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Effects of oral administration of native lactic acid bacteria with probiotic potential on productive parameters and meat quality of fattening guinea pigs (*Cavia porcellus*)

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ABSTRACT

Background: The use of probiotics in guinea pig farming has emerged as an alternative to antibiotics because proper probiotic administration provides beneficial effects to the host without the risks associated with antibiotics. However, few studies have reported the significant effects of probiotics on guinea pig production and meat quality.

Aim: This study aimed to evaluate the effects of the oral administration of different lactic acid bacteria with probiotic potential on the productive parameters and meat quality of fattening guinea pigs (*Cavia porcellus*).

Methods: A total of 72 guinea pigs from a fattening line weaned at 14 days post-birth with an initial average weight of 248.6 ± 42.2 g were distributed into six pens ($n = 12$ each). They received oral administration of 3 ml of native lactic acid bacteria: Treatment 1 (T1): *Enterococcus gallinarum*, Treatment 2 (T2): *Exiguobacterium sp.*, Treatment 3 (T3): *Lactococcus lactis*, Treatment 4 (T4): a mixture of the three bacteria, Treatment 5 (T5): addition of Zinc bacitracin, and Treatment 6 (T6): control. After 63 days, final weight, weight gain (WG), dry matter intake, feed conversion ratio, carcass yield (CY), economic merit (EM), and meat quality were determined.

Results: The addition of lactic acid bacteria did not significantly affect the final weight ($p = 0.242$). However, differences were observed in WG ($p = 0.04$), specifically between T1 and T3 ($p = 0.039$). No significant differences were observed in dry matter intake ($p = 0.99$) or feed conversion ratio ($p = 0.72$). The CY was similar across all treatments ($p = 0.093$), as was EM ($p = 0.157$). Sensory analysis indicated better acceptance of meat from animals treated with probiotics, although no significant differences were found ($p > 0.05$).

Conclusions: The oral administration of the native probiotic bacteria *Enterococcus gallinarum*, *Exiguobacterium sp.*, and *Lactococcus lactis* did not affect the productivity or meat quality of guinea pigs.

Keywords: *Lactococcus lactis*, *Exiguobacterium sp.*, *Enterococcus gallinarum*, Growth promoter antibiotic, Huanuco.

Introduction

Commercial guinea pig production is carried out due to the high market appreciation of guinea pig meat, valued for its flavor, texture, high protein content, low-fat content, and high-quality amino acids (Enriquez, 2019; Herrera *et al.*, 2022; Aphrodita *et al.*, 2024). In Peru, production is limited by lower technological

advancements and diseases caused by *Salmonella sp.*, *E. coli*, *Streptococcus sp.*, *Klebsiella sp.*, and *Bordetella sp.* (Obregón *et al.*, 2018; Angulo-Tisoc *et al.*, 2021; Mendoza-Rodriguez, 2022). The use of growth-promoting antibiotics improves guinea pig production; however, their use can alter the gut microbiota, leave antibiotic residues in the meat and some viscera (Ampuero-Riega and Morales-Cauti,

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2021), and contribute to the emergence of resistant bacteria (Ripon *et al.*, 2023).

One alternative is the application of probiotics, which contain beneficial live microorganisms. Proper dietary administration of probiotics can regulate the gastrointestinal microbial population and provide beneficial effects to the host (FAO/WHO, 2001). Probiotic intake reduces pathogen populations through competition and/or the production of antimicrobial molecules. Additionally, they enhance the intestinal barrier against pathogens and strengthen the immune system to prevent infectious diseases (Raheem *et al.*, 2021; Fijan, 2023; Mousa *et al.*, 2023). The most recognized bacterial genera with probiotic capacity include *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Lactococcus*, *Pediococcus*, and *Leuconostoc* (Fijan, 2014; Lee *et al.*, 2019; Alayande *et al.*, 2020; Chen *et al.*, 2021; Goicochea-Vargas *et al.*, 2024).

