Journal of Animal Prod. 31 (2), https://revistas.reduc.edu.cu/index.php/rpa/article/view/e2796

Effect of Efficient Microorganisms on Bioproductive Indicators of Pre-fattening Pigs

Alex Valdés Suárez, Víctor Manuel Álvarez Villar*, Alain Legrá Rodríguez*, Narcys Margarita Bueno Figueras***

*** Agroforestry Faculty, University of Guantánamo, Cuba**

**** Faculty of Agriculture, University of Granma, Bayamo, Cuba**

alexvs@cug.co.cu

ORCID: https://orcid.org/0000-0001-5265-045X

ABSTRACT

Background: At present, additives are used as an alternative to increase animal productive yields. The aim of this paper was to evaluate the effect of efficient microorganisms on bioproductive indicators of pre-fattening pigs.

Methods: A total of 80 Yorkshire/Landrace x CC21 animals (38 days old and 7.8 kg) were used under a completely randomized design in four groups of 20 animals each: control; treatment 1 (60 mL EM/5 kg of feed); treatment 2 (same as treatment 1, plus 1 mL EM/5 L of water); and treatment 3 (1 mL EM/5 L of water). The initial and final weights, weight gain, mean daily gain, food conversion, mortality, morbidity, and viability were evaluated for 49 days. The results were compared by covariance analysis, with IW as covariable; multiple comparison tests were made for comparison of means.

Results: The final weight, weight gain, mean daily gain, and food conversion showed significant differences ($P \leq 0.05$) between the treatments and the control, with the exception of mean daily gain in the third treatment. Mortality, morbidity, and viability were significantly different in the three groups treated in relation to the control.

Conclusions: The best results were observed in the first treatment, thus the inclusion of efficient microorganisms in the diet of pre-fattening pigs improved the bioproductive indicators.

Key words: *pigs, biopreparates, productive parameters, probiotic*

INTRODUCTION

The constant growth of world population has increased the demands of animal protein, namely meat, eggs, and milk (Bajagai, 2016). This calls for production increases of livestock breeding systems in order to supply the demand, in keeping with sustainable and environmentally friendly practices (Valdovska *et al.*, 2014). Today, additives are being used as an alternative to increase the animal productive yields (biocatalysts, enzymes, essential oils, plant bioactive compounds and seeds, and probiotics) in the daily diet (Sathyabama, Ranjith-kumar, Brunthadevi, Vijayabharathi and Brindha, 2014; Rodríguez *et al.*, 2016).

Post-weaning in intensive breeding is critical, since final success or failure will depend on the measures and decisions made in this stage. The stress produced has effects on the intestines. Some of the immediate negative effects consist of a sharp reduction of metabolic activity, and morphological and physiological changes of enterocytes, with an ensued drop in nutrient uptake. This damage increases with the ingestion of dried feeds. Another effect is raised susceptibility to enteric diseases, caused by a sudden separation from sows and union to new litters under different environments (Galeano, Herrera, and Suescún, 2015).

One alternative to diminish these effects is to include diets based on compounds that improve intestinal health, such as probiotics (Zhao *et al.*, 2014), which were first mentioned by Russian scientist Metchnikoff (Lama, 2014), and date back to the Twentieth Century. They are based on of live microorganisms with beneficial effects on the intestinal tract of the host, since they help maintain and strengthen defense mechanisms against pathogens, without disturbing their physiological and biochemical functions (Guevara, 2011).

Efficient microorganisms are a complex mix of microorganisms that live in nature. They are used to create a technology that collects all nature's potential to be utilized as organic additives to feed animals and treat sewage water (Higa, 2004).

In Cuba, research and development works at Indio Hatuey Pasture and Forages Station resulted in a biopreparate based on efficient microorganisms (EM) which act like probiotics, registered under the name IHplus®. It comprises a group of microorganisms produced by spontaneous fermentation, some of which are lactic bacteria (Suárez *et al*., 2011). The utilization of EM in swine breeding systems has brought about health benefits as well as improvements in zootechnical results (Contino *et al.*, 2008).

The Integrated Swine Farm named Bombi, at Coronel Arturo Lince Gonzalez Mountain Agroforestry Company, on Bayate-Manati road, km 4 ½, Salvador municipality, province of Guantánamo, Cuba, reports low bioproductive indicators mainly caused by the high incidence of gastrointestinal diseases in weaned pigs.

Therefore, the aim of this paper was to evaluate the effect of efficient microorganisms (EM) on the bioproductive indicators of pre-fattening pigs.

MATERIALS AND METHODS

This study was done in March-April, 2018. The food supplied consisted in pre-starting and starting feeds, in keeping with the standards of the Military Agricultural Union.

