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Article

Effect of Agro-Industrial Substrates Fermented with Lactic Acid Bacteria and Yeasts on the Intestinal Microbiota of Guinea Pigs (*Cavia porcellus*)

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Abstract: The aim of the study was to evaluate the impact of probiotics obtained from agro-industrial substrates fermented with lactic acid bacteria and/or yeasts on the health and changes in the microbiota of the digestive tract of guinea pigs. Eighty male guinea pigs, Kuri breed, 30 days old and 250 g live weight, were randomly selected and divided into four groups of 20 animals each. T0, Control, T1, *Lactobacillus acidophilus* and *L. bulgaricus*. T2, *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* and T3. *L. acidophilus*, *L. bulgaricus*, *S. cerevisiae* and *K. fragilis*. T1, T2 and T3 contained molasses-vinasse substrate in their base, the dose administered was 0.75 mL/animal orally. The parameters evaluated were weight gain, occurrence of diarrhea and mortality, blood profile, macroscopic lesions in the digestive tract organs and changes in the microbiota of the stomach, caecum, small and large intestine. Treatments T1, T2 and T3 improved weight gain ($p < 0.05$) and reduced the presence of guinea pigs with diarrhea ($p < 0.05$) and there was no mortality; hematic indices did not vary significantly ($p > 0.05$) between treatments; animals in the control group presented a greater amount ($p < 0.05$) of macroscopic lesions in the digestive tract organs; in the T1, T2 and T3 group there was an improvement in the natural microbiota. It is concluded that the inclusion of microbial additive in young guinea pigs improves intestinal health, consequently, improves weight gain, reduces diarrhea and deaths, and normalizes the natural microbiota of the gastrointestinal tract.

Keywords: probiotic; production; intestinal health; performance; intestinal flora

1. Introduction

In the Andean countries over the last decade, the consumption of non-conventional animal proteins such as guinea pig meat (*Cavia porcellus*) has been increasing, which has led to a significant increase in the production of this species in the rural sector in Ecuador [1]. In Ecuador, in the last two decades, per capita consumption of guinea pig meat has increased from 0.7 to 2.5 kg/year/person). The increase in demand for this meat has been due to its nutritional value [2,3]. However, the feed efficiency of guinea pigs reared in Ecuador is still not ideal, because producers in this sector have not yet implemented feeding strategies to improve animal performance [1,3].

In most developing countries, the use of additives such as antibiotic growth promoters still persist in animal production, with the aim of improving the health and increasing the productive parameters of animals [2,4]; however, the inappropriate use of these products has led to bacterial resistance, attributed to antibiotic residuals in the carcass, which creates a public health problem [2,5]. Probiotics consist of lactic and non-lactic bacteria, yeasts and fungi, which is important when supplementing monogastric or ruminants [6,7]. Probiotics also have beneficial properties as growth promoters, feed conversion improvers, gut flora modifiers, helping the animal to be more resistant to disease and reducing the use of antibiotics [8,9].

The use of microbial bioactive such as probiotics, prebiotics and symbiotic, emerges as a viable alternative to antibiotic growth promoters [10], with the premise of maintaining meat safety, improving animal welfare, the development of the gastrointestinal tract and the immune system [11], improving carcass yield, without leaving residues in the carcass [12]. Recent studies provide encouraging data on the inclusion of diets with beneficial microorganisms in the ability to balance the microbiota in the different segments of the gastrointestinal tract, which has attracted much attention from researchers [2]. However, scientific information on the positive effect of beneficial microorganisms and the mechanism of action is still insufficient (9, 13).

Previous studies on the use of agro-industrial substrates (molasses-vinasse) fermented with lactic acid bacteria and yeasts have shown significant ($p < 0.05$) increases in weight gain in pre-weaned and post-weaned piglets, chickens and cattle [7,13,14]. It has also been shown to improve health by reducing diarrhea disorders and deaths. However, there is still no scientific data showing a positive action on productive parameters, health and the ability to balance the natural microbiota in the different segments of the organs of the gastrointestinal tract in growing guinea pigs (*cavia porcellus*) [15,16].

However, the impact of these additives differs according to the studies, although there are studies related to the use of probiotics in animals, which report no benefits on the productive parameters of the animals [17]; while other studies attribute them benefits on animal performance, being probiotics the most promising feed additives of the future, postulating themselves as a good alternative for the substitution of antibiotic growth promoters [2,7,18]. Therefore, the aim of the study was to evaluate the impact of probiotics obtained from agro-industrial substrates fermented with lactic acid bacteria and/or yeasts on the health and changes in the microbiota of the digestive tract of guinea pigs.

2. Materials and Methods

2.1. Animal Bioethics

All experimental procedures applied in this study were reviewed and approved by the Commission of Scientific Degrees by Agreement N° 189/13-14. Faculty of Agricultural Sciences, University of Zulia.

The procedures related to the handling, management and health care of live guinea pigs (*cavia porcellus*) complied with the standards applicable to laboratory animals used for scientific purposes and were applied in accordance with the minimum standards for the protection of animals described in Council Directive 2008/120/EC on minimum standards for the protection of pigs (Council of the European Union, 2008) [19].

2.2. Study Location

The experimental study with animals was carried out at the "Irquis" farm, located at Km 20 via Salado-Lentag, Cuenca, belonging to the Universidad de Cuenca.