Probiotics are used to improve the rearing of various livestock species, including chickens (Tsega *et al.*, 2024), pigs (Galli *et al.*, 2024), cattle (Wu *et al.*, 2024), and sheep (Meza-López *et al.*, 2021). Similarly, their administration to guinea pigs, either directly (Carcelén *et al.*, 2021) or mixed with feed (Cano *et al.*, 2016), has shown changes in productive parameters. Probiotics prepared with bacteria of the genus *Lactobacillus*, yeasts, or commercial probiotics have positively impacted final weight, weight gain (WG), feed conversion rate, and/or carcass yield (CY) (Guevara and Carcelén, 2014; Cano *et al.*, 2016; Carcelén *et al.*, 2021; Quijano *et al.*, 2023). However, in some cases, no significant effects were observed (Puente *et al.*, 2019; Valdizán *et al.*, 2019; Andía and Ángeles, 2021). Considering the emergence of new microorganisms with probiotic potential, as well as the small number of reports regarding the successful influence of probiotics on guinea pigs, this study aimed to evaluate the effect of supplementing different lactic acid bacteria with probiotic potential on productive parameters in guinea pig farming, as well as meat quality.

Materials and Methods

Study location

The bioassays were conducted at the Kotosh Experimental Livestock Center of the Universidad Nacional Hermilio Valdizán (UNHEVAL), located in Kotosh at an altitude of 1,894 m above sea level in the Huánuco region, Peru, over a period of 63 days. The recorded average temperature was 20°C, with an average relative humidity of 60%.

Animals, feeding, and management system

A total of 72 guinea pigs (*Cavia porcellus*) belonging to a fattening line of the Kotosh Experimental Livestock Center were used at the weaning stage (14 ± 2 days post-birth). The animals had an average initial weight of 248.6 ± 42.2 g. The diet consisted of fresh forage and balanced feed (BF). The forage was a mixture of alfalfa

(*Medicago sativa*) and corn stover (*Zea mays*), harvested the same day, and provided approximately 27% of their body weight. Additionally, a 6% supplementation of body weight with BF (Coricuy growth concentrate, Corina-Peru) was provided. The BF composition included 65% total digestible nutrients, 18% protein, 2.75 Mcal/kg of digestible energy, 11% fiber, 3% fat, 7% ash, 0.8% calcium, and 0.45% phosphorus. Diet was split into two daily rations: BF was given at 9:00 am and forage at 3:00 pm. The amount of offered feed varied weekly according to the weight of the animals in each pen.

Prior to the animal placement and every 15 days thereafter, the floors and walls of the rearing pens were disinfected with diluted quaternary ammonium. Cleaning was performed every 3 days, mainly to remove feces.

Probiotic preparation

Probiotics were prepared using the following bacterial strains: *Enterococcus gallinarum* (Probiotic 01), *Exiguobacterium sp.* (Probiotic 02), and *Lactococcus lactis* (Probiotic 03). These bacteria, with *in vitro* probiotic potential, were previously isolated from guinea pig feces (Goicochea-Vargas *et al.*, 2024) and preserved in the UNHEVAL Molecular Biotechnology Laboratory collection. Each strain was reactivated by inoculating 100 µl of bacterial stock in 5 ml of De Man, Rogosa, and Sharpe (MRS) broth and incubating at 37°C for 48 hours. The inoculum from each culture was then transferred to MRS broth until reaching a concentration between 10^9 and 10^{11} colony-forming units (CFU)/ml. Each bacterial culture was centrifuged at 4,000 g for 20 minutes, and the supernatant was discarded. The bacterial pellet was resuspended in sterile distilled water containing 0.07% lactic acid to an approximate concentration of 10^7 CFU/ml (Carcelén *et al.*, 2021). Each bacterial suspension was considered a different probiotic product. Additionally, a fourth probiotic (Probiotic 04) was prepared by mixing the three bacterial strains in equal proportions, resulting in a density of 10^7 CFU/ml.