Selection of pre-fattening pigs and treatments

Overall, 80 Yorkland/Landrace x CC21 pre-fattening pigs (38 days old at weaning, mean weight 7.8 kg) were studied. Weight regularity and age were associated to feeding instability and handling malpractice to litters. The animals were distributed in a completely randomized design, in four groups of 20 animals each: control (without EM); treatment 1 (EM included in the feed); treatment 2 (EM in the feed and water); treatment 3 (EM inclusion in water). Two replications were used (pen) in each treatment, and the experimental farm was represented by each individual.

Obtaining a solid state mother

The EM culture was made according to the method described by Díaz *et al*. (2015). The starting material was a 30.0 kg sample of semi-decomposed leaves and organic matters on the ground (1-6 cm from the soil), collected at the Nipe-Sagua-Baracoa mountain range, with predominant tropical climate including mountainous deciduous rain forests and other uncontaminated native species considered virgin; the humidity values were high.

Thirty kg of dry leaves were used for the solid-state fermentation. It was thoroughly mixed with 46.0 kg of cornmeal, then it was humidified to 30-35% with 10 L molasses and 10 L of previously diluted milk whey; humidity was determined by the clenched fist test. Then, microorganic and anaerobic growth were favored by creating appropriate ecological conditions. The mass was highly compressed in the plastic tank (200 L). Then a valve was placed to allow for the exhaustion of gases.

Anaerobic fermentation was made for 21 days, then a semi-solid semi-alcoholic dark product with a fruit-like smell was produced, pH=3.8.

Preparation of liquid-state mother

For the preparation of liquid fermentation, 10 L of whey and 10 l of molasses were poured into the plastic tank (200 L). The rest was filled with non-chlorinated drinking water while the contents were stirred. Then, 10 kg of the primary culture or solid fermentation were placed in a jute sack (knitted fabric) which was closed at the opening. It was dipped in the liquid substrate with a heavy object. When the tank was filled and all the contents were mixed, it was shut and the valve was placed to allow for gas exhaustion. Fermentation lasted 7 days.

Biological control of biopreparates

A sample of EM was sent to the microbiology lab of the Center for Mountain Development (CDM) at the end of the process for biological evaluation, in order to guarantee proper sanitary quality of the product. The final microbial concentration of the product was 10^8 and 10^9 CFU, with a predominance of yeasts. The microbiological tests were performed conforming to standards NC 7440:1986, NC-ISO 6579:2008, NC-ISO 4831:2010, NC-ISO 4833:2011, and NC-ISO 1004:2014

Evaluation of EM effects on bioproductive indicators

The EM was supplied daily at a 60 mL/5 kg feed proportion (treatment 1); 60 mL/5 kg feed plus 1 mL/5 L of water (Treatment 2); and 1 mL/5 L of water (Treatment 3), throughout the experiment. The indicators evaluated were initial weight (IW), final weight (FW), weight gain (WG), mean daily gain (MDG), food conversion (FC), mortality, morbidity, and viability (%). Weighing was made especially in the morning hours before the animals were fed, using a 50 kg ± 0.01 kg precision Salter scale. These indicators were measured weekly for 49 days.

Statistical analysis

A completely randomized design based on IBM SPSS, version 24 was made, to study the dependent variables (FW, WG, MDG, FC, mortality, morbidity, and viability). All the primary data were processed by covariance analysis, in which IW was used as covariable. Previously, variance homogeneity was checked using the Levene test (P=0.244). The independent variables consisted of the EM treatments, which enabled evaluation of inter-group effects, based on multiple comparisons through the MSD (minimal significant difference) adjusted to Bonferroni.

RESULTS AND DISCUSSION

EM effects on the productive indicators

The values of FW, WG, MDG, and FC treatments revealed significant differences (P≤0.05) among them and in relation to the control group, except for variable MDG in the third treatment. No differences were found when compared to the control, as shown by the MSD (minimal significant difference) Multiple Comparison Test (Table 1).