2.3. Design and Dosage of Probiotics Used in the Study

Using a completely randomised design, four treatments, four replicates per treatment, each replicate consisting of 5 animals. The treatments evaluated are shown in Table 1.

Table 1. Treatments used in the study and their formulation.

Treatment	Codification	Variants
Control	T0	basal diet without agro-industrial substrate (vinasse-molasses) fermented with beneficial micro-organisms
Bioadditive 1	T1	Basal diet more 1.00 mL agro-industrial substrate (molasses-vinasse) fermented with <i>L. acidophilus</i> and <i>L. bulgaricus</i> .

Bioadditive 2	T2	Basal diet more 1.00 mL agro-industrial substrate (molasses-vinasse) fermented with <i>S. cerevisiae</i> and <i>K. fragilis</i> .
Bioadditive 3	T3	Basal diet more 1.00 mL agro-industrial substrate (molasses-vinasse) fermented with <i>L. acidophilus</i> , <i>L. bulgaricus</i> , <i>S. cerevisiae</i> and <i>K. fragilis</i>

2.4. Animals and Basal Diet Used

The study used 80 male guinea pigs (*cavia porcellus*), Kury breed, 30 ± 5 days old and 250 ± 30 grams (g) live weight.

The feed offered to the study animals was a mixture of 20, 25, 30, 24.97 and 0.03 % alfalfa (*Medicago sativa*), maralfalfa (*Pennisetum spp*), king grass (*Pennisetum purpureun x P. typhoides*), balanced for guinea pig fattening (15, 4, 12, 7 and 87 % crude protein, fat, crude fibre, ash and dry matter, respectively) and 0.03 % vitamin C, respectively. The diet was designed for three phases: phase I from 30 to 60, phase II from 61 to 90 and phase III from 91 to 120 days of age.

Feed was provided in two equal rations per day (07:00 am and 04:00 pm), as recommended in a previous study by Miranda [13], in accordance with the recommendations described in the NRC [20] that meet the minimum requirements established for guinea pigs. In addition, 50 mL of water was offered daily in automatic waterers (Plasson, SKU: 885B722-8, Argentina).

Table 2. Amount of feed at each stage of production and bromatological composition of the diet used for guinea pigs.

Production stage	A Quantity of food offered, g/animal/day	Nutritional composition*, %.				
		CP	EE	CF	Ash	DM
I	100	19	5.88	12.5	5.85	87.82
II	150	17	5.84	12.8	5.67	88.52
III	200	16	5.85	12.6	5.68	87.64

CP, crude protein. EE, extracte eteri. CF, crude fibre. DM, Dry matter. *, the proximate chemical analysis was carried out in the bromatology laboratory of the Faculty of Agricultural Sciences, Universidad de Cuenca.

2.5. Management and Conditioning System

The biosecurity measures on the farm were conditioned prior to the reception of the guinea pigs, as recommended by Vivas [21], which allowed animal health control of the animals during the study. Site disinfection was performed at a dose of 3 cc/L with glutaraldehyde, quaternary ammonium and isopropyl alcohol (Viroguard® Lima, Peru), as recommended by the manufacturer.

Guinea pigs were housed in group cages of 1.50×1.00 m², with five animals per cage. The temperature of the house was maintained at 14 ± 2 °C. The cages for each treatment were placed 1.50 m apart on both sides of the aisle to avoid self-inoculation. All animals subjected in the study received the relevant veterinary care according to the guinea pig (*Cavia porcellus*) husbandry manual [21].

2.6. Obtaining and Administering Microbial Bioadditives to Guinea Pigs

All the bioadditives evaluated in the study were obtained following the methodology described by Miranda [22]. T1, agro-industrial substrate (molasses-vinasse) fermented with *L. acidophilus* and *L. bulgaricus*. T2, agro-industrial substrate (molasses-vinasse) fermented with *S. cerevisiae* and *K. fragilis*. T3, agro-industrial substrate (molasses-vinasse) fermented with *L. acidophilus*, *L. bulgaricus*, *S. cerevisiae* and *K. fragilis*.

The administration of the microbial bioadditives under study was carried out according to the dose and group indicated in Table 1, the first dose was in single doses orally, and from the second dose onwards it was administered every 3 days, according to the assigned group, while the control group received 0.25 mL of distilled water.

2.7. Productive Indicators

The guinea pigs (*cavia porcellus*) under study were weighed on a digital scale (Camry, China) of 5.00 kg capacity with an error of ± 5 g, at the beginning (30 d of age) and at 60, 90 and 120 days of age, with this information the weight gain (WG) was calculated.

2.8. Continuous Physical Evaluation of the Animals

All guinea pigs under study were monitored daily for macroscopic lesions, behavioural changes and health status. The presence of diarrhea and deaths were recorded daily on an individual basis, as animals were identified by a code on the eartag. This information was used to determine the presence of diarrhea and percentage mortality as described by Thrusfield [23].

2.9. Slaughter and Scalding of the Animals

At the end of the study (90 days), six animals from each treatment were fasted for a period of 12:00 h, prior to slaughter, as recommended in the methodology used by Cornejo [24]. At the end of this period the guinea pigs were transferred to the slaughter room. Sanitary slaughter of the animals was performed with prior stunning using the denudation technique at the atlanto-occipital joint, according to the methodology described by Sánchez [25], previously established in the Mexican Official Standard NOM-033-ZOO-1995, Humane Slaughter of Domestic and Wild Animals (Humane Slaughter Association, 2016) [26].

