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Effects of Additives PROBIOLACTIL® and IHPLUS® on Suckling Piglets

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Abstract

Context: Lactation is one on the most critical stages of swine breeding due to the frequent presence of diseases.
Aim: To evaluate the probiotic effect of biopreparations PROBIOLACTIL® and IHPLUS® on suckling piglets.
Method: The study relied on a completely randomized experimental design, which included three treatments: I. Basal diet (Control); II Basal diet + PROBIOLACTIL®; III. Basal diet + IHPlus®. Productive indicators like live weight, weight gain, mean daily gain, food conversion, and health indicators were evaluated.
Results: The additives improved all the indicators evaluated and brought about benefits ($P<0.05$) in live weight (6.88, 7.63, 7.77 kg); mean daily gain (0.207, 0.281, 0.299 g); weight gain (1.06, 1.38, 1.50 kg); and feed conversion (0.29, 0.25, 0.20). Additionally, there was a drop in the occurrence of diarrhea (33.33, 3.88, 2.77%) observed in the animals treated.
Conclusions: The results confirm the probiotic potential of these biopreparations when supplied to the suckling piglets.

Keywords: *probiotics, Lactobacillus salivarius, swine litters.*

Introduction

Intensive swine production in Cuba started with the beginning of the revolution, which included the design of genetic breeding policies. In the 1980s, the sector consolidated (over 102 400 t a year), though, the so-called Special Period caused a huge impact with a marked decline to 15 000, by 1993. Overtime, new recovery strategies were laid out, but the supply still fell short to the demands of society. The figures are evident: of 65 000 t by 2005, the current production accounts for 130 000 t, and by 2020, the goal is to reach the 220 000 t (Orta, 2014).

Swine farming in Cuba constitutes one of the most relevant items of the economy; it looks to balance the protein consumption needs through the amounts of meat produced. This animal species, like no other, has distinctive features that make it the top choice for many farmers. In that sense, the heterogeneity of the diet consumed by this species stands out, along with remarkable conversion, adaptability, high proliferation, and high carcass yields, which includes high protein and lipid levels (Ayala et al., 2014; Beruvides, 2019).

However, one of the main risks affecting yields is mortality, which in many cases results from the incidence of stressing factors that lead to gastrointestinal disorders, the presence of diseases, and immunodepression (GRUPOR, 2017).

To address these issues, diets are supplemented with antibiotics with favorable results in terms of reducing diarrhea or as promoters of animal growth (Milián et al., 2017). These seemingly satisfactory variants caused complications, such as the presence of harmful residues for consumers and the environment; a fall in the efficacy of human antimicrobials due to the appearance of anti-bioresistant bacteria (Errecalde, 2004). Therefore, the International Organization of Epizootias (OIE, 2014), and the Food and Agriculture Organization/World Health Organization (FAO/WHO, 2001) are working together to introduce new products and technologies into animal production systems, to offer healthy foods from high, safe productions, and proper economic sustainability.

Among these products are probiotic additives, which represent a potentially significant and safe therapeutic improvement (Flores-Mancheno et al., 2016).

The University of Matanzas and the Animal Science Institute (ICA), implemented several research projects for the development of economically viable products that improve animal yields and health (Pérez, 2000; Milián, 2009). One of the probiotics is PROBIOLACTIL® (Rondón, 2009), which was evaluated in birds and calves, and showed promising results in terms of weight increases, improvements in feed conversion, and a reduction of diseases (Rondón et al., 2020).

In Cuba, the Indio Hatuey Station for Pastures and Forages conducted a research-development study produce a biopreparation (IHplus®) whose microbial composition has similar actions to that of probiotics. It contains a group of efficient microorganisms (ME) generated through spontaneous fermentation, part of which is composed of lactic bacteria (Suárez et al., 2011).

Traditionally, swine nutrition relies on feedstuffs and alternative feeds, such as harvest stalks, roots, and tubers. In Cuba, they are also given NUPROVIM (protein combination of vitamins and minerals) + type B molasses (GEGAN-Swine Division, 2018). However, there are problems with the low quality of these feeds, which creates physiological, productive, and health problems to different swine stages (Hernández et al., 2015; Pérez et al., 2015). Nevertheless, the common practice does not include the administration of additives to address this issue. Accordingly, the aim of this paper was to evaluate the effect of PROBIOLACTIL® and IHPlus® on the production and health parameters of suckling piglets.

Materials and Methods

Treatments and experimental conditions The experiment was conducted at the EL Valle Basic Company (UEB), from the Agricultural Company at the Ministry of Interior (MININT) in the province of Matanzas, Cuba, located, on Chirino Road, the Yumuri Valley.