Table 1. Results of the MSD Multiple Comparison Test performed to treatments

Effect data		Variables studied											
		FW (kg)			WG (kg)			MDG (g)			FC (kg)		
Intergroup comparison Treatments		Sig	CI (95%)		Sig	CI (95%)		Sig	CI (95%)		Sig	CI (95%)	
			SL	IL		SL	IL		SL	IL		SL	IL
Treat. 1	Treat. 2	*	.179	.272	*	.271	.178	*	.007	.003	*	-.026	-.048
	Treat. 3	*	2.981	3.107	*	3.106	2.980	*	.085	.079	*	-.526	-.556
	Control	*	3.824	3.898	*	3.899	3.824	*	.080	.077	*	-.752	-.770
Treat. 2	Treat. 1	*	-.272	-.179	*	-.178	-.271	*	-.003	-.007	*	.048	.026
	Treat. 3	*	2.779	2.858	*	2.858	2.779	*	.079	.075	*	-.494	-.513
	Control	*	3.577	3.695	*	3.696	3.577	*	.077	.071	*	-.710	-.738
Treat. 3	Treat. 1	*	-3.107	-2.981	*	3.696	-3.106	*	-.079	-.085	*	.556	.526
	Treat. 2	*	-2.858	-2.779	*	-2.779	-2.858	*	-.075	-.079	*	.513	.494
	Control	*	.739	.894	*	.896	.741	NS	1.846 E-5	-.007	*	-.202	-.238
Control	Treat. 1	*	-3.898	-3.824	*	-3.824	-3.899	*	-.077	-.080	*	.770	.752
	Treat. 2	*	-3.695	-3.577	*	-3.577	-3.696	*	-.071	-.077	*	.738	.710
	Treat. 3	*	-.894	-.739	*	-.741	-.896	NS	.007	-1.84	*	.238	.202

6E-5

Legend: FW=final weight; WG=weight gain; MDG=mean daily gain; FC=food conversion; CI=confidence interval; IL= inferior limit; SL=superior limit; NS=not significant differences; *(P<0.05)

The best results were observed in the experimental group (T1), which only consumed the biopreparate in the feed (Table 2). Although it was not the one with the highest EM load, the bioproductive indicators improved considerably in relation to the control and the other treatments. On the contrary, treatment 2 (60 mL/5 kg of feed plus 1 mL/5 L of water) showed lower values than the first treatment, though the microbial load was higher. The third treatment showed a response similar to the control group, demonstrating that the administration of that product in the water had a very low effect. This may be associated to the organoleptic characteristics of the water used, the state of water conduits, and residual chlorine concentration (Díaz *et al.*, 2015).

Table 2. Behavior of the variables studied per treatment and their significance

Variables	Experimental groups								
	Control		Treatment 1		Treatment 2		Treatment 3		Sig.
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	
FW (kg)	20.89	0.021	24.74	0.02	24.52	0.01	21.70	0.02	0.00
WG (kg)	12.99	0.021	16.86	0.015	16.63	0.014	13.81	0.022	0.00
MDG (kg)	0.266	0.001	0.344	0.001	0.339	0.001	0.262	0.001	0.00

FW: final weight; WG: weight gain; MDG: mean daily gain; FC: food conversion; SE: Standard error

Moreover, the variability of the results may be linked to the administration method of EM in each group, which may have influenced the inclusion of microorganisms for probiotic action in the intestines of the host. The efficacy of this procedure will only be possible when the number of microorganisms required to colonize the intestines and express the benefits of these products add to their own individual stimulating potential (Delgado, Barreto, and Rodríguez, 2014).

The absence of significant differences of the dependent variable (MDG) between the third treatment and the control was associated to the type of statistical analysis used and the dispersion of data processed, since MDG is directly linked to the rest of the productive indicators evaluated.

These results coincided with the studies conducted by Blanco *et al.* (2017) using a product (IHplus®) with similar features to the one studied; it was possibly caused by the differences in processes, technologies to produce biopreparates, and the sources of microbial inoculates and their compositions. No significant differences were observed in the 40 mL/animal/day doses as to productive indicators (FW, MDG, and FC), proving that the 40 mL dose of IHplus®/animal/day had no effects on the productive behavior of animals. According to Palomo (2015), this result was possibly caused by the effects of low microorganism concentration and the fast transit speed of digests at early ages, which does not favor colonization in the digestive system.

Furthermore, Blanco *et al.* (2017) said that the animals treated with the highest doses (80 and 120 mL of IHplus®) were observed to have a significantly higher zootechnical performance (P<0.05) in comparison to T1 (control) and T2 (40 mL). The results were even better in the 120 mL group. Interestingly, the 14.4

and 31.8 g/day increase observed in mean daily gain for T3 (80 mL) and T4 (120 mL), respectively, compared to the control, and was associated to FC improvements. Groups T3 and T4 consumed between 83 and 66% of feed to gain one kg of live weight, compared to T1 and T2 (Blanco *et al.*, 2017).

Quemac (2014) said that the inclusion of 5, 10, and 15 mL doses per kg of weight of a microbial biopreparate in the diet of weaned pigs increased the final weight in 1.1, 3.82, and 5.56 kg per animal, respectively, when compared to the control, which coincided with the results of this investigation.