Exsanguination was performed after stunning, exsanguination was performed by unilateral cutting of the jugular vein and carotid artery, according to the methodology described by Sanchez [25]. In a surgical cauldron of 15 L capacity, 12 L of water was tempered to 70 °C. The previously obtained guinea pig carcasses were immersed for 20 seconds in cranio-cutaneous position. Finally, all fur was removed.

2.10. Evisceration of the Animals

Prior to evisceration, the atlanto-occipital joint and cervical vertebrae, as well as the carpal-metacarpal and tarsal-metatarsal joints were cut until a carcass without autopods and head was obtained. Evisceration was performed by laparotomy to isolate the organs of the gastrointestinal tract.

Digestive segments (pancreas, liver, stomach, small and large intestines), lungs, spleen, thymus and kidneys were carefully separated from the mesentery, stomach, small and large intestines were washed with sterile distilled water after removal of digesta contents and finally weighed on a digital electric scale (KAMRY, model EK5055-11, Hong Kong, China) with a capacity of 5 ± 0.1 kg.

2.11. Gross Pathological Examination of the Organs of the Gastrointestinal Tract

In lesions of the stomach, small intestine, colon and cecum the following parameters were assessed: thickness of the intestinal wall, using a tape measure, presence of circulatory disorders in the mucosa (oedema, congestion, haemorrhages), consistency of the intestinal contents (watery, mucous, foamy) and pH was measured with a digital pH meter (Hanna®, HI 99163. USA).

2.12. Collection of the Intestinal Mucosa

After isolation of the organs of the gastrointestinal tract, a 2 cm² longitudinal incision was made in the stomach, small intestine, cecum and colon after washing with sterile distilled water and saline phosphate buffer (BFS) (NaCl 8, 0 g, KCl 0. 2 g, Na₂HPO₄-2H₂O 1.44 g, 0.2 g, KH₂PO₄, in 1.0 L sterile distilled H₂O) with 0.01% gelatin pH 7.4 according to the methodology used by Cueto [27].

The previously obtained fragments were deeply scraped with the help of a 75 mm spatula to obtain 2.00 mL of mucus, which were collected in 15 mL Falcon plastic tubes (Henso, Germany) with sterile screw cap and 5 mL of BFS were added according to the methodology described by Kandler

and Weiss [28]. Finally, they were centrifuged in a digital centrifuge (Yingtai, China) at $4582 \times g$ at 8°C for 10 minutes and the supernatant was removed; this procedure was performed three times.

2.13. Microbial Growth on Selective Culture Media

1.0 mL of the previously obtained mucus content was taken, added to a 150 mL erlenmeyer flask containing 50 mL of nutrient broth and MRS separately. It was then incubated at 37°C for 6 h in an incubator with an orbital shaker (Inkubationshaube TH 15, Germany) at 15 rpm. After this time, 5.0 mL of each culture was taken and homogenised with physiological saline at a ratio of 1/10 (v/v), followed by serial dilutions of 1/10, (v/v) to the 0.5 scale of the MacFarland scheme.

The microbial culture was then processed on the surface of Petri dishes containing sterile selective media (MRS and M17 agar, Sabouraud Dextrose agar and MacConkey agar) and a general medium (nutrient agar), using the streaking method. They were then incubated at 37°C and at 30°C for AS. Petri dishes containing MRS and M17 were incubated under anaerobic conditions in a GasPak Plus™ jar with 5% CO_2 . After this time, the process of identification of typical colonies was carried out. In addition, Gram staining was performed and observed with a binocular optical microscope (BA310 MOTIC, China) to differentiate morphosynthesis characteristics according to the Manual of Systematic Bacteriology [28].

2.14. Biochemical Tests

Catalase and coagulase tests of scraping samples from digestive tract organs were performed with apiWeb version 5.1. Colonies grown on agar (MRS, M17, AN, AS and MacConkey) from subcultures were inoculated into miniaturised biochemical test kits: API® 50 CHL, API® 20 E and API® ID 23 C (BioMerieux), which were incubated for 24 and 48 h at 37°C and 30°C for bacteria and yeast, respectively, according to the manufacturer's recommendations. Genus confirmation and species definition were performed with the commercial API 20NE system (BioMerieux, St Louis, MO, USA), following the manufacturer's instructions. After this time, the carbohydrate fermentation profile of each strain was compared with the database provided by the manufacturer.

2.15. Statistical Analysis

Experimental data were processed with the statistical package Statgraphics plus ver. XV. II for Windows. Experimental variables such as weight gain, relative weight of digestive tract organs and microbial load ($\text{CFU} \cdot \text{mL}^{-1}$) obtained in the culture media were subjected to a simple rank analysis of variance (ANOVA) according to a completely randomised design [29]. When the P-value was <0.05 , Duncan [30] comparison test was applied to discriminate differences between treatments.

For the variables occurrence of diarrhoea and percentage mortality, a multiple comparison analysis of proportions was performed in the statistical package SAS version 17.