The study relied on a completely randomized experimental design, which included three treatments: GI-Control, the animals were only supplied with the basal diet; GII Basal diet + PROBIOLACTIL®; and GIII Basal diet + IHPlus®.

The experiment was evaluated between March and September 2019. During that period, the behavior of temperatures was as follows: mean temperature (24 ºC ± 5); maximum temperature (29 ºC ± 1); minimum temperature (18 ºC ± 3). The average mean humidity was 78% ± 3, based on the reports of the Provincial Weather Facility in Matanzas (Weather Station, 2019).

Additives evaluated: PROBIOLACTIL® was prepared using the methodology described by Rondón (2009). The dose used was 5 mL (10^9 CFU.mL^{-1}) by feed kg, according to Socorro (2016). IHPlus® was prepared from a mother solution, according to Rodríguez et al. (2013). The IHPlus, whey, and molasses were mixed in a 2-hL tank (12, 10, and 10 liters, respectively). Upon homogenization, chlorine-free water was added, leaving just a small empty space (approximately 12 cm off the edge), then it was capped. The tank was kept in a fresh place (28 ºC-30 ºC). After two weeks, a sour-sweet product was obtained, as of lactic fermentations, pH below 3.5. The biopreparation contained a population of over 10^5 CFU.ml^{-1}, coinciding with the recommendations of Díaz-Solares et al. (2020). A 25 mL dose was administered to each animal per day.

The number of pigs included in the experiment totaled 180 (crossbred York-Land x Large Whit Landrace and L-35 breeding pigs), under the same feeding and handling conditions. The feed was supplied as a meal (corn-soybean), according to the NRC (2012). It was administered in small quantities every hour, and it was prepared in two sections. One in the morning, the other at noon; the additive was included depending on the experimental group, and it was left to rest for at least half an hour before the administration.

The experiment began seven days before farrowing, supplying the sows with the additives according to the treatment type. The biopreparations were supplied at the moment the feedstuff was administered, three times a day, in the morning, at noon, and in the afternoon. It was repeated until farrowing, then it was administered to the litters orally on the eighth day of birth, when they started to consume small amounts of the feedstuff.

The experiment design included 18 litters from the first day of life on, until 33 days of age, descendants from two-parous sows with same-size adjusted litters (10 piglets), with three treatments and six litters (60 piglets or replicas) each.

Feeding and handling conditions. From the eighth day on, in addition to the colostrum and the mother's milk, all the piglets were given feedstuffs (Table 1) until the end of the lactation stage, averaging 0.06 kg/animal/day.

Sanitary conditions were created in the housing facility, according to the Technical Manual for breeding these animals on floor (IIP, 2008). Each pen lodged 10 pigs (10 pens total). Water was supplied *ad libitum*, and feed consumption was limited according to the Consumption standards established for this category (GRUPOR, 2017).

Table 1. Formulation and bromatological composition of the diet supplied to the piglets

Raw materials	Inclusion %
Corn	72.40
Soybean	24.29
NaCl	0.50
Carbonate	1.50
Pre-mix I*	0.25
Choline	0.07
Phosphate	0.90
Cu phosphate	0.09

Bromatological composition of the feedstuff

DM (%)	CP (%)	ME (MJ.kg^{-1})	Ca (%)	P (%)	C (%)
90.81	19.00	18.97	0.61	0.49	4.55

*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.

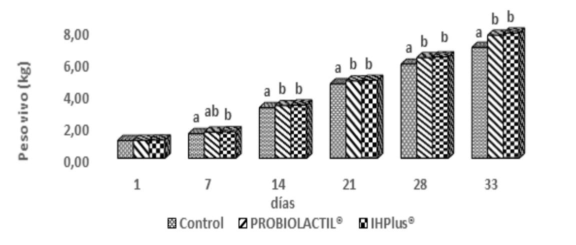
DM: Dry matter; CP: Crude protein; ME: Metabolizable energy; Ca: calcium; P: phosphorous; C: ashes.

Statistical processing INFOSTAT, version 2012 was used for statistical analysis (Di Rienzo et al. 2012). The simple analysis of variance model was used for the productive variables before checking the normal data distribution and the homogeneity of variance through the Shapiro & Wilk (1965) and Levene (1960) tests. The differences between groups were checked through Duncan's multiple rank comparison test (1955). The results of the effects of diarrhea were analyzed through the ComparPro version 1 software (Font et al., 2007).

Results and discussion

Fig 1. shows the behavior of live weight in the animals under PROBIOLACTIL® and IHPlus® during lactation. There was an increase in the live weight ($P<0.05$) of piglets treated with the biopreparations, compared to the control. This rise occurred at 14 days of application. From that age and until 33 days old, there was an apparent weight increase in the animals treated with the biopreparations, compared to the control group.

