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Original

Effect of bioadditives on bioproductive indicators of nulliparous guinea pigs (*Cavia porcellus*) and their offspring

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ABSTRACT

Objective. Evaluate the effect of bioadditives on the bioproductive indicators of nulliparous guinea pigs (*Cavia porcellus*) and their offspring. **Materials and methods.** A total of 40 improved nulliparous guinea pigs, 125 days old, 1450 g live weight, were used, divided into four groups of 10 guinea pigs each. T1, Control (basal diet without additive); T2, bioadditive with *Lactobacillus acidophilus*. T3, bioadditive with *Kluyveromyces fragilis* and T4, bioadditive with *L. acidophillus* and *K. fragilis*. A completely randomized design was used where weight gain during pregnancy, age at first delivery, percentage of fertility, conception index, health, and values of hemoglobin, hematocrit and mean corpuscular volume were evaluated. **Results**. Animals that consumed bioadditive T4, weight gain during pregnancy was greater (p<0.05); the age at first delivery was reduced (p<0.05); the fertility percentage and the conception index were better (p<0.05) and the occurrence of diarrhea was lower (p<0.05) in the T4 group. Hematological values improved in all groups that consumed biopreparations. **Conclusions**. The additives with *L. acidophilus* and *K. fragilis* improved the productive and reproductive indicators in primiparous guinea pigs. In addition, it intervenes in the improvement of health and hematological values.

Keywords: *Lactobacillus acidophilus*; *Kluyveromyces fragilis*; reproductive indicators; hemogram; health (Source: Tesauro de biología animal IEDCYT)

RESUMEN

Objetivo. evaluar el efecto de bioaditivos sobre los indicadores bioproductivos de cobayas (*Cavia porcellus*) nulíparas y sus crías. **Materiales y Métodos**. Se emplearon un total de 40 cobayas nulíparas mejoradas, con 125 días de edad, 1450 g de peso vivo, repartidas en cuatro grupos de 10 cobayas cada uno. T1, Control (dieta basal sin aditivo); T2, bioaditivo con *Lactobacillus acidophilus*. T3, bioaditivo con *Kluyveromyces fragilis* y T4, bioaditivo con *L. acidophillus y K. fragilis*. Mediante un diseño completamente aleatorizado se evaluó: ganancia de peso durante la gestación, edad al primer parto, el porcentaje de fertilidad, índice de concepción, salud y los valores de hemoglobina,

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hematocrito, y volumen corpuscular media. **Resultados.** En los animales que consumieron bioaditivo T4, la ganancia de peso durante la gestación fue superior (p<0.05); la edad al primer parto se redujo (p<0.05); el porcentaje de fertilidad e índice de concepción fue mejor (p<0.05) y la ocurrencia de diarrea fue menor (p<0.05) en el grupo T4. Los valores hematológicos se mejoraron en todos los grupos que consumieron biopreparados. **Conclusiones.** Aditivos con *L. acidophilus* y *K. fragilis* mejoraron los indicadores productivos y reproductivos en cobayas primíparas. Además, interviene en el mejoramiento de la salud y los valores hematológicos.

Palabras clave: *Lactobacillus acidophilus*; *Kluyveromyces fragilis*; indicadores reproductivos; hemograma; salud (Fuente: Tesauro de biología animal IEDCYT).

INTRODUCTION

The production of guinea pig meat in the Andean countries (Bolivia, Ecuador and Peru) increased significantly in the last decade, the increase in consumption of this product in the local population, as well as its growing export to the United States of America (USA) and Europe are the reasons for this increase (1,2). According to Jurado et al (3) the consumption of guinea pig meat in Ecuador is close to 13 million animals per year, with 2.1 kg live weight, which represents 26,590 tons (t) of meat consumed per year. According to Canto et al (2) and Núñez (4) in Ecuador, the per capita consumption of guinea pig meat is 700 - 800 g per person per year.

