutri. Hosp. 22 :14

Guerin, C., Meslin, J., Chambard, A., Charpiliense, A., Relano, P., Bouley, C., Cohen, J. & Andrieux, C. 2001. Food supplementation with milk fermented by *Lactobacillus casei* DN - 114 001 protects suckling rats from rotavirus associated diarrhea. J. Nutr., 131: 111

Higgins, S.E., Erf, G.F., Higgins, J.P., Henderson, S.N., Wolfenden, A.D., Gaona-Ramirez, G. & Hargis, B.M. 2007. Efecto del tratamiento prebiótico en pollos de engorde sobre el número de macrófagos intestinales y la fagocitosis de *Salmonella enteritidis* por células abdominales exudadas. Poult. Sci. 86: 2322

Iñiguez, C., Jiménez, R., Vázquez, L., Ramos, G. & Acedo, E. 2011. Proteincarbohydrate interactions between *Lactobacillus salivarius* and pig mucins. J. Nutr. 132:110

Jacela, Y.J., De Rouchey, J.M., Tokach, M.D., Goodband, R.D., Nelssen, J.M., Renter, D. G. & Dritz, S. S. 2010. Feed aditives for swine: fact sheets-prebiotics. J. Swine Health Prod. 18: 132

Korhonen, J.M., Sclivagnotis, Y. & von Wright, A. 2007. Characterization of dominant cultivable lactobacilli and their antibiotic resistance profiles from faecal samples of weaning piglets. J. Appl. Microbiol. 103:2496

Lata, J., Jurankova, J., Doubek, J., Pribramska, V., Fric, P., Dite, P., Kolar, M., Scheer, P., & Kosakova, D. 2006. Labelling and content evaluation of commercial veterinary probiotics. Acta Veterinaria. BRNO 75: 139

Liu, P., Piao, X.S., Kim, S.W., Wang, L., Shen, Y.B., Lee, H.S. & Li, S.Y. 2008. Effects of oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of *Escherichia coli* and Lactobacillus in weaning pigs. J. Anim. 86: 2609

Ma, Y.L., Xu, Z.R. & You, P. 2004. Adhesion of some bacteria to broiler intestinal mucus. ACTA Microbiol. Clín. 44:361

Marín, A., García, A., Marrero, L., Manso, M. J. & González, M. 2007. Estudio del efecto en lechones lactantes del probiótico de biomasa proteica obtenida por la tecnología de cultivo de Lactobacillus y levaduras en miel B. Livestock Res. Rural Devel. 19:195

McDonald, P., Edwards, R.A., Greenhalgh, J.F. & Morgan, C.A. 2006. Nutrición animal. 6ta. Ed. Zaragoza. Ed. Acribia S.A. p. 58

Mejía, W., Rubio, J., Calatayud, D., Rodríguez, A. & Quintero, A. 2007. Evaluación de dos probióticos sobre parámetros productivos en lechones lactantes. Zootécnia Trop. 25:301

Mroz, Z., Jongbloed, A., Partanen, K., Vreman, K., Kemme, P. & Kogut, J. 2000. The effects of calcium benzoate in diets with or without organic acids on dietary buffering capacity, apparent digestibility, retention of nutrients, and manure characteristics in swine. J. Anim. Sci. 78:2622

Nahashon, S.N., Nakaue, H.S. & Mirosh, L.W. 1994. Production variables and nutrient retention in single comb white leghorn laying pullets fed diets supplemented with direct fed microbials. Poult. Sci. 73: 1699

Nazef, L., Belguesmia, Y., Tani, A., Prévost, H. & Drider, D. 2008. Identification of lactic acid bacteria from poultry feces: evidence on anti-Campylobacter and anti-Listeria activities. Poult. Sci. 87: 329

Nemcova, R., Laukova, A., Gancarcikova, S. & Kastel, R. 1997. *In vitro* studies of porcine lactobacilli for possible use. Berl. Munch. Tieraztl. Wschr. 110:413

Nomoto, K. 2005. Prevention of infection by probiotics. J. BioSci. Bioengineering 100:583

