INFLUENCE OF A MICROBIAL ADDITIVE ON THE PRODUCTIVE BEHAVIOR OF PREGNANT SOWS, AS WELL AS, HEMATOCHEMICAL AND DIARRHEAL INCIDENCE IN THEIR OFFSPRING[1]

[INFLUENCIA DE UN ADITIVO MICROBIANO EN EL COMPORTAMIENTO PRODUCTIVO DE CERDAS GESTANTES, ASÍ COMO, HEMATOQUÍMICA Y INCIDENCIA DIARREICA EN SUS CRÍAS]

J.E. Miranda-Yuquilema[1, 2*], A. Marin-Cárdenas[2], H. Oliva-Bello[2], D. Baño-Ayala[3], M. Barros-Rodríguez[4], H. Jácome-Vargas[4] and D. Villamarín-Barragán[4]

[1]Becario de instituto de fomento al talento humano, SENESCYT, Ecuador.
[2]Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas, Cuba. Email: efra_miranda@outlook.com
[3]Facultad de Ciencias Pecuarias, Escuela Superior Politécnica de Chimborazo, Ecuador
[4]Facultad de Ciencias Agropecuarias, Universidad Técnica de Ambato, Carretera Cevallos-Quero, 180350 Cevallos, Tungurahua, Ecuador
**Corresponding author*

SUMMARY

The objective of this study was to evaluate the productive behavior in sows, as well as the hematochemical profiles and the incidence of diarrhea in piglets when using a microbial preparation fermented in byproducts of the agroindustry. We used 14 breeding sows, CC21 hybrids (Yorkshire - Landrace/L35 Duroc) and their offspring, divided into two groups: T1, Control and T2, microbial preparation A. T2, contained *Lactobacilus acidophilus* y *Kluyveromyces fragilis* L-4 (UCLV). The microbial additive was developed in a substrate composed of molasses, torula yeast and soy milk. 20 ml of microbial preparation were applied to the sows from the last third of gestation until weaning; and the piglets were supplied with 1 ml in mono dose for 2 weeks and subsequently increased to 1.5; 2 and 3 ml per animal respectively. A completely randomized design was used. The productive performance of sows and piglets was evaluated, such as weight gain, average daily gain, feed conversion, protein efficiency ratio, incidence of diarrhea and mortality, and blood profile and blood chemistry. With the use of microbial preparation weight gain, weight gain, average daily gain, feed conversion and protein efficiency ratio varied (P<0.05) between treatments, indices of diarrhea and mortality were lower (P <0.05) in T2. Blood profiles and blood biochemistry differed (P>0.05) between treatments (T1 and T2). In conclusion, the use of microbial preparation in the diet of pigs improves the productive parameters, decreases the incidence of diarrhea and blood profiles vary in some cases in the categories studied

Keywords: probiotic, waste agro-industry, piglets, health.

RESUMEN

El objetivo de este estudio fue evaluar el comportamiento productivo en cerdas madres, así como los perfiles hematoquímicos e incidencia de diarreas en lechones al emplear un preparado microbiano fermentado en subproductos de la agroindustria. Se emplearon 14 cerdas reproductoras, híbridos CC21 (Yorkshire – Landrace/ L35 Duroc) y su descendencia, distribuidas en dos grupos: T1, Control y T2, preparado microbiano A. T2 contenía *L. acidophillus* y *K fragilis* L-4 (UCLV). El aditivo microbiano fue desarrollado en un sustrato compuesto por melaza, levadura de torula y leche de soja. Se aplicó 20 mL a las madres desde el último tercio de la gestación hasta el destete; y a las crías 1 mL en mono dosis durante 2 semanas y posteriormente fue aumentado a 1.5; 2 y 3mL por animal respectivamente. Se utilizó un diseño completamente aleatorizado. Se evaluaron, comportamiento productivo a las madres y las crías, ganancia de peso, ganancia media diaria, conversión alimenticia, relación eficiencia proteica,

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incidencia de diarrea y mortalidad, perfiles hemáticos y bioquímicas sanguíneas. Con el uso del preparado microbiano, la GP, GMD, CA, y PER varío (P>0.05) entre tratamientos, los índices de diarrea y muertes fue menor (P<0.05) en el T2. Los perfiles hemáticos y bioquímica sanguínea difirieron (P>0.05) entre tratamientos (T1 y T2). En conclusión, el uso del preparado microbiano se logra mejorar los parámetros productivos, disminuye la incidencia de diarrea y los perfiles hemáticos varían en algunos casos en las categorías estudiadas.