3. Results

Figure 1 summaries the responses in weight gain in guinea pigs. When diets containing agro-industrial substrate fermented with lactic acid bacteria and yeasts were included, weight gain increased, but the animals in T3 gained more weight ($p < 0.05$) in relation to the other treatments (T0, T1 and T2) in the evaluations carried out at 30, 60 and 90 days of study, which shows that probiotic microorganisms have a positive action on the utilization of the diet offered.

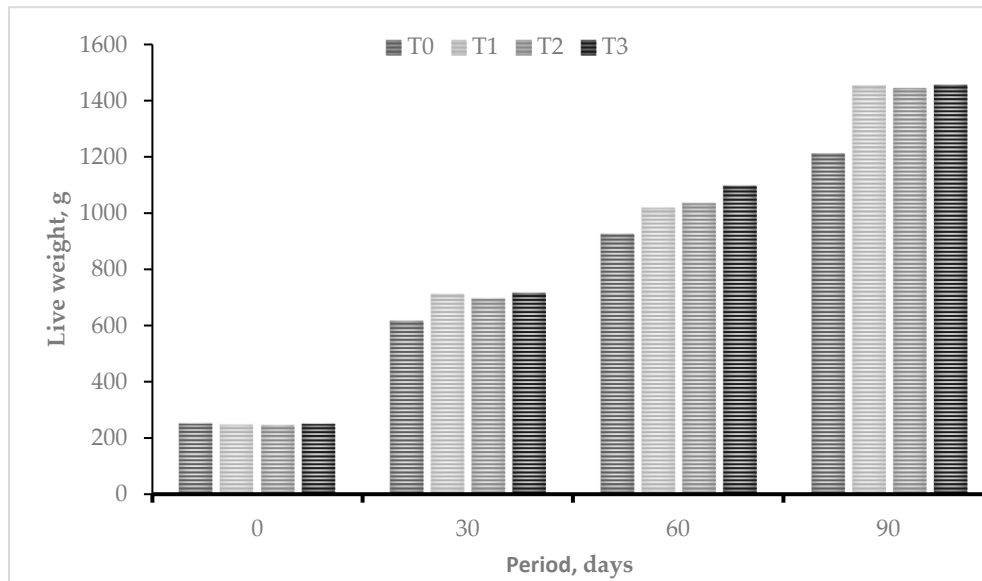


Figure 1. Weight gain of guinea pigs in the evaluations carried out at the beginning, and at 30, 60 and 90 days of the study, when including in the diet agro-industrial substrate (molasses-vinasse) fermented with lactic bacteria and yeasts. **T0**, Control. **T1**, *L. acidophilus* and *L. bulgaricus*. **T2**, *S. cerevisiae* and *K. fragilis*. **T3**, *L. acidophilus*, *L. bulgaricus*, *S. cerevisiae* and *K. fragilis*.

The animals that consumed diets with microbial additives presented less ($p < 0.05$) number of animals with diarrhoea, of these the treatments T1 and T2, were with less ($p < 0.015$ and $p < 0.014$) occurrence of diarrhea, in the evaluation carried out at 30 and 60 days respectively. However, the aetiology of the possible agents associated with the occurrence of diarrhea was not identified. In terms of deaths, animals in the control group had a higher number ($p > 0.05$) of animals dying from diarrheal disorders at 30 and 60 days of the study, but there were no guinea pig deaths at 90 days of the experiment (see Table 3).

Table 3. Percentage of diarrhoea and mortality in guinea pigs, evaluated at 30, 60 and 90 days of study, when fed a diet with agro-industrial substrate (molasses - vinasse) fermented with bacteria and yeasts.

Period evaluated, d	Indicator, %	Treatments				SEM	p-valor
		T0	T1	T2	T3		
30	Incidence of diarrhea	4.08 ^a	1.08 ^c	0.9 ^c	0.3 ^b	2.2	0.015
	Mortality	0.77	0.59	-	-	-	-
60	Incidence of diarrhea	2.62 ^b	1.40 ^c	0.72 ^a	0.19 ^b	2.5	0.014
	Mortality	1.01	0.08	-	-	-	-
90	Incidence of diarrhea	1.03	-	-	-	-	-
	Mortality	-	-	-	-	-	-

^{a,b,c} superscript different in the same column differ at $P < 0.05$ (Duncan, 1955). **T0**, Control. **T1**, *L. acidophilus* and *L. bulgaricus*. **T2**, *S. cerevisiae* and *K. fragilis*. **T3**, *L. acidophilus*, *L. bulgaricus*, *S. cerevisiae* and *K. fragilis*. **SEM**, standard error. **d**, day.

Table 4 shows the general weight status of the digestive tract organs of guinea pigs at 120 days of age. The relative weight of the small intestine with cecal contents, liver, lungs and kidneys was higher ($p < 0.05$) in animals consuming diets containing agro-industrial substrate fermented with lactic acid bacteria and yeast. However, in the other organs evaluated there were no significant differences ($p > 0.05$).

Table 4. Relative organ weights of the digestive tract of 120-day-old guinea pigs fed a diet containing agro-industrial substrate (molasses-vinasse) fermented with bacteria and yeast.

