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Original

Effects of a Biopreparation Containing Efficient Microorganisms on the Bioproductive and Hematological Parameters of Pig Litters

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ABSTRACT

Background: Efficient microorganisms offer a broad spectrum for application in crop and animal production. **Aim**: To evaluate the effect of a biopreparation based on autochthonous efficient microorganisms in Guantanamo (MEAG), Cuba, on swine litters' bioproductive and hematologic parameters. **Methods:** A total of 96 animals (Duroc/CC21) weighing 1.55±0.27 kg were included in the experiment from birth, and distributed in four groups with 24 repetitions, following a completely randomized design. The treatments consisted of the control and addition of the biopreparation, in 2.0, 10.0, and 20.0 $mL.kg^{-1}$ doses live weight/day, orally. The experiment lasted 33 days, and the final weight, weight gain, mean daily gain, feed conversion, mortality, morbidity, hemoglobin, hematocrit, total leukocytes, eosinophils, lymphocytes, and monocytes were analyzed. A simple analysis of variance was performed, and the mean differences were detected using the multiple range test. **Results:** The results were better ($p<0.05$) in the 2.0 mL dose of the MEAG/kg live weight biopreparation associated with final live weight and weight gain (0.59; 0.70, and 0.66 kg), and mean daily gain; whereas it was lower in feed conversion in the control and treatments two and three. In morbidity alone, differences between the control group and treatment three were observed, though no differences were detected between the two remaining groups. The hematological values rose with larger doses but within the normal range. **Conclusions:** The

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utilization of MEAG as a feed additive improves swine herds' bioproductive and hematological parameters.
Keywords: microbial additive, pigs, productive response (*Source*: *AIMS*)

INTRODUCTION

Modern animal production relies on a highly productive density, regardless of the species. Consequently, animals are under constant stressful conditions that may lead to a higher frequency of disease outbreaks and the ensuing reduction in production levels (Beruvides *et al*., 2018). Pigs are characterized by a very high mortality percentage in comparison to other species (cattle, sheep, horses). Although pig farming uses one of the most modern technologies in animal production, mortality accounts for approximately 10-15% of born litters. The suckling pigs are born with marked physiological deficiencies that jeopardize neonate adaptation to the new environment in the first 24-72 h hours of life (Ayala *et al*., 2008).

Today, one alternative to raise animal productive performance is the inclusion of additives, such as biocatalyzers, enzymes, probiotics, essential oils, and plant and seed bioactive compounds in the daily diet (Sathyabama *et al*., 2014; Rodríguez-Fernández *et al*., 2016). Another sustainable choice comes from efficient microorganisms (EM), which apart from sharing similar features with the above, enable a broader application spectrum in crop and animal production (Barreto Argilagos *et al*., 2017). Generally, they are defined as a mixed culture of beneficial microorganisms, with no genetic modification, which is present in natural ecosystems, and are physiologically compatible to one another (Luna and Mesa, 2016).

In Cuba, research facilities, like the Indio Hatuey Experimental Station of Pastures and Forages, in the province of Matanzas, and the University of Camagüey, developed microorganism mixtures from dead leaves and other organic matters from chemical fertilizer and herbicide-free areas and were applied in pig production systems. These biopreparations had beneficial effects on animal health, as well as an increase in zootechnical results (Barreto Argilagos *et al*., 2015; Montejo-Sierra *et al*., 2017; Rodríguez *et al.*, 2021).

In the eastern Cuban provinces, like Guantanamo, there is no sufficient scientific evidence related to the dosage of efficient microorganisms to be applied in newborn pigs. Hence, this study aims to evaluate the effect of a biopreparation containing autochthonous efficient microorganisms in Guantanamo (MEAG), Cuba, on swine litters' bioproductive and hematologic parameters.

MATERIALS AND METHODS

The research was conducted at AZUMAT Swine Facility, part of the same company, located on Jamaica Road, km 5 ½, the municipality of Manuel Tames, Guantanamo, with an epizootiological quadrant of 102-147-17. The annual average precipitation is 746 mm, with a mean temperature of 25 ºC, and average relative humidity of 77%.