Blanco et al. (2012) developed IHplus®, whose effectiveness was demonstrated by enhancing the animal response when used as a digestive activator in the diet of ruminants and monogastric animals. Rodríguez (2017) also evaluated the efficacy of efficient microorganisms, using 90 three-day old piglets in three groups, with three repetitions of 10 each (30 per treatment). Groups 1 and 2



a, b, c. Columns with unequal scripts differ from $P<0.05$ (Duncan, 1955). 1 day SE ±0.02, P=0.2; 7 days, SE ±0.009, P=0.2; 14 days SE ±0.01, P=0.04, 28 days SE ±0.01, P=0.05 and 33 days. SE ±0.098, P=0.001.

Fig. 1. Behavior of live weight during the experiment

Groups I and II (on PROBIOLACTIL® and IHPlus®) showed no differences; though they were different from the control, increasing the piglets' live weight. Rondón et al. (2013) demonstrated that the utilization of this biopreparation in suckling piglets increases ($p \leq 0.05$) the live weight of the animals treated (9.46 kg), in comparison to the control group (8.02 kg) in five weeks. It also increased weight and mean daily gain (MDG). There was also a decrease of diarrhea. The literature refers that several species of genus *Lactobacillus* are commonly handled to make nutritional additives with probiotic effects on swine production (Arce, 2017; Wang et al., 2018), such as Lactobacillus salivarius, L. fermentum, L. acidophilus and L. plantarum, which are generally found in the soil, feces, and gastrointestinal tract of animals (Dowarah et al., 2017; Masumizu et al., 2019).

Socorro (2016) evaluated the effect of PROBIOLACTIL® (C65 *Lactobacillus salivarius* culture), SUBTILPROBIO® (C31 *Bacillus subtilis* culture) and the mixture of the two additives in production and health parameters of suckling and pre-weaning pigs. The additives and their mix enhanced every indicator compared to the control, with greater effects on the pigs that consumed PROBIOLACTIL®. This biopreparation was beneficial to the animals, as they improved the gastrointestinal tract eubiosis, increasing ($p<0.05$) live weight (27.15/25.59 kg), mean daily gain (445.27/408.65 g), weight increase (18.70//17.160 kg), and feed conversion (0.29/0.24). Moreover, there was a reduction of diarrhea in the animals treated.

Table 2 shows the behavior of other production indicators using both biopreparations. There were no difference between the piglets that consumed PROBIOLACTIL® and IHPlus®, as to WI and MDG. However, an improvement was observed in feed conversion in group III. In that sense, Díaz-Solares et al. (2020) noted that IHplus® has a broad range of application, whose effect lies in the diverse microbial composition, a mixed population that contains photosynthetic bacteria (*Rhodopseudomonas* sp.), actinomycetes (*Streptomyces* sp.), molds (*Aspergillus* sp, *Mucor* sp.), yeasts (*Saccharomyces* sp., *Candida* sp.), and acid-lactic bacteria (*Lactobacillus* sp. and *Streptococcus* sp.); *Saccharomyces* sp, *Lactobacillus* sp. and *Streptococcus* sp have proven their efficiency as probiotics.

Table 2. Effect of the biopreparations on the productive behavior of weaning pigs

Indicators	G I	G II	G III	P SE
WI (kg)	1.06_a	1.38_b	1.50_b	0.001 0.042
MDG (kg)	0.207_a	0.281_b	0.299_b	0.001 0.084
FC	$O.29_a$	0.25_b	0.20_c	0.001 0.078

G I. Control; G II. PROBIOLACTIL®; G III. IHPlus®
WI: Weight increase; MDG: Mean daily gain; FC: Feed conversion. Note: values with unequal subscripts indicate significant differences.

Table 3. Effect of diarrhea on piglets supplemented with PROBIOLACTIL® and IHPlus® for 33 days

Indicators	Treatments	No. of diarrheal episodes	%	SE± Sign
Effect of diarrheal episodes	Control	60	33.33_a	0.46 P<0.001
	PROBIOLACTIL®	7	3.88_b	
	IHPlus®	5	2.77_b	
Total of animals	180	72	-	

[a,b] Percentages with unequal scripts differ for P<0.001 (Duncan, 1955).