Experimental design and treatment application

The experimental units were distributed in a completely randomized design with 12 animals per pen. The pens were constructed from wood and metal mesh and were 1.48 m long, 1.42 m wide, and 0.5 m high.

Six treatments (T1–T6) were evaluated: T1: Basal diet + 3 ml Probiotic 01; T2: Basal diet + 3 ml Probiotic 02; T3: Basal diet + 3 ml Probiotic 03; T4: Basal diet + 3 ml Probiotic 04; T5: Basal diet + 300 ppm Zinc Bacitracin; and T6: Basal diet (Control).

For T1–T4, probiotics were administered twice, with the first administered from days 1 to 10 (weaning stage). The second dose was administered for 5 days starting on day 34 (growth stage). A 1-ml syringe was used to orally administer 3 ml/animal/day of probiotics before feeding (Valdizán *et al.*, 2019). The growth-promoting antibiotic (GPA) Zinc Bacitracin 10% (Zinbax 10%,

Pro-Premix Nutrition Sac, Peru) was used only in T5. GPA was mixed with BF at a concentration of 300 ppm and supplied throughout the trial (Carcelén *et al.*, 2021). In addition, animals in T5 and T6 received 3 ml/animal/day of a 0.07% lactic acid solution in sterile distilled water, administered simultaneously with the other treatments.

Evaluation of productive parameters

Final live weight (FLW)

At the end of the bioassay, animals from each treatment group were weighed in a fasting state using an electronic balance with a sensitivity of 1 g.

Weight gain (WG)

Individual weights (g) were recorded at the beginning of the bioassay and weekly before feeding. Total WG was calculated by subtracting the initial weight from the weight recorded in week 9. Weekly WG per treatment was calculated as the difference between consecutive weekly weights.

Weekly dry matter intake (WDMI)

The weight of the offered feed was recorded weekly, along with the weight of leftover BF and forage. The dry matter content of the forage and its residues was determined weekly by placing 100 g of each in an oven at 70°C for 60 hours and weighing them afterward. For BF, dry matter was determined using the same procedure at the beginning and end of the experiment (Valdizán, 2018). WDMI was calculated using the following formula:

$$\text{Dry matter intake} = \text{Weight of administered feed} \times (\text{Dry matter content of feed}) - \text{Weight of residual feed} \times (\text{Dry matter content of residual feed}).$$

Weekly feed conversion ratio (WFCR)

Weekly feed conversion was calculated by establishing the ratio between WDMI and weekly live WG.

Carcass yield (CY)

Four guinea pigs per treatment were sacrificed using cervical dislocation and bleeding techniques. The animals were weighed beforehand, and afterward, their fur, head, legs, and viscera were removed, leaving only the carcass, which was subsequently weighed. Yield was calculated by dividing carcass weight by live weight and multiplying by 100% (Canto *et al.*, 2018; Pedemonte-Córdova and Peña-Arias, 2018).

Economic merit (EM)

Economic returns were calculated based on the difference between the product of the final carcass weight (kg) and the price per kg of guinea pig meat (S/.), and the total cost associated with BF, forage, probiotic preparation, and GPA addition. The EM was determined by dividing the economic return by the total cost of each treatment.

Sensory evaluation

The thighs of the euthanized animals were seasoned only with salt and fried in oil for 15 minutes. For each frying batch, fresh oil was used. A sensory evaluation of

the meat was conducted by 20 panelists, who assessed color, odor, flavor, juiciness, and tenderness. A scale from 1 to 4 was used, with increasing preference levels (Pedemonte-Córdova and Peña-Arias, 2018).

Statistical analysis

Results are presented in a table as mean $\pm$ SD. Tables and graphs were generated using Microsoft Excel version 365 (Microsoft Corporation, Redmond, WA, USA). Correlational statistical analyses were conducted using SPSS Statistics for Windows, version 26.0 (IBM Corp, Armonk, NY, USA). The normal distribution of data for each variable was assessed using the Shapiro–Wilk test, assuming normality when $p > 0.05$. Levene’s test was applied to determine variance homogeneity among groups when $p < 0.05$. The results of both tests guided the selection of parametric and nonparametric tests for evaluating variable differences.