Experimental design and treatment

A completely randomized design with 24 repetitions was used, which included control and addition of the efficient microorganisms biopreparation in 2.0, 10.0, and 20.0 $mL.kg^{-1}$ live weight (LW)/day doses administered orally, using plastic syringes between the 7^{th} and the 33^{rd} day, following adaptation to a single-dose additive between days zero and six. Three cages were used per treatment, and every animal was an experimental unit. The animals were labeled with consecutive numbers using the notch pattern commonly utilized on genetic facilities (IIP, 2016).

Animals and feeding and handling system

A total of 96 Duroc/CC21 animals were used from their birth, with an average live weight of 1.55±0.27 kg (49 females and 47 males). The animals were placed along with their mothers in standard Flat-Deck farrowing units (1.60 m x 2.40). The diet included pre-starting feed (fish meal and corn), according to the nutritional requirements for this category (Rostagno *et al*., 2017), and the Cuban technical procedures (IIP, 2016). It was supplied five times in special troughs for the category, beginning at 7:00 am after the corresponding MEAG biopreparation dose administration to animals. The consumption of milk from the mother was estimated through the double-sampling method (Mercanti, 2018), in 21 kg/animal, including total solids (TS) 4.11 kg, with an average feedstuff of 1.23 kg dry matter (DM). Then the consumption of metabolizable energy (ME) and crude protein (CP) was determined. The pigs were treated with the MEAG for 33 days and weighed every week to adjust the dose administered. The water was supplied *ad libitum* in troughs with nipples placed 8.0 cm from the floor. The experiments complied with the IIP (2016) instructions.

MEAG biopreparation characteristics and formulation

The culture of MEAG biopreparation was made according to the methodology described by Tellez-Soria and Orberá-Ratón (2018). At the end of the process, the new product had a sour-sickly sweet smell, as of a lactic fermentation, the characteristics are shown in Table 1. For evaluation of the biopreparation in pigs, three containers (600 L) remained in the facility for as long as the experiment lasted. The physical characteristics were determined according to the methodologies used by Miranda (2018); the microbiological attributes were determined to homogeneous samples from the three containers, at the Provincial Veterinary Diagnostic Laboratory, using the Cuban Standards (CN), by triplicate. The methods of serial dilutions and Petri dish culture in MRS agar medium (incubated for 24-72 h, at 37 ºC were used for lactic bacterial concentration. The pH was measured using a digital pH meter CRISON® BasiC 20,40*H 110 (USA).

Table 1. Physical and microbiological attributes of the MEAG biopreparation evaluated in pre-fattening pigs

Parameter	Reference	Mean (n=3)	SD	VC (%)
Viable yeasts, $CFU.mL^{-1}$	NC-ISO 1004:2016	$1.6x10^{10}$	0.16	9.88
Filamentous fungi, $CFU.mL^{-1}$		$2.7x10^{6}$	0.18	6.68
Fecal and total coliforms, $CFU.mL^{-1}$	NC-ISO 4831:2010	Negative	----	----
Salmonella in 25 mL, $UFC.mL^{-1}$	NC-ISO 6579:2008	Negative	----	----

Lactic acid bacteria (LAB), CFU.mL^{-1}	4.2x10^{9}	0.38	9.07
pH	3.40	0.06	1.86
Color	Brown	----	----
Odor	Sour-sickly sweet	----	----
Flavor	Sickly sweet	----	----
Texture	Liquid	----	----
CFU: Colony Forming Units of: SD: standard deviation; VC: Variation coefficient			

Experimental procedure to evaluate the MEAG effect

The parameters evaluated were DM, ME, and CP consumption, initial live weight (IW), final weight (FW), weight gain (WG), mean daily gain (MDG), feed conversion (FC) of DM, ME, and CP, hemoglobin, hematocrit, total leukocytes, eosinophils, lymphocytes, monocytes, morbidity, mortality, and their causes. These parameters were determined as follows:

The DM, ME, and CP consumption was calculated according to the chemical composition of the feed, and it was determined as follows: Feeds supplied (kg) - rejected feed (kg).

The final live weight was quantified at 33 days, using a 50 kg Salter balance (±0.01 kg precision).

Live weight gain was calculated as the difference between the final live weight (FW) and the initial live weight (IW), as WG=PF-PI.

The mean daily gain was calculated using this formula: MDG = (FW-IW) / evaluation time

Feed conversion was calculated using this formula:

FC, of DM = kg of DM consumed + of milk TS /kg LW gain.

FC, of ME = Megajoule (MJ) of ME consumed/kg LW gain.

FC, of CP = g of CP consumed/kg LW gain.