On the continent, Peru is the country in the region with the largest export of guinea pig meat to the world (71.3%); while Ecuador registers 28.7% of exports (5.6). The high migratory movement of South Americans to the United States and Spain opened the market for this product and the consumption of this meat is gradually increasing. Currently in the US approximately 105.7 t/year is consumed, while in Spain 188.8 t annually (7). However, the production of guinea pig meat in the countries that produce this species is still low (5.067.749 animals/year), since most of this production only partly supplies the local demand (8,9,10).

Livestock producers in order to produce a greater number of animals (cattle, pigs, chicken, guinea pigs, among others) per area, thus shoveling productive demand. In most of the livestock industries, growth promoter antibiotics (APC's) were used, because these additives helped improve weight gain, and in turn reduced morbidity and mortality in animals, becoming one of the economic alternatives in animal production (2.11). However, the excessive use of these products caused a problem for the health of the final consumer, since various studies (6,9,12) confirm possible residues of antibiotics present in final products of animal origin such as milk, meat and eggs (13), which could generate resistance to certain antibiotics that are used to treat people (2). This generates alteration of the intestinal microbiota, by causing a decrease in bacteria that compete with pathogens, which increases the risk of disease (11), on the other hand, it is also associated with allergic and toxic problems (14). Most of the APC's residues are generally stored in the liver, muscles, kidneys and in the subcutaneous tissue (shells) (7,15).

One of the alternatives to replace the APC's in the livestock industries is the use of microorganisms with probiotic action, because these partially fulfill the function of a natural growth promoter, they are also capable of reducing the symptoms of stress, evidencing a clear advantage over APCs, and have no withdrawal time (16). In this sense, some studies (11,17), show positive effects on the productive index of guinea pigs (11,12,15), especially in the control of pathogenic agents and mortality (10), while, during lactation the pups improve weight gain, which would be indirectly related to the increase in milk production in the mother (7,8,12). The following objective was set in the study, to evaluate the effect of bioadditives on the bioproductive indicators of nulliparous guinea pigs (Cavia porcellus) and their offspring.

MATERIALS AND METHODS

Area of study. The study was developed in the livestock farm "La Caldera", Sidcay parish, Cuenca Canton, Azuay Province, Ecuador. The place is located at 2° 49′ 29.66″LS longitude 78° 58′ 16.19″LW, 2,548 meters above sea level, average temperature 15ºC, annual relative humidity 78% and annual rainfall 300– 600mm

Experimental Management system. Conditioning of the shed: Prior to receiving the animals, extreme biosecurity measures were taken based on the methodology used by N'Goran et al (18), which allowed the zoosanitary control of the guinea pigs during the study. For this purpose, glutarhaldeide, quaternary ammonium and isopropyl alcohol (Viroguard®/ Lima, Peru) were used in doses of three cubic centimeters (cc) / liters (L) as disinfectant, as described by Sánchez et al (19).

Animals used. A total of 40 commercial primiparous guinea pigs were used, live weight (LW) 1450 ± 50 grams (g) weighed on a 5 kg scale (Camry, China) with error ± 0.25 g capacity, and 125 ± 5 days of age.

Activation of microorganisms and obtaining Bioadditive. The ATCC (American Type Cultures Collection, USA) strains were used to ferment the substrates: *L. acidophilus* and *K. fragilis*. For the activation of the strains that came in lyophilized format and subsequent obtaining of the microbial biomasses, what was described by Miranda et al (20) was followed.

The substrates obtained from agroindustrial residues from the Agroindustry Laboratory, Faculty of Agricultural Sciences, University of Cuenca, received physical treatment prior to mixing. The bioadditives under study (Treatment 2, 3 and 4) were obtained by mixing, composed of: 30% sugar cane molasses, 60% vinasse must and 10% biomes previously obtained by Miranda et al (20). After making the mixture in the aforementioned percentages, it was homogenized and then fermented for 48 hours at 37 ± 2ºC, according to the indications described by Miranda et al (20). In treatment T2, substrate fermented with *L. acidophilus*. T3, substrate fermented with *K. fragilis* and T4, substrate fermented with *L. acidophilus* and *K. fragilis.*
Experimental design and treatments: a completely randomized design with 10 repetitions was used. Breeding guinea pigs were divided into four groups of 10 animals each. The bioadditives with probiotic action were supplied to the reproductive females of treatments T2, T3 and T4 daily inoculated in the basal diet at 07:00, as described in table 1.