Organs of the digestive tract, g	Treatments				SEM	p-valor
	T0	T1	T2	T3		
Stomach with luminal contents	52.06	52.23	54.12	54.51	2.11	0.084
Stomach without luminal contents	8.15	8.24	8.73	8.97	1.28	0.059
Small intestine with luminal contents	40.21 ^b	43.32 ^a	42.43 ^{ab}	43.42 ^a	0.18	0.012
Small intestine without luminal contents	25.64	24.76	25.87	26.32	0.12	0.081
Large intestine with luminal contents	54.12	55.02	54.87	55.15	0.25	0.073
Large intestine without luminal contents	22.09	21.98	23.45	22.97	0.10	0.342
Cecum with luminal contents	85.13	86.02	86.03	86.05	0.15	0.061
Cecum without luminal contents	22.86	23.07	23.26	23.44	0.21	0.842
Liver	34.02 ^c	34.56 ^c	34.98 ^b	35.23 ^a	0.54	0.012
Lungs	13.87 ^b	14.24 ^b	14.87 ^a	15.34 ^a	0.22	0.008
Kidneys	10.02 ^c	10.24 ^c	10.67 ^b	11.64 ^a	0.10	0.022

^{a,b,c} superscript different in the same column differ at $P < 0.05$ (Duncan, 1955). **T0**, Control. **T1**, *L. acidophilus* and *L. bulgaricus*. **T2**, *S. cerevisiae* and *K. fragilis*. **T3**, *L. acidophilus*, *L. bulgaricus*, *S. cerevisiae* and *K. fragilis*. **SEM**, standard error.

Table 5 shows the results of macroscopic lesions of the digestive tract organs in 120-day-old guinea pigs. Animals in the control treatment (T0) showed a higher number of animals with lesions at the intestinal level. However, guinea pigs that consumed the agro-industrial substrates fermented with lactic acid bacteria and yeast (T1, T2 and T3), did not present macroscopic lesions to be considered physiological alterations at the level of the digestive tract organs.

Macroscopic changes in the stomach, small intestine, colon and cecum showed significant changes in relation to the thickness of the intestinal wall in the animals of the control group (T0) compared to guinea pigs consuming agro-industrial substrates fermented with lactic acid bacteria and yeast. A similar situation occurred with circulatory disorders at the level of the intestinal mucosa and contents in the T0 treatment, as can be seen in Table 5.

Table 5. Macroscopic lesions observed (*post-mortem*) in 120-day-old guinea pigs fed a diet containing agro-industrial substrate (molasses - vinasse) fermented with bacteria and yeasts.

Gross lesions	Stomach				Small intestine				Colon				Cecum			
	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
Gut wall thickness																
Altered	1.08	WI	WI	WI	0.87	WI	WI	WI	1.06	WI	WI	WI	2.08	WI	WI	WI
Circulatory disorders of the mucosa																
Edema	1.02	WI	WI	WI	1.21	WI	WI	WI	0.28	WI	WI	WI	0.24	WI	WI	WI
Congestion	0.15	WI	WI	WI	0.89	WI	WI	WI	0.06	WI	WI	WI	0.36	WI	WI	WI
Haemorrhage	0.08	WI	WI	WI	0.31	WI	WI	WI	SL	WI	WI	WI	0.18	WI	WI	WI
Intestinal contents																
Aqueous	0.15	WI	WI	WI	0.09	WI	WI	WI	0.09	WI	WI	WI	0.31	WI	WI	WI
Mucous	0.10	WI	WI	WI	0.24	WI	WI	WI	0.18	WI	WI	WI	0.25	WI	WI	WI
Frothy	0.18	WI	WI	WI	0.23	WI	WI	WI	0.37	WI	WI	WI	0.33	WI	WI	WI
pH	2.9	1.8	1.7	1.8	4.9	4.8	4.7	4.8	5.88	5.67	5.65	5.71	6.3	5.98	5.97	5.98

T0, Control. **T1**, *L. acidophilus* and *L. bulgaricus*. **T2**, *S. cerevisiae* and *K. fragilis*. **T3**, *L. acidophilus*, *L. bulgaricus*, *S. cerevisiae* and *K. fragilis*. **WI**, without injury.

Table 6 presents the microbial load cultured on MRS, M17, Nutrient, Sabouraud Dextrose and MacConkey Agar. In stomach contents samples (*post-mortem*) cultured on MRS, M17, Nutrient and Sabouraud Dextrose agar, there was no significant growth ($p > 0.05$) between treatments. However,

on MacConkey agar, higher microbial growth ($p=0.003$) was observed in samples from the control treatment (T0) compared to the other treatments (T1, T2 and T3).

Samples of small intestine, colon and cecum scrapings from animals consuming diets containing agro-industrial substrates fermented with lactic acid bacteria and yeast (T1, T2 and T3), showed increased microbial growth ($p<0.05$) when cultured on Petri dishes containing MRS, M17, Nutrient and Sabouraud Dextrose agar, compared to guinea pigs from the control treatment. In contrast, on MacConkey agar, higher microbial growth was observed in the control treatment samples compared to T1, T2 and T3 treatments (see Table 6).