Throughout the experimental stage, the number of animals that suffered diarrhea or died was recorded to determine the morbidity/mortality ratio.

At the end of the experiment, eight animals were chosen at random from every treatment, and blood was drawn from the orbital vein with California-type needles. The samples were placed in tubes impregnated with disodium EDTA (1.0 mg/mL of blood), and were processed at the Provincial Veterinary Diagnostic Laboratory, in keeping with the methodologies described by Coffin (1966).

Statistical analysis

The experimental data were processed through STATISTICA version 10 DE StatSoft, Inc. 1984-2011. A simple analysis of variance (ANOVA), was performed based on the Kolmogorov-Smirnov normality assumptions, and the variance homogeneity assumptions between groups Levene (1960). The differences between means were detected using Duncan's multiple range test (1955), with $p<0.05$. The initial weight, feed conversion of DM, ME, and CP, hematocrit, hemoglobin, lymphocytes, and total leukocytes did not match the above assumptions, so the Kruskal and Wallis (1952) multiple comparison test of independent samples was applied, which permitted the

evaluation of their intergroup effects. The magnitude of the differences between the medians was determined through Z mean range comparison of $p<0.05$ (Siegel and Castellan, 1988). The morbidity and mortality caused by digestive disorders were evaluated through proportions analysis, using the Chi-square test (χ^2), with a $p<0.05$ significance.

RESULTS

Table 2 shows the results of the productive parameters in litters supplemented with the MEAG biopreparation at 33 days of the experiment, with differences ($p<0.05$) between the treatments, except for the initial weight. The results were better ($p<0.05$) in the 2.0 mL dose of the MEAG/kg live weight in the final live weight and weight gain (0.59; 0.70, and 0.66 kg), and mean daily gain(17.8; 21.1, and 20.0 g), respectively; whereas it was lower in feed conversion of DM, ME, and CP in the control and treatments two and three, respectively.

Table 2. Effect of the MEAG biopreparation on the productive parameters of 33 day-old pig litters

Parameters	**MEAG dose, mL.kg$^{-1,}$ LW**				**H†**	**p**
	Control	**2.0**	**10.0**	**20.0**		
Initial weight, kg	1.49 (1.56)	1.51 (1.56)	1.57 (1.55)	1.53 (1.56)	0.428	0.934 NS
Feed conversion of DM + TS, kg/kg	0,93^{b} (0.96)	0,85^{a} (0.86)	0.95^{b} (0.99)	0,96^{b} (0.97)	11.64	0,009**
Conversion of ME, MJ.kg^{-1} LW	6.65^{b} (6.81)	6.04^{a} (6.11)	6.75^{b} (7.03)	6.81^{b} (6.89)	11.64	0.009**
Conversion of CP, g.kg^{-1} LW	79.48^{b} (81.41)	72.25^{a} (72.97)	80.68^{b} (84.08)	81.34^{b} (82.33)	11.64	0.009**
Parameters	**MEAG dose, mL.kg$^{-1,}$ LW**				**±SE**	**p**
	Control	**2.0**	**10.0**	**20.0**		
Final weight, kg	7.25^{b}	7.84^{a}	7.13^{b}	7.17^{b}	0.105	0.021*
Weight gain, kg	5.69^{b}	6.28^{a}	5.58^{b}	5.62^{b}	0.088	0.021*
Mean daily gain, g	172.5^{b}	190.3^{a}	169.2^{b}	170.3^{b}	2.684	0.021*

†Kruskal and Wallis (1982), ^{a,b} Medians with unequal scripts differ (Z (Siegel y Castellan, 1988). ^{a,b} Means with unequal scripts in the same row differ from p≤ 0.05 (Duncan 1955)‡, SE: Standard Error Means within parentheses, *p<0.05, **p<0.01, NS (non-significant).

Table 3 shows the results of the health parameters evaluated. No differences were observed between the groups in the mortality proportion. Meanwhile, morbidity was higher in the control group. This group differed from treatment three, with no differences between the two groups with the 2.0 and 10.0 mL doses of MEAG/kg LW/day. The digestive disorders were the main cause of animal death in the first two treatments.