The biopreparations protected the animals from pathogenic microorganisms. Swine litters are known to be immunodeficient at birth, since there is no antibody transference in the uterus; their immunity is passive, through the colostrum and mother's milk are needed for survival (Masumizu et al., 2019; McCormack et al., 2019a). During the first days of life, the animals are susceptible to environmental pathogens, and may be exposed to infections caused by type A Clostridium perfringens, or enterotoxigenic E. coli (ETEC), which causes enteric infections that produce diarrhea in pigs (Barreto et al., 2020). Contamination may take place at any productive stage, though the highest incidence occurs when the animal lacks a developed immune system, or when the piglets are exposed to environmental stressing factors (Gardiner et al., 2020).

Sayan et al. (2018) used newborn pigs and administered a biopreparation of *L. salivarius*. They also challenged the animals with enterotoxigenic *Escherichia coli* F4+ (10^8 cfu.mL^{-1}). Consequently, the animals that used the probiotic gained weight and improved their intestinal health, reducing the occurrence of diarrhea and improving the intestinal morphology. *L. salivarius* adheres to the intestinal mucosa, so it can act as an intestinal barrier against pathogenic microorganisms, activate the immune system, and improve the host's health (Tuomola et al., 2001).

L. salivarius is known to stimulate the immune system of animals, contributing to greater protection against infectious agents that cause diarrhea. Xia et al. (2021) studied the effects and mechanism of *L. salivarius* LI01 in the immune modulation and metabolic regulation through the monocolonization of germ-free Sprague-Dawley (SD) rats with *L. salivarius* LI01 The GF rats were split into two groups, then the bacterial culture was administered through a catheter, whereas the control group received the same amount as saline solution. The levels of serum biomarkers and interleukin (IL)-1α, IL-5, and IL-10 were restored, indicating the activation of Th0 cell differentiation toward immune homeostasis.

Lactobacillus salivarius is a lactic acid bacterium with a probiotic potential (Wang et al., 2018). Robredo & Torres (2000) acknowledged that it produces lactic acids and bacteriocins that inhibit the development of pathogenic microorganisms that cause diarrhea in animals. Organic acids reduce intestinal pH and prevent the colonization by undesirable bacteria that do not proliferate in the presence of the probiotic (Van der Wielen et al., 2000). In turn, bacteriocins are peptides that destroy the integrity of the cytoplasmic membrane by forming pores, causing the exit of small compounds or a change in proton or nucleic acid drive (Chikindas et al., 1993).

Blanco-Betancourt et al. (2017) and Valdés-Suárez et al. (2019) used IHplus® in pigs and pointed out that these animals showed a significantly higher zootechnical behavior (P < 0.05) than the control. One of the major problems of postweaning is thought to be a disruption in the normal microbiota of the gastrointestinal tract, with changes in the intestinal flora of the cecum, thus increasing the number of enterobacteria while decreasing the acid-lactic bacteria that proliferate in suckling piglets. Hence, the addition of proper doses of this type of lactic bacteria (such as the ones found in EM), regenerate the digestive balance of these animals (Giraldo-Carmona et al., 2015).

Ojeda-García et al. (2016) also observed improvements upon the application of a biopreparation containing efficient microorganisms (IHplus®) during a research study under production conditions in fattening crossbred pigs. A total of 144 animals were included, with a starting LW of 27.0 ± 0.5 kg and 76 days of age (36 piglets per treatment). The experimental period lasted 132 days. The piglets that did not consume IHplus® showed the worst productive indicators (90.4 ± 1.6 kg; 0.478 ± 0.011 kg and 4.06 ± 0.01 kg for the LW, MDG, and FC, respectively), whereas the 40 mL dose produced the best results (98.3 kg; 0.583 kg and 3.64 kg), with a 15.4% gain increase. The authors concluded that the inclusion of IHplus® generateed greater income.

The evidence showed that the manipulation of the intestinal microbiota enhance immunity in pigs, which contributed to intestinal health. Therefore, some authors note that further studies might potentially be directed to the manipulation of the intestinal microbiome to enhance nutritional efficiency in pigs. If successful, this procedure could reduce the costs of production and the environmental impact on swine production. (McCormack, et al., 2017; McCormack, et al., 2019b).

Conclusions

The results confirm the probiotic potential of biopreparations PROBIOLACTIL® and IHPLUS® to improve the productive and health indicators of suckling piglets.

Author contribution statement

Yurién Ojito López: Research, analysis of the results, redaction of the manuscript.

Ana Julia Rondón Castillo: Research planning, analysis of the results, redaction of the manuscript, final review.

Marlen Rodríguez Oliva: Research planning, analysis of the results, redaction of the manuscript, final review.

Grethel Milián Florido: Research planning, analysis of the results, redaction of the manuscript, final review.

Agustín Beruvides Rodríguez: Research planning, analysis of the results, redaction of the manuscript, final review.

Conflict of interest statement

No conflicts of interest have been stated whatsoever.

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