Table 6. Microbial load in each segment of the digestive tract, cultured in Petri dishes with culture media (MRS, M17, Nutrient, Sabouraud Dextrose and MacConkey Agar, respectively) of samples from stomach, intestine, colon and cecum scrapings from 120-day-old guinea pigs (*post-mortem*).

organs of the digestive system	Culture medium for microbial growth	Treatments **				SEM	<i>p</i> -valor
		T0	T1	T2	T3		
Stomach	MRS agar	6.22	6.31	6.32	6.42	0.32	0.841
	M17 agar	6.47	6.48	6.50	6.72	0.55	0.836
	Nutrient agar	6.90	6.87	6.85	6.77	0.28	0.965
	Sabouraud Dextrose Agar	2.80	2.65	2.57	3.50	0.26	0.077
	MacConkey agar	5.97 ^a	5.59 ^a	4.47 ^b	3.45 ^b	0.29	0.003
Small intestine	MRS agar	3.77 ^b	6.51 ^a	6.62 ^a	7.07 ^a	0.46	<.001
	M17 agar	3.72 ^b	6.97 ^a	7.07 ^a	7.15 ^a	0.14	<.001
	Nutrient agar	6.72	6.58	6.72	6.70	0.27	0.081
	Sabouraud Dextrose Agar	3.05 ^b	5.98 ^a	6.10 ^a	6.15 ^a	0.26	<.001
	MacConkey agar	6.20 ^a	3.08 ^b	3.42 ^b	2.85 ^b	0.23	<.001
Colon	MRS agar	3.40 ^b	7.08 ^a	6.95 ^a	7.12 ^a	0.37	<.001
	M17 agar	4.92 ^b	7.02 ^a	7.05 ^a	6.97 ^b	1.15	0.030
	Nutrient agar	6.85	6.66	6.85	6.85	0.61	0.510
	Sabouraud Dextrose Agar	2.27 ^b	5.98 ^a	5.57 ^a	5.85 ^a	0.12	<.001
	MacConkey agar	6.45 ^a	4.15 ^b	3.95 ^b	3.98 ^b	0.10	<.001
Cecum	MRS agar	4.07 ^b	7.18 ^a	7.07 ^a	7.15 ^a	0.10	<.001
	M17 agar	3.47 ^b	6.97 ^a	7.15 ^a	6.95 ^a	0.23	0.002
	Nutrient agar	6.37	6.52	6.12	6.97	0.20	0.505
	Sabouraud Dextrose Agar	2.37 ^c	5.83 ^a	5.61 ^b	5.97 ^a	0.15	<.001
	MacConkey agar	6.87 ^a	3.43 ^b	3.30 ^b	3.52 ^b	0.24	0.007

^{a,b,c} superscript different in the same column differ at $P<0.05$ (Duncan, 1955). **T0**, Control. **T1**, *L. acidophilus* and *L. bulgaricus*. **T2**, *S. cerevisiae* and *K. fragilis*. **T3**, *L. acidophilus*, *L. bulgaricus*, *S. cerevisiae* and *K. fragilis*. **SEM**, standard error. **, these values correspond to logarithmic scales (\log^{-1}).

Table 7 reports the main mycoorganisms detected in the different organs of the digestive tract in guinea pigs at 120 days of age. In the samples from animals that consumed diet containing agro-industrial substrates fermented with lactic acid bacteria and yeasts (T1, T2 and T2), a higher presence of microorganisms with numerical profiles corresponding to *L. acidophilus*, *L. bulgaricus*, *Saccharomyces* spp. and *K. fragilis* was observed, compared to animals in the control group.

In samples from control treatment animals, a higher presence of numerical profiles corresponding to pathogens such as *E. coli* was observed.

In the small intestine scraping samples, a higher presence of numerical profiles of microorganisms known as probiotics was observed mainly in the T1, T2 and T3 treatments, compared to samples from animals in the control group, as shown in Table 7.

Table 7. Main microorganisms detected in samples from the scraping of the digestive tract of 120-day-old guinea pigs (*post-mortem*) and associated with bacteria and yeasts introduced with the diet, identified using the API (Miniaturized Biochemical Test Kits) biochemical identification system.

API	Numerical profile	Microorganism	Stomach				Small intestine				Cecum			
			T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
API 50 CHL	4356101	<i>Lactobacillus</i> spp.	***	**	**	**	*	***	***	***	*	**	**	**
	3322230	<i>Lactobacillus</i> spp.	*	**	***	**	*	***	***	***	*	**	**	**
	1552137	<i>L. bulgaricus</i>		**	**	**	*	***	***	***	*	**	**	**
	1231576	<i>L. lactis</i>		**	**	**	*	*	*	*		*	*	
	1269781	<i>P. acidilactici</i>	*	*	*	*	*	**	**	**	*	*	*	
	4356135	<i>L. acidophilus</i>		*	*	*		**	**	**	*	*	*	
	1184227	<i>L. bulgaricus</i>		**	**	**		***	***	***		*	*	*
	4356101	<i>S. thermophilus</i>		*	*	*		*	*	*		*	*	
	2530294	<i>L. paracasei</i>	*		*		*	*	*	*	*		*	
	5310336	<i>L. rhamnosus</i>	*			*	*	*	*	*	*		*	
	8042697	<i>Pediococcus</i> spp.	*			*	*			*	*	*	*	
API 20 E	1427157	<i>E. coli</i>	***	*	*		***	*	*		**		*	*
	1431430	<i>E. coli</i>	**	*	*	*	***	*	*	*	*	*	*	*
	1429274	<i>E. coli</i>	*	*	*	*	**	*	*		*	*	*	*
	1177524	<i>E. coli</i>	**		*	*	**	*	*	*	*	*		
	4313717	<i>P. multivida</i>	*	*	*		*	*	*					
	1313837	<i>S. dysenteriae</i>	**	*		*	*			*	*	*		
API ID 32 C	5764734	<i>Saccharomyces</i> spp.	*	**	**	**	*	**	***	***		**	**	***
	4026727	<i>Saccharomyces</i> spp.	*	**	**	**	*	**	**	**	*	*	**	
	9763534	<i>S. cerevisiae</i>	*	**	**	***	*	**	***	***	*	*	*	**
	7401236	<i>S. boulardii</i>	*	*	*	*	*	*	**	**		*	**	
	2009655	<i>Kluyveromyces</i> spp.		*	**	***		*	***	***		*	***	***
	2229633	<i>K. marxianus</i>	*	*	*	*		*	***	***	*	*	**	**
	4653746	<i>K. fragilis</i>			**	***			***	***			***	***
	3659028	<i>Candida</i> spp.	*	*		*	*		*	*	*	*		