Palabras clave: probióticos, residuos de agroindustria, lechones, salud

INTRODUCTION

In the last decade, the swine industry has been interested in improving the efficiency of production by increasing the number of offspring per female at lower cost, with the reduction of days of lactation, in the stage of the physiological development of the pig. This situation leads to the late establishment of the natural microbiota in the digestive tract, which causes the consequent appearance of enteric diseases, product of the rapid colonization of pathogenic microorganisms in the host (Betscher *et al.,* 2010; Rondón *et al.,* 2013; Ciro *et al.,* 2015). Diarrhea is a complex syndrome in newborn piglets, whose causes may be due to a transfer of passive, epidemiological, etiological, host and environmental immunity. Clinically, this syndrome occurs from the first 12 hours of life, is characterized by the excretion of watery and profuse stools, progressive dehydration, acidosis and in severe cases causes death, mainly when there are primary or secondary bacterial infections by endotoxins (Diaz *et al.,* 2013).

Inclusion of microbial additives in the diet of pigs could be an alternative to improve health and increase production yields (Sun *et al.,* 2015). Its consumption favors: a) improving the immune system in a non-inflammatory way, b) reducing distention problems due to gases, c) improving digestion and absorption of nutrients (Escobar *et al.,* 2015), d) producing chain fatty acids short, e) participates in the synthesis of vitamins, mainly B complex (Datt et al., 2011), f) produces its own bacteriocins, a family of bioactive peptides with bacteriostatic activity that exerts its function on Gram-positive germs, g) improves intestinal immunity and prevents the development of *Rotavirus, E. Coli, Salmonella* spp., among others, organisms that cause diarrheal processes (Jurado *et al.,* 2013), h) reduces the micellar solubilization of cholesterol (Pajarillo *et al.,* 2014) and i)) improves the productive parameters in the animal (Haffner *et al.,* 2016). The objective of this study was to evaluate the productive behavior of the mother sow, as well as the hematochemical profile and the incidence of diarrhea in their offspring

MATERIALS AND METHODS

Place of the study

The microbial preparation was obtained in the laboratory of fermentation of Centro de Investigaciones Agropecuarias (CIAP), laboratory of microbiology of the facultad de ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas. The field experiment was developed in the swine production unit UEB "Javierito", city of Mataguá, located at 22 ° 09'00 "LN 79 ° 58'35" LO, 230 meters above sea level (masl), with an annual rainfall: 115 mm, temperature average 24.2 ° C and annual relative humidity 82.97%.

Animal's accommodation and basal diet

Used 14 sows of the third calving CC21 (Yorkshire-Landrace / L35 Duroc) and their descendants, distributed in two groups of seven animals each (control T1 and Probio-A T2). The sows were housed in cement floor cubicles, with a density of 1.8 m2 per animal from 80 to 110 d of gestation. From there to weaning they were placed in individual cages (maternity), this was maintained at 28 ° C during the first two weeks after calving, then reduced by 1.5 ° C every week until weaning. The sows were fed a commercial diet (Table 1) adjusted to the nutritional requirements according to NRC (1998) twice a day, at 08:00 and 16:00 h. (food and water *ad libitum*). The photoperiod was controlled with 12 h of light and 12 h of darkness. The litters of each treatment were located distant from each other (with a cage on both sides of the aisle) to avoid self-inoculation. The piglets at birth received the corresponding veterinary attention. At weaning the piglets of each of the treatments (without altering the groups of origin) were grouped into groups of 20 little pigs, these were housed in collective pens of 6 x 6.5 m. Piglets from 10 days of age until the completion of this experiment received a commercial diet and water *ad libitum* that meets the nutritional requirements according to the NRC (1998) for each stage of the physiological development of piglets.

Microbial preparations

The strains used in the microbial culture were: *Kluyveromyces fragilis* (L-4 UCLV) from the Microorganisms Bank of the Universidad Central "Marta Abreu" of Las Villas (Villa Clara, Cuba) and the strain *Lactobacillus acidophilus* from the Villa

Clara strains bank. These were activated in skim milk at 37 °C for 24 h. To obtain the microbial preparations, a mixture of sugarcane molasses, torula yeast and soy milk was used as a substrate and fermented at 37 ºC for 24 h according to the methodology described by Miranda *et al.* (2017). In the microbial preparation A. *L. acidophilus* and *K. fragilis* (L-4 UCLV) were used. The chemical composition and microbial concentration of the microbial preparation contains: 20% dry matter (DM); 16% crude protein (CP); 13.5% true protein (TP); 3.1% ether extract (EE); 3.2% ash (Cz); 3.88 pH; $9x10^7$ UFC/mL microbial concentration; 0.73 mmol/ml lactic acid and 94% viability.