Table 3. Morbidity/mortality proportion in the animal groups evaluated

Parameter	Treatments	Number of animals	Value (%)	χ^2	±SE	p
Mortality	Control	1	4.17	2.04	2.91	0.563 NS
	T1	1	4.17			
	T2	0	0.00			
	T3	0	0.00			
Morbidity	Control	16	66.7^{b}	18.4	9.62	0.004^{**}
	T1	8	33.3^{ab}			
	T2	5	20.8^{ab}			
	T3	3	12.5^{a}			

[a, b] Proportions with unequal scripts in the same column differ significantly (p<0.05), SE: Standard Error. T1, T2, and T3, 2.0, 10.0, and 20.0 mL doses of MEAG/kg live weight/day, respectively, χ^2: Chi-square value, [**]p<0.01, NS (non-significant).

The values of the hematological parameters of the 33 day-old pigs are shown in Table 4. These values rose with a higher dose. However, all the values are within the different ranges considered as normal, as reported by Perri *et al.* (2017), except for a moderate leukopenia in the control group.

Table 4. MEAG effect on the productive parameters of 33 day-old pig litters

Parameters	RR	MEAG dose, mL.kg$^{-1,}$ LW				H†	p
		Control	2.0	10.0	20.0		
Hematocrit,%	26.0-41.0	29.0^{c} (28.37)	30.0^{abc} (29.63)	30.0^{abc} (30.87)	33.0^{a} (32.62)	17.36	0.0006^{***}
Hemoglobin, g.dL^{-1}	8.80-14.0	9.65^{c} (9.45)	10.0^{abc} (9.86)	10.0^{abc} (10.27)	10.8^{a} (10.76)	16.41	0.0009^{***}
Lymphocytes, x10^{9}.L^{-1}	2.22-16.0	4.20^{c} (4.20)	4.50^{bc} (4.51)	4.90^{ab} (4.97)	6.20^{a} (6.26)	19.29	0.0002^{***}
Total leukocytes, x10^{9}.L^{-1}	8.70-37.9	8.7^{b} (8.39)	10.35^{ab} (9.62)	11.15^{ac} (11.37)	13.4^{c} (13.24)	27.24	0.0000^{***}
Parameters	**RR**	**MEAG dose, mL.kg$^{-1,}$ LW**				**±SE**	**p**
		Control	**2.0**	**10.0**	**20.0**		
Monocytes, x10^{9}.L^{-1}	0.00-5.00	0.31^{d}	0.44^{cd}	0.55^{bc}	0.85^{a}	0.047	$<0.0001^{***}$
Eosinophils, x10^{9}.L^{-1}	0.00-1.80	0.37^{c}	0.52^{c}	0.82^{b}	1.19^{a}	0.066	$<0.0001^{***}$

†Kruskal and Wallis (1982), [a,b,c] Medians with unequal scripts differ (Z (Siegel and Castellan, 1988). [a,b,c,d] Means with unequal scripts in the same row differ from p≤ 0.05 (Duncan 1955)‡, SE: Standard Error, Means within parentheses, [***]p<0.001.RR: Referential ranges by Perri *et al.* (2017).

DISCUSSION

The behavior of productive and health parameters observed in this study (Tables 2 and 3), may be linked to the multifunctional action performed by the microbial additives in the gastrointestinal tract of the animals. Mostly, these additives can stabilize and protect the gastrointestinal ecosystem, improve nutrient digestion and absorption, and modulate the immune system (Sosa, García and

Dustet, 2018). They noted that the activity will depend on the microbial species in the product. Giraldo-Carmona, Narváez-Solarte and Díaz-López (2015), and Fouhse, Zijlstra, and Willing (2016) said that the response in terms of intestinal health, wellbeing, and pig productivity will depend on the quantity, microbiological concentration, and proper time of application that ensures the balance of the intestinal microbiota.

Another element that enables a favorable state of eubiosis to the animal is the production of antimicrobial substances, vitamins, and other nutrients. In that sense, Beruvides (2020) highlighted the role of lactic bacteria in the production of organic acids and antimicrobial substances, like bacteriocins. Moreover, Barreto Argilagos *et al.* (2015) stressed that thanks to these cultures, the production of organic acids (especially lactic acid), and short-chain fatty acids (acetate, propionate, and butyrate) can modify the intestinal lumen pH (pH<4.0 which is not tolerated by certain enteropathogens). Likewise, additives can increase the production of enzymes associated with digestive processes, such as *β*-galactosidase, which stimulates gastrointestinal peristalsis and promotes apparent nutrient digestibility (Zhao and Kim, 2015; Beruvides, 2020). An improvement in the above process effects on the animal nutritional state can manifest through the hematological parameters (Fernández *et al*., 2014), as determined in this study.