One-way analysis of variance was used to determine differences between treatments for FLW, WG, WDMI, and WFCR (Awad *et al.*, 2024), with Tukey’s post hoc test. For CY, EM and sensory attributes (color, odor, flavor, tenderness, and juiciness) were analyzed using a one-factor Kruskal–Wallis nonparametric analysis of variance with multiple pairwise comparisons to assess differences between treatments. Both tests were conducted at a 95% confidence level, with significant differences at $p < 0.05$.

Ethical approval

All animal procedures performed in this study were performed according to the protocol for handling animals for research approved by the Bioethics Committee of Veterinary Medicine and Zootechnics faculty of UNHEVAL, Peru (N°52-2021-UNHEVAL-FMVZ).

Results

The effects of probiotics and GPA supplementation on key productive parameters in guinea pigs are presented in Table 1.

Final live weight

Compared with other treatments, guinea pigs treated with Probiotic 03 (T3) exhibited the highest mean FLW (908.33 ± 59.39 g), although no significant differences were found ($p = 0.242$).

Weight gain

Comparison of WG after the trial revealed the highest mean in T3 (641.67 ± 37.45 g) and the lowest in T1 (550.09 ± 39.70 g). However, statistical analysis showed significant differences between treatments ($p = 0.04$), with differences found only between T3 and T1 ($p = 0.039$). Weekly WG differences among treatments became evident from the third week of evaluation ($p < 0.05$), although no treatment showed superior WG in all weeks of the trial (Fig. 1).

Weekly dry matter intake

Animals in the T3 group exhibited the highest mean WDMI (396.21 ± 116.20) compared with the other groups. However, the statistical analysis indicated that the observed differences were not significant ($p = 0.99$).

Table 1. Effect of probiotic supplementation on FLW, WG, WDMI, WFCR, CY, EM, and Guinea pig meat organoleptic properties.

	Treatments					
	T1	T2	T3	T4	T5	T6
Productivity parameters						
Number of individuals (n)	11	11	9	11	8	10
IW (g)	246.08 ± 59.09	235.00 ± 29.59	260.17 ± 25.08	251.91 ± 39.58	253.50 ± 48.79	245.33 ± 46.18
FLW (g)	798.64 ± 97.02	860.36 ± 94.18	908.33 ± 59.39	844.73 ± 104.67	884.13 ± 97.18	874.44 ± 139.98
WG (g)	550.09 ± 39.70 ^b	625.00 ± 65.43 ^{ab}	641.67 ± 37.45 ^a	586.09 ± 73.01 ^{ab}	614.38 ± 71.24 ^{ab}	619.33 ± 98.10 ^{ab}
WDMI (g)	367.27 ± 98.05	373.53 ± 100.22	396.21 ± 116.20	367.53 ± 102.59	387.04 ± 99.52	387.78 ± 98.95
WFCR	6.37 ± 1.98	5.71 ± 1.81	7.32 ± 3.93	6.26 ± 2.40	8.02 ± 6.20	6.83 ± 3.37
CY (%)	47.75 ± 1.89	50.00 ± 1.15	50.25 ± 0.25	49.75 ± 2.22	49.00 ± 0.82	50.75 ± 1.50
EM (%)	122.35 ± 35.89	141.64 ± 28.19	133.12 ± 8.26	135.86 ± 20.44	155.27 ± 6.20	172.36 ± 39.98
Organoleptic properties						
Color	3.22 ± 0.65	3.56 ± 0.62	3.31 ± 0.62	3.53 ± 0.61	3.70 ± 0.47	3.45 ± 0.76
Odor	3.28 ± 0.75	3.39 ± 0.70	3.44 ± 0.63	3.26 ± 0.81	3.45 ± 0.51	3.40 ± 0.60
Flavor	3.39 ± 0.61	3.78 ± 0.43	3.25 ± 0.58	3.21 ± 0.63	3.55 ± 0.51	3.25 ± 0.91
Tenderness	3.50 ± 0.62	3.67 ± 0.59	3.25 ± 0.68	3.26 ± 0.65	3.55 ± 0.51	3.25 ± 0.85
Juiciness	3.39 ± 0.78	3.28 ± 0.67	3.56 ± 0.63	3.11 ± 0.66	3.70 ± 0.47	3.45 ± 0.69