Table 1. Bromatological characteristics of the food used for piglets

Indicators	Crude protein %	Ethereal extract %	Crude fiber %	Ash %	Dry matter %
Preweaning	19.0	4	3	6	90
Weaning	17.8	4	4	5	88
Increase	16.7	4	4	6	85

Supply of microbial additives to the animals

The sows of the treated group (T2) received 20 ml of the microbial culture, every 3 days from 80 d of gestation until weaning. The piglets descended from the breeding sows of group T2 continued to receive the same additive as their mothers. First dose applied to the piglets was at 10 d of age. Dosage, orally, varied according to age: 1 ml of 13 to 33 d of age; 2 ml from weaning to 50 d of age, from there to the end of the experiment, 3 ml per animal. Control group (T1) received physiological saline in the same amount as the treated groups (T2). Microbial additive is administered at 07:00 h.

Response variables

Weighing of animals and productive indexes

The weighing of the breeding sows was carried out at the beginning (80 d of gestation) of the experimental period, at parturition and finally at weaning, by means of a Roman scale (URKO) of 500 Kg capacity, previously calibrated with an error of ± 0.50 g . The piglets of each litter were weighed at birth and at 14; 33; 50 and 70 d of age. For the weighing of the piglets, two Roman manual scales (URKO) of 20 and 50 kg were used for each period, previously calibrated. With this information, we calculate the weight gain (WG), the average daily weight gain (ADG), food conversion (FC) and the protein efficiency ratio (PER), the latter, from weaning.

Experimental procedure for the taking and analysis of blood samples

Nine piglets of each treatment were selected at random, these were immobilized and extracted 8 mL of blood from the eye vein, using a California type needle, at 33 and 70 d of age. The blood was collected in Vacutainer test tubes, with and without ethylenediaminotetraacetic (EDTA) and were transferred to the laboratory within the first hour after the extraction of the blood for further processing. The evaluation of the blood profile consisted of the determination of hemoglobin, hematocrit and total leukocytes, by means of the methodology described by Colina *et al.* (2010). Indicators of the blood biochemistry were determined total proteins, albumin and total cholesterol, by means of the methodology described by Londoño & Parra (2015).

Control of diarrhea and mortality

All the animals under study were subjected to a rigorous clinical control to detect behavioral changes or diarrheal disorders, being able to analyze each animal independently. Information was recorded daily in all the animals that were subjected to the experiment.

Statistical Analysis

Experimental data were processed with the statistical package Statgraphics plus 15.1 for Windows. Analysis of variance was performed according to the completely randomized experimental design, and in the necessary cases Duncan's comparison test (1955) was applied to discriminate differences between means at $p<0.05$.

RESULTS

Productive behavior of sows

Table 2, shows the productive behavior of the sows. Weight gain in the last third of gestation was greater in T2. The live weight of the piglets at birth and the weaning weight was higher ($P<0.05$) in the animals that consumed the microbial culture (T2). Weight loss in mothers during lactation was lower ($P=0.0012$) in T2 (10.03 kg). The average weight of the litter and the number of live born piglets was higher ($P=0.0023$ and $P=0.0011$ respectively) in T2.

Productive behavior and health of piglets

In Table 3, the productive behavior of the piglets is reported. WG and ADG of the piglets in all the measurements (14, 33, 50 and 70 d of age) was higher (P <0.05) in the T2 with respect to the animals of the control group. In the period of 50-70 d of age there was higher WG and ADG in the animals that received the microbial preparation. With respect to FC and PER, the differences were different (P <0.01) between treatments (see Table 3).

Table 2. Productive parameters of the sows when adding microbial preparation in gestation and lactation.

Indicators	Treatments		SEM	P
	T1	T2		
Weight at the start, kg (80 days gestation)	168,15	167.95	0.23	0.2822
Weight at birth, kg	174,20b	176,45a	0,12	0.0251
Weaning weight, kg	162,25b	166,42a	0,23	0.0012
Weight gain in the last third of gestation, kg	6,05b	8,50a	1,23	0.0123
Weight loss in breastfeeding, kg	11,95b	10,03a	0.14	0.0012
Average litter weight, kg	1,05b	1.54a	0.24	0.0023
Piglets born alive per litter, U	10.82b	11.85a	1.25	0.0011

a,b different superscripts in the same row differ P< 0,05 (Duncan, 1955)

Table 3. Productive behavior of piglets descended from mothers who consumed microbial preparation.