Table 1. Treatments evaluated in the study.

Groups	Variants of treatments
T1	Basal diet without probiotic
T2	Basal diet plus 1.00 mL of bioadditive *with L. acidophillus* (7.4 x10^6 CFU/mL)
T3	Basal diet plus 1.00 mL of bioadditive with *K. fragilis* (7.4 x10^6 CFU/mL)
T4	Basal diet plus 1.00 mL of bioadditive with *L. acidophillus* and *K. fragilis*

Accommodation and basal diet. Guinea pigs were housed in collective pens of 2.00 square meters (m2), with a cement floor and a bed of rice husks (Oryza sativa), with a density of 10 females per m2. The basal diet used for the guinea pigs was composed of 20% alfalfa + 30% maralfalfa + 25% king grass and 25% barley grains plus 0.03 g of Vitamin C per animal. Each breeding guinea pig will receive 200 g of previously formulated basal diet; as indicated by Szendrő and Dalle (21). The rations were offered twice a day in the same proportion, between the hours of 07:00 and 16:00. The bromatological composition of the diet offered to the animals is described in table 2; according to the recommendations described in the NRC (22) that meet the minimum requirements established for guinea pigs. 50 mL of water was also offered daily in automatic drinkers (Plasson, SKU: 885B722-8, Argentina).

Table 2. Bromatological composition of the food offered to the animals.

Components (% DB)	Basal diet
dry matter	60
Crude protein	18.35
True protein	14.25
Energy (MJ/Kg) 12.65	12.65
Crude fat	3.52
Ashes	3.35

DB: dry basis

Environmental management. The ambient temperature of the house and the room was maintained at 16 and 18°C, respectively. Site lighting was controlled with 12 h of light and the same amount of darkness. The relative humidity of the building was maintained at 58%. The pools of each treatment were separated from each other, with a distance of two meters, to avoid interference between treatments. All guinea pigs and their offspring in the study received appropriate veterinary care as described by N'Goran et al (18).

Evaluated variables. productive indicators: the nulliparous guinea pig mothers under study were weighed at the beginning, also at 15, 30 and 45 days of gestation and at delivery, with this information the weight gain (GP) was calculated. The animals were weighed on a digital scale (Camry, China) with a capacity of 5.00 kg with an error of ± 3 g.

Reproductive indicators. Breeding females were evaluated for age at first observed heat, age at first conception, and age at first calving; in addition, the percentage of fertility and conception rate. Likewise, the number of kits born alive and stillborn, the total number of pups born per breeder, the weight of the litter and the weight of the male and female kits at birth were recorded.

Diarrheal cases and number of deaths. mother guinea pigs and their offspring of all groups (T1, T2, T3 and T4) underwent strict clinical control, as described by Szendrő and Dalle (21), behavioral changes such as diarrheal disorders and deaths were detected independently, because all the animals were identified with earrings. Hematological indicators: The blood sampling of the guinea pigs was performed at the beginning and at 30 d of gestation, the seven guinea pigs of each treatment were selected through a completely randomized design. With prior immobilization of the animals, 2.00 mL of blood were extracted from the lateral saphenous vein. Blood extraction was performed with a 22-gauge hypodermic needle, with a depth of 2.5 inches (in) in diameter, the blood was deposited in vacutainer tubes, with and without ethylenediaminetetraacetic acid (EDTA, PEDTM, china). The samples were transferred to the Clinical Laboratory of the Faculty of Agricultural Sciences, University of Cuenca, in the first three hours after collection, which were subsequently analyzed. The evaluation of the blood profile consisted of the determination of hemoglobin (Hb), hematocrit (HCT), and mean corpuscular volume (MCV), as described by Jurado et al (14).

Statistical analysis. The data obtained in the study were analyzed using the statistical package SPSS v. 26, windows (23). Using the completely randomized design, the analysis of variance was performed and, where necessary, Duncan's comparison test was applied to differentiate between the means at $p<0.05$ (24).