Patterson, J.A. & Burkholder, K.M. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82: 627

Pérez, M., Laurencio, M., Rondón, A.J., Milian, G., Bocourt, R. & Arteaga, F. 2011. Actividad antimicrobiana de una mezcla probiótica de exclusión competitiva y su estabilidad en el tiempo. Rev. Salud Animal 33:147

Pieper, R., Janczyk, P., Schumann, R. & Souffrant, W. B. 2006. The intestinal microflora of piglets around weaning – with emphasis on lactobacilli. Arch. Zootech. 9: 29

Riboulet-Bisson, E., Sturme, M.H.J., Jeffery, I.B., O'Donnell, M.M., Neville, B.A., Forde, B.M., Claesson, M.J., Harris, H., Gardiner, G.E., Casey, P.G., Lawlor, P.G., O'Toole, P.W. & Ross, R.P. 2012. Effect of *Lactobacillus salivarius* bacteriocin Abp118 on the mouse and pig intestinal microbiota. Plos One. 7: 31113

Roberfroid, M. B., Mäkeläinen, H., Saarinen, M., Stowell, J., Rautonen, N. & Ouwehand, A. C. 2007. Xylo-oligosaccharides and lactitol promote the growth of *Bifidobacterium lactis* and Lactobacillus species in pure culture. Beneficial Microbes. p. 139

Rondón, A.J. 2009. Obtención de biopreparados a partir de lactobacilos autóctonos del tracto digestivo de pollos y evaluación integral de las respuestas de tipo probióticas provocadas en estos animales. PhD Thesis. Instituto de Ciencia Animal. La Habana. Cuba

Rondón, A.J., Samaniego, L.M., Bocourt, R., Rodríguez, S., Milián, G., Ranilla, M.J., Laurencio, M. & Pérez, M. 2008. Isolation, identification and partial characterization of the probiotic properties of *Lactobacillus spp*. strains obtained from the gastrointestinal tract of broilers. Cienc. Tecnol. Aliment. 6:17

Schneider, R., Rosmini, M., Eherman, M. & Vogel, R. 2000. Aplicación de técnicas de RAPD y análisis del 16S rDNA para la identificación de bacterias lácticas componentes de la microbiota aislada de terneros criados en condiciones artificiales. VI Congreso Latinoamericano de Microbiología de los Alimentos. Buenos Aires. Argentina. p.75

Schneitz, C., Kiskinen, T., Toivonen, V. & Nasi. M. 1998. Effect of BROILAC on the physiochemical conditions and nutrient digestibility in the gastrointestinal tract of broilers. Poult. Sci. 77:426

Segura, A. & De Bloss, M. 2000. La alternativa a los promotores del crecimiento. III Congreso Nacional de Avicultura. Memorias. Centro de Convenciones Plaza América. Varadero. Cuba. p. 37

Seifert, S. & Watzl, B. 2007. Inulin and oligofructose: review of experimental data on immune modulation. J. Nutr. 137: 2563S

Simon, O., Vahjen, W. & Scharek, L. 2003. Microorganisms as

feed additives. 9th International Symposium on Digestive Physiology in Pigs.1:295

Tsai, C.C., Hsih, H.Y., Chiu, H.H., Lai, Y.Y., Liu, J.H., Yu, B. & Tsen, H.Y. 2005. Antagonistic activity against Salmonella infection *in vitro* and *in vivo* for two Lactobacillus strains from swine and poultry. Intern. J. Food Microbiol. 102: 185

Weber, T.E., Schinckel, A.P., Houseknecht, K.L. & Richert, B.T. 2001. Evaluation of conjugated linoleic acid and dietary antibiotics as growth promotants in weanling pigs. J. 79: 2542

Yeo, J. & Kim, K. 1997. Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. Poult. Sci. 76: 381

Zhang, J., Deng, J., Wang, Z., Che, C., Li, Y. & Yang, Q. 2011. Modulatory effects of *Lactobacillus salivarius* on intestinal mucosal immunity of piglets. Current Microbiology 62:1623

Received: April 24, 2013