Periods	Indicators	Treatments		SEM	P
		T1	T2		
Start	Birth weight, kg	1,05b	1.54a	0,21	0.0002
0-14 days	Weight gain, kg	3.16b	3.51a	0,13	0.0241
	Average daily gain, g	225b	250a	0.10	0.0011
14-33 days	Weight gain, kg	7,12b	8.16a	0,11	0.0127
	Average daily gain, g	368b	429a	1.21	0.0012
33-50 days	Weight gain, kg	6.11b	7.16a	0,13	0.0025
	Average daily gain, g	359b	421a	1.02	0.5621
	Feed conversion	1.26a	1.07b	2.12	0.0024
	Protein efficiency ratio	0.321b	0.378a	1.21	0.0214
50-70 days	Weight gain, kg	8.89b	9.52a	0.03	0.0121
	Average daily gain, g	444b	476a	0.12	0.0245
	Feed conversion	2.13a	1.99b	3.21	0.0124
	Protein efficiency ratio	0.508b	0.544a	2.12	0.0012

[a, b] different superscripts in the same row differ P< 0,05 (Duncan, 1955)

Table 4 shows the incidence of diarrhea and percentage of mortality. In all measurements (0-70 d of age), the incidence of diarrhea and percent mortality was lower (p <0.05) in the animals that consumed the microbial culture (T2) than in the control group. In the period between 33-50 d of age there was a greater number of animals with diarrhea and deaths, however, in T2 it was lower (P <0.05) with respect to the animals of group T1. In Table 5, the hematochemical behavior of the piglets is observed. In the evaluation performed at 33 and 70 d of age, the hemoglobin indices varied between treatments (P <0.05), however, the values of hematocrits and total leukocytes did not vary between groups, in both measurements. The total proteins, total cholesterol and albumin differed (P <0.05) between treatments, of these the highest (P <0.05) was in the animals of the control group (T1) with 33 and 70 d of age.

DISCUSSION

Results obtained with respect to the productive parameters of the mother sows of the present study

(Table 2) possibly were due to the use of the culture mix developed in by-products of the agro-industry, in effect to a greater process of the digestion and absorption of the nutrients, together with the gestational anabolism in the sows, which allowed a better use of their nutrients, favoring the growth of the fetus in the final stage of gestation, being the cause of the results obtained in terms of fetal growth, absence of abortions, fetuses momeficados and deaths in offspring (Betscher *et al.*, 2010, Ciro *et al.*, 2015). All the above, could have led to a better productive behavior in pregnancy and lactation, in the latter, managed to reduce weight loss in animals. Similar results were reported by Ayala *et al.* (2015), by adding probiotics obtained from lactic acid bacteria in the rations of sows in the last stage of gestation, which corroborates our hypothesis that the use of mixed cultures of lactic acid bacteria plus yeast positively influences the manifestation of a better productive behavior. In relation to this last Brea *et al.* (2014) observed piglets with higher birth weight when the sows in pregnancy consumed a mixed culture of *L. acidophilus* and *L. rhamnosus*. However, Liu *et al.* (2013) when *Bacillus subtilis* was used at twenty-one days before calving, they did not find differences in the number of pigs born alive.

Table 4. Sanitary behavior of the piglets that consumed the microbial preparation in the different productive stages

Age (days)	Indicators	Treatments		SEM	P
		T1	T2		
0-14	Incidence of diarrhea	28.05b	8.93a	0,13	0.0241
	Mortality, %	8.22b	1.22a	1.23	0.0175
14-33	Incidence of diarrhea	34,45b	12.33a	0,11	0.0273
	Mortality, %	8.23b	3.67a	0.11	0.0123
33-50	Incidence of diarrhea	48.75b	23.54a	0,13	0.0022
	Mortality, %	18.35b	7.65a	3.01	0.0012
50-70	Incidence of diarrhea	29.06b	15.83a	0.03	0.0121
	Mortality, %	9.65b	4.39a	0.24	0.0024

[a,b] distinct letters in the same row differ P< 0,05 (Duncan, 1955)

Table 5. Behavior of hematochemical indicators of piglets that consumed probiotic