Ethical aspects. The selection and use of the biological material used (primiparous guinea pigs, biopreparations with probiotic action) was carried out in a timely manner and which allowed the reduction of generating a harmful effect on the environment. On the other hand, there was strict compliance with animal bioethics during the handling and maintenance of biological media.

RESULTS

Table 3 summarizes the values obtained in regard to the productive parameters in primiparous guinea pigs. In the evaluation carried out at 15 d of gestation, there was no difference ($P>0.05$) in live weight between the treatments; while, in the weighing carried out at 30 and 45 days of gestation and at delivery, the females that consumed biopreparations with probiotic action from the groups (T2, T3 and T4) obtained better weight gains, and of these the one with the highest ($p<0.05$) weight was T4.

Table 3. Live weight changes of guinea pigs during pregnancy with the inclusion of probiotics.

Gestation days, g	Treatments				EE	p-valor
	T1	T2	T3	T4		
Beginning	1410	1430	1420	1390	0.10	0.554
15	78.3	80.6	82.3	84.3	0.12	0.542
30	112.7^{c}	113.3^{bc}	120.3^{b}	130.4^{a}	0.11	0.024
45	118.3^{c}	135.4^{cb}	145.3^{b}	160.4^{a}	0.03	0.031
Parto	520.3^{c}	540.5^{bc}	542.3^{b}	612.4^{a}	0.09	0.012
WG, gestation	740.3^{c}	752.4^{cb}	760.5^{b}	818.3^{a}	0.11	0.001

^{a,b,c} different superscript letters in the same row differ at $p<0.05$ (Duncan 1955). **T1,** basal diet control without additive. **T2**, basal diet + bioadditive with *L. acidophilus*. **T3**, basal diet + bioadditive with *K. fragilis*. **T4**, basal diet + bioadditive with *L. acidophilus* and *K. fragilis*. **EE**, standard error. **WG**, weight gain. **g**, grams. **kg**, kilogram.

The values of reproductive indicators in primiparous guinea pigs when biopreparations with probiotic action are included in the animals' diet are summarized in Table 4. The age at first heat, first conception and first parturition were significantly reduced ($p < 0.05$) in the primiparous guinea pigs that consumed the bioadditives with probiotic action (T2, T3 and T4), of these the T4 group was the one with the greatest days of reduction with respect to the other treatments and the control ($p<0.05$).

Fertility percentage and conception rate were higher ($p<0.05$) in T4 treatment (5%) compared to control group animals and 1% compared to the other groups (T2 and T3) under study. Regarding the number of guinea pigs born alive per litter, there were no significant changes ($p>0.05$) between the treatments under study. The weight of the litter was higher ($p<0.005$) in the T4 treatment (+83 g), over when compared to the control group and higher by 50 and 60 g of live weight over the T2 and T3 groups, respectively. The weight of the female and male offspring at birth was higher ($p<0.05$) in the T4 group (40 and 35 g), compared to the group of animals that did not consume the bioadditives with probiotic action.

The indicators corresponding to the number, percentage and weight of male and female guinea pigs individually and the weight of the litter at weaning were higher ($p<0.05$) in the group of animals treated (T2, T3 and T4) with the bioadditives (Table 4), within the treated ones, the pups from the mothers that consumed the T4 treatment, were the ones with the best behavior. Table 5 reports the behavior of health (occurrence of diarrhea and % mortality) and blood changes in primiparous guinea pigs during the gestation period. In animals of the groups (T2, T3 and T4); that consumed diets containing bioadditives with probiotic action had a lower occurrence of diarrheal disorders compared to animals in the control group. In all study groups there were no deaths in this period. The hematic profile (Hb, HTO, VCM) of the guinea pigs, at 15 d of gestation did not show significant differences. With the inclusion of microbial preparations, in the measurement made at 30 days of gestation, hemoglobin and hematocrit values improved ($p<0.05$) compared to the control group. Regarding the MCV values, they did not differ between the groups of animals (T1, T2, T3 and T4) treated in the measurements made (at the beginning and at 30 days of gestation).

Table 4. Reproductive indicators of primiparous guinea pigs when including probiotics in the diet.