T0, Control. T1, *L. acidophilus* and *L. bulgaricus*. T2, *S. cerevisiae* and *K. fragilis*. T3, *L. acidophilus*, *L. bulgaricus*, *S. cerevisiae* and *K. fragilis*. *, presence. **, presence in less than two samples. ***, presence in more than three samples.

4. Discussion

4.1. Productive Behaviour

The use of probiotics as growth promoters in animal feed is mainly due to the described improvement in production associated with increased digestion and nutrient absorption [8,31]. In fattening guinea pigs fed agro-industrial substrates fermented with lactic acid bacteria and yeast in the diet, a significant increase in weight gain was observed. On the other hand, [9,18,32] observed no significant variations in weight gain, feed intake and feed conversion in broilers fed a commercial probiotic in their diet. In pigs receiving probiotics based on different *Lactobacillus* species, a significant increase in daily weight gain, a reduction in the incidence of diarrhea compared to the control group [33], and an increase in nutrient digestibility [32] were observed.

The increase in nutrient digestibility could be due to the increased enzyme activity in the gut caused by the administered probiotics [10]. Research has reported increased amylase enzyme activity

when *Lactobacillus* is added to piglets diets [14], and increased sucrase and lactase activity when *Lactobacillus* is added to pig diets [7]. *Bacillus amyloliquefaciens*, which has been used as a probiotic in animal feed, produces extracellular enzymes such as amylase, cellulase, proteases and metalloproteases, which can improve nutrient digestion [34]. It has also been reported that probiotic consumption increases the height of intestinal villi in rabbits, increasing the area of nutrient absorption [35].

Probiotics reduce the symptoms of lactose maldigestion. This effect was observed in people who ingested fermented dairy products [36]. This effect is due, on the one hand, to the fact that the bacteria contained in these products possess lactose-metabolising enzymes such as β -galactosidase (lactase) [37]. On the other hand, these strains increase intestinal enzyme activity (intestinal lactase) [11].

In other species evaluated, production parameters improved with the inclusion of probiotics in the diet, as is the case reported by Cornejo [24] evaluating the probiotic Bioplus 2B in rabbits. While Miranda [7], found a 12% improvement when including *Lactobacillus* in the diet of piglets. This result is probably due to the increase in enzyme activity in the intestinal tract caused by probiotics, which results in an increase in nutrient digestibility, in addition, the consumption of agro-industrial substrates fermented with lactic acid bacteria and yeast increases the ratio of villus height/crypt depth and duodenal villus height [6,18], this improvement increases the area of nutrient absorption and translates into higher weight gain in guinea pigs (5, 11).

4.2. Health and Diarrhea

One of the most studied yeasts has been *Saccharomyces*, which reduced the risk of diarrhoea. Other studies show a reduction in diarrhoea caused by *Clostridium difficile*, although this was only significant in puppies [9,11]. Therefore, its use can be considered with a strong recommendation (based on a moderate level of evidence).

In the mucous membranes, the epithelium plays a barrier function that prevents the entry of toxic substances and pathogens [16]. To this end, the intestinal epithelium synthesises mucins to form a physical mucus barrier [17]. Probiotics protect the epithelium through a cytoprotective effect and have the ability to increase the expression of mucins by cells in the ileum and colon, which is a highly effective mechanism in the antibacterial fight against bacteria [6,38].

Other reports agree with the data obtained in the present study, with the inclusion of different species of microorganisms (usually *Lactobacillus* alone or in combination with *bifidobacteria*, *enterococci* or *Saccharomyces boulardii*) [4,8]. The use of mixed cultures of lactic acid bacteria and yeasts decreases the risk of *E. coli* associated diarrhoea [7]. Although overall positive results were found with all probiotics used, it is recognised that there is significant heterogeneity in the studies, so there is insufficient evidence to say whether the effect varies systematically between populations, including the probiotic preparation usedb [8,16,27].

In controlled studies in which probiotic bioaditoves fermented with *Lactobacillus* spp, *L. bulgaricus* and *Streptococcus thermophilus* were administered, the incidence of *Clostridium* diarrhoea was reduced and is therefore recommended, although the level of recommendation would be weak with low evidence [31,33].