EM effects on epidemiological indicators

The results of epidemiological indicators evaluated in the experimental stage are shown in Table 3. The experimental groups (Treatments 1, 2, and 3) which consumed EM (water and/or feed), clearly showed low morbidity and mortality values in relation to the control group, with the best viability results.

Table 3. Results of evaluation of epidemiological indicators in pre-fattening pigs

Indicators	Experimental groups							
	Control		Treatment 1		Treatment 2		Treatment 3	
	X	%	X	%	X	%	X	%
Mortality	1	5.00	0	0	0	0	0	0
Morbidity	11	55.37	4	20.00	4	20.00	5	25.00
Viability	19	95.00	20	100.0	20	100.0	20	100.0

In that sense, one of the main postweaning problems was a disruption in the normal microbiota of the intestinal tract, with changes in the bacterial flora of the cecum. An increase in the number of enterobacteria was observed along with a decrease in lactic acid bacteria, which are abundant in suckling pigs. Hence, the addition of optimum doses of this kind of lactic bacteria (like the ones in EM) reinstated the balance of the digestive tract of these animals (Giraldo-Carmona, Narváez-Solarte, and Díaz- López, 2015).

Similar results were reported by Miranda and Marín (2018), who used two types of biopreparates from molasses and orange vinasse, fermented with *Lactobacillus acidophilus*, *L. bulgaricus*, *Streptococcus thermophilus* (T1), and (T2) same as before, plus *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* (L-4 UCLV), in 2.5 mL/animal/day doses, which were supplied in the diet of post-weaned pigs. Accordingly, the animals which consumed the biopreparates (T1 and T2) showed the lowest ($P<0.05$) diarrheal episodes and death rates of all the treatments, compared to the control. Between 28-35 days of age, the control animals underwent the highest ($P<0.05$) percentage of diarrheal disorders and death rates in relation to T1 and T2, without differences between these two.

Authors like Rodríguez, Barreto, Bertot, and Vázquez (2013) used a commercial biopreparate known as MAM (multipurpose autochthonous microorganisms) in recently weaned pigs, and were able to reduce intestinal disorders like diarrhea and low growth yields. This beneficial effect was mainly attributed to the capacity of microorganisms to improve animal intestinal health, modulate their immune system, and therefore, have a favorable influence on productive yields, with economic advantages.

CONCLUSIONS

The inclusion of efficient microorganisms in the diet of pre-fattening pigs improved the bioproductive indicators, leading to the best results in the first treatment.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to professors Roberto Vázquez, Yoel Rodríguez, and Osmani Lafita for their unconditional advisory on statistical analysis.

REFERENCES

Blanco-Betancourt, Dairom, Félix Ojeda-García, Luis Cepero-Casas, Lázaro Jesús Estupiñan-Carrillo, Luis Miguel Álvarez-Núñez, and Giraldo Jesús Martín-Martín. "Efecto del bioproducto IHplus® en los indicadores productivos y de salud de precebas porcinas." *Pastos y Forrajes* 40, no. 3 (2017): 201-205.

Contino, Y.; Ojeda, F.; Herrera, R.; Altunaga, N.; Pérez, M G. y Hernández, K. (enero de 2008). *Evaluación del Microben (microorganismos benéficos) como aditivo, su influencia en el comportamiento productivo y hematológico en precebas porcinas*. [cd-rom]. Congreso Panamericano de las Ciencias Veterinarias, PANVET. Guadalajara, México.

Delgado, R.; Barreto, G. y Rodríguez, H. (2014). La antibiosis, génesis y componente de los probióticos; dos conceptos imperecederos. (Nota técnica). *Revista de Producción Animal, 26* (3), 51-53.

Díaz, A.; Urgelles, I.; Abreu, N.; Abreu, A.; Martín, J. y Herrera, D. (2015). *Lactofermentos, guía técnica para su elaboración y aplicación en la producción agropecuaria*. Guantánamo, Cuba: Editorial Oxfam Solidaridad.

BAJAGAI, Y. S.; KLIEVE, A. V.; DART, P. J. y BRYDEN, W. L. (2016). *Probiotics in Animal Nutrition: Production, Impact and Regulation*. FAO.

GALEANO, J. A. C.; HERRERA, A. L y SUESCÚN, J. P. (2015). La adición de cepas probióticas modula la secreción de mucinas intestinales en íleon de cerdos en crecimiento. *CES Medicina Veterinaria y Zootecnia, 10* (2), 150-159.

GIRALDO-CARMONA, J.; NARVÁEZ-SOLARTE, W. y DÍAZ-LÓPEZ, E. (2015). Probióticos en cerdos: resultados contradictorios. *Revista Biosalud 14* (1), 81-90.