Indicators		Treatments				EE	p-valor
		T1	T2	T3	T4		
Age at the beginning of the study, d		125	124	125	122	0.10	0.587
Age at 1st heat observed, d		142^{a}	131^{bc}	132^{bc}	128^{c}	0.26	0.041
Age at first conception, d		150^{a}	139^{b}	139^{b}	134^{c}	0.12	0.042
Age at first calving, d		215^{a}	206^{b}	206^{b}	201^{c}	0.11	0.012
Fertility, %		94^{b}	98^{a}	98^{a}	99^{a}	0.08	0.048
Conception rates, %		91^{c}	95^{b}	96^{b}	99^{a}	0.10	0.751
Number of live born pups, A		2.8^{b}	2.9^{b}	2.7^{c}	3.1^{a}	0.03	0.031
Number of pups per litter, U		2.6	2.6	2.7	3.0	0.12	0.571
Litter weight at birth, g		402^{c}	424^{b}	435^{b}	485^{a}	0.11	0.005
Weight of guinea pigs at birth	Males, g	109^{c}	116^{b}	120^{b}	149^{a}	0.18	0.001
	Females, g	102^{c}	107^{b}	109^{b}	137^{a}	0.09	0.012
Number of pups weaned, A		2.2^{c}	2.9^{b}	3.5^{ab}	3.8^{a}	0.08	0.024
Percentage of pups weaned, %		68^{c}	78^{b}	88^{ab}	95^{a}	0.12	0.012
Weaning weight	Males, g	280^{c}	340^{b}	350^{b}	414^{a}	0.07	0.002
	Females, g	242^{c}	318^{b}	329^{b}	384^{a}	0.10	0.021
Weaning litter weight, g		650^{d}	968^{c}	1225^{b}	1560^{a}	0.08	0.011

$^{a, b, c}$ different superscript letters in the same row differ at $P < 0.05$. **T1,** basal diet control without additive, **T2,** basal diet + bioadditive with *L. acidophilus*. **T3,** basal diet + bioadditive with *K. fragilis*. **T4,** basal diet + bioadditive with *L. acidophilus* and *K. fragilis*. **EE**. standard error. d, days. **U**. unit. g, grams. %, percent.

Table 5. Health behavior (occurrence of diarrhea and mortality) and hematological changes of primiparous guinea pigs during gestation when including probiotics in the basal diet.

Indicators		Treatments				EE	*p-valor*
		T1	T2	T3	T4		
Health,							
Occurrence of diarrhea		5.51^a	1.11^b	1.15^b	0.81^b	0.16	0.004
Mortality		-	-	-	-	-	-
Hematic Indicators							
Hemoglobin, g/L	Inicio	135.9	135.7	138.8	134.25	0.02	0.021
	30 d	138.8^b	149.5^a	150.2^a	151.1^a	0.05	0.032
Hematocrit, L/	Inicio	0.37	0.32	0.31	0.32	0.03	0.0511
	30 d	0.38^a	0.31^b	0.32^b	0.31	0.01	0.0401
VCM, fL	Inicio	79.80	79.70	78.54	79.54	0.02	0.0541
	30 d	76.52	81.02	82.52	82.03	0.01	0.0512

$^{a, b, c}$ different letters in the superscript of the same row differ at $P < 0.05$, by comparing mean proportions. **T1,** basal diet control without additive, **T2,** basal diet + bioadditive with *L. acidophilus*. **T3,** basal diet + bioadditive with *K. fragilis*. n, basal diet + bioadditive with *L. acidophilus* and *K. fragilis*. **EE**, standard error. **%**, percent. **g/L,** grams per liter. **L/L,** liter per liter. **fL** phyctoliter.