The microbial additives can also influence the changes produced in the morphophysiology of the intestinal mucosa (Knap *et al*., 2011). In that sense, Trevisi *et al.* (2017) administered some biopreparation and observed that the animals tended to augment the number of mitotic cells in the villi and crypts and to reduce the number of apoptotic cells in comparison to the control group. An increase in the nutrient absorption surface of the intestinal mucosa, along with the efficiency induced in digestibility enhances the productive parameters.

Similar productive performances were reported by Montejo-Sierra *et al.* (2017), who evaluated the effect of IHpus microbial additive, similar to MEAG, in suckling pigs. Furthermore, Beruvides (2018 and 2020), and Suárez, Buitrago, and Rondón-Barragán (2019) found that the pigs gained more weight at weaning, along with greater mean daily gain and feed conversion, compared to the control group, when supplying microbial additives containing lactic bacteria and yeasts.

Moreover, the application of mixed cultures containing different microbial species or multi strains favors the exclusion of enteropathogens that compete over adhesion sites and nutrients for growth (Betancur *et al*., 2021). This mechanism is mainly attributed to bacteria capable of adhering to the intestinal epithelium through molecules or receptors that enable the process and block enteropathogen ligands, an essential step that promotes colonization and the further release of enterotoxins (Barreto Argilagos *et al*., 2015). The exclusion process is more complex in swine litters as they are under a complete microbial succession (Fouhse, Zijlstra, and Willing, 2016). However, the inclusion of a beneficial biota contributes to the protection of the gastrointestinal tract and the strengthening of the immune system.

The lactic acid bacteria and yeasts present in bioproducts are capable of increasing the number of plasmatic cells in the gastrointestinal tract, and in response, it improves the production of specific

circulating antibodies against pathogenic bacteria (Mishra *et al*., 2014). This mechanism is linked to the reduction of the occurrence of diarrhea caused by digestive disorders in pigs, and the ensuing mortality. This effect was observed in this study (Table 4) and was reported by Miranda-Yuquilema, Marin-Cárdenas, and González-Pérez (2018), when administering 2.5 mL/animal/day doses of two biopreparations containing *Lactobacillus acidophilus, L. bulgaricus, Streptococcus thermophilus, Saccharomyces cerevisiae,* and *Kluyveromyces fragilis* strains in the diet of weaning pigs. Similarly, Betancur *et al.* (2021) reported a reduction in the incidence of diarrheal suckling pigs during the first weeks of life, and fewer deaths of pigs/litters, when their mothers were given 10.0 mL/animal/day doses of a microbial biopreparation containing *Lactobacilos plantarum* CAM-6 to the lactating sows.

Herrera, Galeano, and Parra (2016) reported improvements in the unspecific defense mechanisms of the host, and the stimulation of blood cell production associated with the innate or adaptative immune response (lymphocytes, monocytes, and granulocytes), related to the utilization of microbial additives in suckling pigs, with no excessive or harmful action to the host. Furthermore, by interacting with antigens, the cells possibly secrete specific pro and anti-inflammatory cytokines (interleukine-1, interleukine-2, interleukine-4, interleukine-6, interleukine-10, gamma-interferon, alpha-tumor necrosis factor, beta-transforming growth factor), which regulate the function of regulatory T-cells. It permits the formation of an effective immunological system and decreases the susceptibility to various inflammations and allergies (Laskowska, Jarosz, and Grądzki, 2017). Perhaps a higher number of somatic cells in the animals that consumed MEAG indicates the immunomodulating and bioprotective effects of the compounds present in the biopreparation.

CONCLUSIONS

The utilization of MEAG as a feed additive improves the bioproductive and hematological parameters of swine litters, and showed better results with the administration of the 2.0 $mL.kg^{-1}$ live weight/day dose.

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AUTHOR CONTRIBUTION

Conception and design of research: AVS, VMÁV, YGH, PS, YRV, EPP; analysis and interpretation of data: AVS, VMÁV, YGH, PS, YRV, EPP; redaction of the manuscript: AVS, VMÁV, YGH, PS, YRV, EPP.

CONFLICT OF INTERESTS

The authors declare the existence of no conflicts of interest.