GUEVARA, J. (2011). *Probióticos en nutrición animal. Sistema de revisiones en investigación veterinaria de San Marcos (Srivs)*. Perú: Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos. Retrieved on May 2018, from http://www.unmsm.edu.pe/veterinaria/files/Articulo_ guevara_probioticos.pdf

HIGA, T. (junio de 2004). *La Tecnología de los Microorganismos efectivos "EM"*. Conferencia dictada en el Real Colegio de Agricultura, Cirencester, Reino Unido.

LAMA, J. M. (2014). Papel de los probióticos en la microbiota intestinal porcina y su repercusión en los rendimientos reproductivos. *Cría y salud. 22* (1), 60-66.

MIRANDA, J. E. y MARÍN, A. (2018). *Obtención de biopreparados, a partir de melaza-vinaza fermentadas, con acción probiótica en cerdas primíparas y crías*. Tesis en opción al grado científico de Doctor en Ciencias Veterinarias, Facultad de Ciencias Agropecuarias, Universidad central "Marta Abreu" de las Villas, Santa Clara, Cuba.

NC 7440:1986. (1986). *Determinación de microorganismos proteolíticos viables*. La Habana, pp 5.

NC-ISO 1004:2014. (2014). *Microbiología de Alimentos de Consumo Humano y Animal. Guía general para la enumeración de hongos y levaduras. Técnica de placa vertida*. La Habana, pp 5.

NC-ISO 4831:2010. (2010). *Microbiología de alimentos de consumo humano y animal Método horizontal para la detección y enumeración de coliformes técnica del número más probable* (ISO 4831: 2006), pp 5.

NC-ISO 4833:2011. (2011). *Microbiología de Alimentos de Consumo Humano y Animal. Guía general para la enumeración de microorganismos aerobios mesófilos viables. Técnica de placa vertida a 30oC*. La Habana, pp 4.

NC-ISO 6579:2008. (2008). *Método Horizontal para la Detección de Salmonella spp*. La Habana, pp 6.

PALOMO, A. (abril de 2015). Resumen XIII Symposium Internacional sobre Fisiología Digestiva en Porcino. Kliczków, Poland: Warsaw University of Life Sciences, Freie Universität Berlin.

QUEMAC, M. (2014). *Evaluación de tres dosis de probiótico* (Rhodopseudomona spp, Lactobacillus spp, Saccharomyces spp) *en la alimentación para el engorde de cerdos*. Tesis de Ingeniería, Universidad Estatal Politécnica del Carchi, Tulcán, Ecuador.

RODRÍGUEZ, H. C.; BARRETO, G.; BERTOT, A. y VÁZQUEZ, O. (2013). Los microorganismos eficientes como promotores del crecimiento en los cerdos hasta el destete. *REDVET. Revista Electrónica de Veterinaria, 14* (9), 1-7.

RODRÍGUEZ, J.; MÉNDEZ, V.; CALERO, I.; PEÑA, K.; MARTOS, D. y KUKURTCU, B. (2016). Evaluation of the nutritional supplement VIUSID vet powder on the productive behaviour of sows and boars. *Journal of Environmental Science and Engineering., 5 (número especial)*, 432-439.

SATHYABAMA, S.; RANJITH-KUMAR, M.; BRUNTHADEVI, P.; VIJAYABHARATHI, R. y BRINDHA, V. (2014). Co-encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment. *LWT - Food Science and Technology, 57* (1), 419-425.

SUÁREZ, J.; MARTÍN, G. J.; SOTOLONGO, J. A.; RODRÍGUEZ, E.; SAVRAN, V. y CEPERO, L. (2011). Experiencias del proyecto BIOMAS-CUBA. Alternativas energéticas a partir de la biomasa en el medio rural cubano. *Pastos y Forrajes, 34* (4), 473-496.

VALDOVSKA, A.; JEMELJANOVS, A.; PILMANE, M.; ZITARE, I.; KONOSONOKA, I. y LAZDINS, M. (2014). Alternative for improving gut microbiota: use of Jerusalem artichoke and probiotics in diet of weaned piglets. *Journal of Veterinary Sciences, 17* (1), 61-69.

Zhao, Y.; Weaver, A. C.; Fellner, V.; Payne, R. L. y Kim, S. W. (2014). Amino acid fortified diets for weanling pigs replacing the use of fish meal and whey protein concentrate: Effects on growth, immune status, and gut health. *J. Anim. Sci. Biotechnol*. *5* (1), 57-60.

.

Received: 2-8-2019
Accepted: 3-26-2019