DISCUSSION

Productive behavior: the greater weight gain in primiparous females during pregnancy is possibly due to the inclusion of biopreparations obtained from substrates of agroindustrial waste fermented with lactic acid bacteria and yeasts with probiotic action in the diet of these animals, which that could intervene in the activation of the main structural amino acids present in the basal diet, the action of microorganisms, in addition to regenerating atrophied microvilli, improves the absorption of the main nutrients available in the intestinal lumen, which helps improve physiological development of the animal (1,5,10). The results reported in this study coincide in part with the values obtained by Portocarrero et al (7), who reported improvement in GP in young animals, achieving support in the physiological development of the animal. In this sense, Torres et al (25) demonstrated that the inclusion of up to 2.00 mL of probiotic obtained from L. acidophilus in the basal diet improved GP up to 80 g during gestation in guinea pigs. Studies reported by Valdizán et al (17); Cano et al (15) and Núñez et al (4), show a significant improvement in the productive behavior of animals when using probiotics obtained from fermentation with lactic acid bacteria and yeasts. For his part, Guevara et al (8) with the use of yeasts in the diet found improvements in the productive performance. However, despite consuming less (1.00 mL), the animals managed to obtain greater weight gain (100 g), consequently increasing the productive profitability in primiparous guinea pigs. Criollo et al (11) indicated that guinea pigs fed probiotics obtained greater weight gain from the fifth week of gestation, which is similar to the values obtained in the study.

Reproductive behavior: the primiparous breeding guinea pigs of the groups (T2, T3 and T4) of animals that consumed the biopreparations reduced the age of presenting the first heat, the first conception and the age of parturition, with respect to the animals of the control group. This effect was possibly due to the beneficial action of the microorganisms (L. acidophilus and K. fragilis) used in the diet of the animals. The days of presence of first heat, first conception and parturition were also reduced; which may be due to the increase in the efficiency of food utilization at the level of the intestinal lumen (2, 3, 11), which contributes to improving the body condition of the animal at delivery, and with it the reproductive indicators (15, 17 , 25).

The weight at birth in the present study was higher in the animals that consumed the biopreparations with probiotic action. Similar results to those reported by Xicohtencatl et al (12) and Mínguez et al (10) who found higher weight than control groups at birth with the use of probiotics in the diet of reproductive females.

For their part, the values obtained by Castro et al (5) agree in part with the results reported in the present study. While Rodríguez et al (9) reported average birth weights lower than those obtained in this study.

Sanitary indices: the lower occurrence of diarrhea in the animals of the treated groups (T2, T3 and T4), is associated with the efficient action of the bioadditives used in the study, because these natural products are capable of acting positively on the movement of Na+ and K ions, since these act on the intestinal wall, which improves the osmotic gradient; In addition, they are capable of normalizing the immune system, consequently, improving the health of the digestive tract and preparing to positively face possible aggressions from pathogens.

Valdizán et al (17) and Cano et al (15), by including microorganisms with probiotic action (bacteria and yeasts) in the diet of the different species of young animals, improved health, mainly of the gastrointestinal tract. For their part, Guevara and Carcelén (8) with the application of 2.00 mL of probiotic developed with bacteria of the genus Lactobacillus in the diet of guinea pigs reported lower values than those achieved in the present study. Although the dose applied in our research was lower (1.00 mL) compared to reports in other studies, the results achieved were higher than those obtained by other researchers (4, 5, 25).

The improvement of the health of guinea pigs could be given by the inclusion of bioadditives fermented with lactic acid bacteria and yeasts, because these organisms are capable of producing organic acids (lactic and acetic acid) as a secondary metabolite product, these are capable of varying the pH values at the level of the gastrointestinal tract that limit the presence of pathogenic agents that cause diarrhea processes (Salmonella spp.) in young guinea pigs. Several studies (9,11,15) report improvements in animal health when using microbial additives.

Cano et al (15) with the use of probiotics obtained from lactic acid bacteria significantly reduced ($p<0.05$) the presence of diarrheal disorders and improved the productive parameters in young guinea pigs. While, Rodríguez et al (9) did not show differences between the animals that consumed probiotics developed from the different species of lactobacilli, but the reported values are lower than those achieved in this study. While the indices reached by Jurado et al (3) who included a mixture of Lactobacillus, are similar to those achieved in this work.

In conclusion, the biopreparations included in the diet of primiparous guinea pigs improve the productive and reproductive indexes during pregnancy and lactation. Reproductive parameters were also improved in the groups that consumed the probiotics. Likewise, it is possible to reduce diarrheal disorders and mortality in the offspring.

Interest conflict

There is no conflict of interest.

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