Age (days)	Parámetros	Indicators	Treatments		SEM	P
			T1	T2		
33		Hemoglobin, g/L	110.21b	111.23a	2.12	0.0125
	Hematics	Hematocrit, L/L	0.382	0.384	1.13	0.2251
		Total leukocytes, x10^9/L	12	12	1.23	0.1254
	Biochemicals	Total proteins, g/L	54.10a	51.71b	1.21	0.0012
		Albumin, g/L	43.11a	41.25b	2.31	0.0032
		Total cholesterol, mmol/L	1.28a	1.11b	0.34	0.0151
70		Hemoglobin, g/L	112.12b	113.21a	0.23	0.0021
	Hematics	Hematocrit, L/L	0.375	0.382	0.12	0.0623
		Total leukocytes, x10^9/L	13	13	1.20	0.1251
	Biochemicals	Total proteins, g/L	58.21a	57.26b	0.12	0.0125
		Albumin, g/L	44.12a	43.04b	1.12	0.1251
		Total cholesterol, mmol/L	1.28a	1.18b	2.31	0.0112

[a,b] distinct letters in the same row differ P< 0,05 (Duncan, 1955)

The results of the productive parameters in the piglets probably occurred due to the microorganisms used, since the use of these probiotic organisms acts as growth promoters in the pigs, which could contribute to a greater assimilation of the nutrients, thus improving the nutritional conversion (Datt *et al*., 2011). At weaning it can reduce the stress caused by changes in diet, as a result of the above, it helped to improve the weight gain in all the stages studied (Miranda *et al.*, 2017). At the same time, the use of different microorganisms added to the diet could act in different ways, as is the case of yeasts that act through the symbiosis of bacteria, as well as, in the hydrolysis of disaccharidases and inducing the effects adhesives and antagonists against pathogens, consequently improved the productive performance of piglets supplemented with microbial preparation at different ages. Similarly, Datt *et al.* (2011), Miranda *et al.* (2017) observed greater weight gain in response to the use of probiotics in the diet of weaned piglets; which indicates that efficient microorganisms act as growth promoters in piglets in the different productive stages (Escobar *et al.,* 2015). Flores *et al.* (2015) also reported an increase in WG and ADG by adding lactic acid bacteria developed in agro-industry byproducts in the basal diet of young pigs. Similarly, Etleva *et al.* (2010), Sun *et al.* (2015) observed the improvement of WG and FC when the piglets consumed foods containing probiotics obtained from Lactobacillus and Enterococcus, supplemented every 3 days, under tropical conditions. However, Navas *et al*. (1995) did not observe the weight gain in the piglets in the growth stage.

Lower presence of diarrhea and deaths in piglets that consumed microbial preparation (Table 4) could be due to the improvement of the immune system, the inhibition of the development of pathogenic microorganisms in the gastrointestinal tract of the piglet, preserving its function in the protective barrier by inhibiting cellular of organisms such as *E. coli* and *Salmonella* spp., agents that cause diarrhea and deaths in the host. Ihara *et al*. (2013) reported the production of antimicrobial substances such as bacteriocins and organic acids by probiotic organisms. Similar results were reported by Pajarillo *et al.* (2014) who when adding probiotics in pigs observed the reduction of diarrheal disorders and death in the days after weaning. While Ihara *et al.* (2013) reported an improvement with diarrheal disorders caused by *Salmonella* spp. On the other hand, Jurado *et al.* (2013), Rondón *et al.* (2013) reported a decrease in the amount of pathogens present in the intestinal lumen due to the antimicrobial substances produced by Lactobacillus in young pigs.

Hematological changes and the blood biochemistry of piglets in the present study could be related to ontogenetic changes, the increases in total proteins, as well as the beta/alpha ratio, are congruent with the increase in serum cholesterol, as occurs in pigs with pattern LDL reported by, Londoño & Parra (2015). Ayala *et al.* (2015) observed to improve the balance in the natural microbiota of the gastrointestinal tract causing changes hematic in weaned piglets.

Meanwhile, Liu *et al.* (2013) reported competition for adhesion sites and for nutrients, by influencing the reduction of digestive problems and improving health in pigs. Colina *et al*. (2010), coincide in part with the results obtained in this study, which confirms the beneficial effect of microbial preparations on the performance of the host's natural microbiota against pathogenic microorganisms. In this way, the digestive system cof the animal could be better prepared to face possible pathogens (Ciro *et al.*, 2015).

CONCLUSSION

Under the conditions of this study it can be concluded that the use of a microbial preparation reduces diarrheal and deaths, improves the hematochemical indexes of the blood and the health of the breeding sows and their offspring, all this has an effect on the increase of behavior productive in the different productive stages of the pig.

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