The administration of fermented substrates containing lactic acid bacteria and yeasts to guinea pigs is effective in the prevention of diarrhoea associated with diarrhoea. However, the results are not homogeneous and, in addition, there is a great variability in the strains studied and in the nutritional formulas used [18]. In the present study, different strains have been used, such as *L. acidophilus*, *L. bulgaricus* *S. cerevisiae* and *K. fragilis*. The microorganisms used in the present study have been shown to have a probiotic effect with positive results, only in less than half of the studies were significant benefits observed in terms of reduction of diarrhoea [27,36]. Other probiotics may be effective, but the paucity of studies precludes drawing conclusions [38]. Also, for secondary prevention of recurrent infections by pathogens with a low level of evidence, mixed cultures of lactic acid bacteria and yeasts could be used, although not all authors conclude the same [6,7].

4.3. Modification of the Microbiota and the Environment of the Digestive Tract

One of the mechanisms of probiotics is to change microbial population dynamics, decrease the growth of pathogenic microorganisms and promote the growth of beneficial microflora [1,6]. Beneficial microbial populations in the digestive tract have been associated with increased animal performance, reflecting more efficient digestion and improved immunity [5]. The ability of probiotics to reduce pathogenic microorganisms in the digestive tract may be due to the production of bacteriocins [18], exclusion by competition as probiotics adhere to the intestinal epithelium, change in pH and induction of the immune system response [31].

In guinea pigs that received probiotics as dietary additives, increases in the population of *Lactobacillus* and *Bifidobacteria* decreases in the population of *E. coli* [32] and decreases in the population of *Clostridium* spp. [34] were reported. The bacteria *L. reuteri*, *B. subtilis* and *B. licheniformis*, have shown in piglets the ability to decrease the amount of *E. coli* and *Salmonella* sp. in faeces [36]. This decrease in the excretion of pathogenic microorganisms reduces the risk of infection of other animals in livestock production and cross-contamination. Modification in the digestive tract population has been seen with commonly used probiotic bacteria both gram negative and gram positive, lactic acid bacteria and spore-forming bacteria such as *Bacillus* spp [37,38].

Some of the bacterial species commonly used as probiotics, including lactic acid bacteria such as *Bifidobacterium*, *Bacillus* and *Lactobacillus*, produce substances (bacteriocins) that have the ability to inhibit the growth of pathogenic microorganisms such as *Staphylococcus*, *Enterococcus*, *Listeria* and *Salmonella* in the gut of animals [18,27,31]. Bacteriocins inhibit bacterial cell wall synthesis, resulting in the production of pores on the bacterial surface [1,2,6]. The genera *Lactobacillus* and *Bifidobacterium* produce organic acids such as lactic and acetic acids, which can be taken up by other bacteria, including pathogens, in the gastrointestinal tract, reducing their intracellular pH to lethal levels [7,8,16]. These organic acids can also contribute to the lowering of pH in the digestive tract, creating favorable conditions for the existing microbiota and decreasing the risk of being colonized by pathogenic microorganisms [11,38].

Microorganisms supplied as probiotics could colonise the digestive tract of young and adult animals, preventing colonization by pathogenic microorganisms. Some strains of *Lactobacillus* and *Bifidobacterium* have hydrophobic surface proteins that promote non-specific adhesion to animal cells, cover receptor binding sites and prevent the intestinal epithelium from binding to pathogenic microorganisms [27]. In guinea pig, adhesion of *Lactobacillus* spp. to the intestinal epithelium has been observed, which could exclude pathogenic microorganisms by competition [31].

One of the possible mechanisms to reinforce the integrity of the intestinal barrier and prevent the entry of pathogens and toxins into the animal is to increase the expression of genes involved in the transmission of signals between the tight junctions of the cells of the intestinal epithelium [31,37]. In some probiotics, such as *Lactobacillus*, modulation of genes encoding adhesion proteins within these tight junctions, such as *E. cadherin* and *E-catenin*, has been observed [38].

Some bacteria used as probiotics have the ability to reduce the permeability of the intestinal epithelium, decreasing the translocation of intestinal pathogens from the gastrointestinal tract to other organs such as the liver, spleen and lymph nodes in mice [32] and pigs [7]. For probiotics to effectively contribute to maintaining the barrier function of the intestinal wall, it is crucial that they are administered before pathogens have multiplied in the gastrointestinal tract. This effect depends on the species used as a probiotic and the type of pathogen (virus, bacterium or fungus) [1,6,9,11].

Some bacteria used as probiotics secrete chemical signals called autoinducers that affect the behaviour of other bacteria and even the host (18). This process of bacterial communication is called quorum sensing [27]. These signals sent by probiotic bacteria can have an effect on the virulence of pathogenic bacteria by affecting them.

5. Conclusions

The inclusion of 1.00 mL of agro-industrial substrates fermented with lactic acid bacteria and yeasts tends to improve the productive performance and weight gain of guinea pigs in the growth stage increases significantly. On the other hand, it helps to significantly reduce the occurrence of

diarrhoea and death of the animals, especially in young animals. In addition, there are no macroscopic lesions in the digestive tract organs and, consequently, the digestive organs have a higher weight and a better appearance. Furthermore, an improvement in the presence of beneficial microorganisms in the different segments of the digestive tract is observed in the T1, T2 and T3 treatments.

These results are the basis for the use of bioadditives with probiotic capacity in commercial guinea pig farms.

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