CY = carcass yield; EM = economic merit; FLW = final live weight; T1 = basal diet + *Enterococcus gallinarum*; T2 = basal diet + *Exiguobacterium sp.*; T3 = basal diet + *Lactococcus lactis*; T4 = basal diet + *Enterococcus gallinarum* + *Exiguobacterium sp.* + *Lactococcus lactis*; T5 = basal diet + GPA; T6 = basal diet. IW = initial weight; WDMI = weekly dry matter intake; WFCR = weekly feed conversion ratio; WG = weight gain.

All values are expressed as mean ± standard deviation.

^{a,b}Different letters in the same row indicate significant differences ($p < 0.05$).

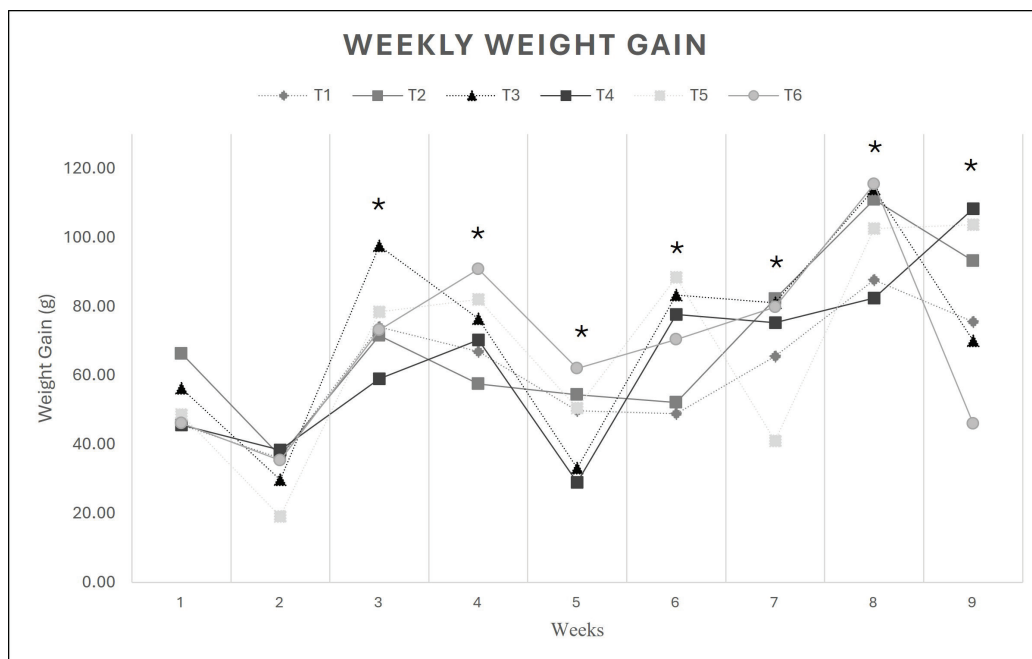


Fig. 1. Effect of probiotics on weight gain (g) throughout 9 weeks of study. T1: Basal diet + Probiotic 01; T2: Basal diet + Probiotic 02; T3: Basal diet + Probiotic 03; T4: Basal diet + Probiotics 01, 02, and 03; T5: Basal diet + GPA; T6: Basal diet. (*) indicates significant differences ($p < 0.05$).

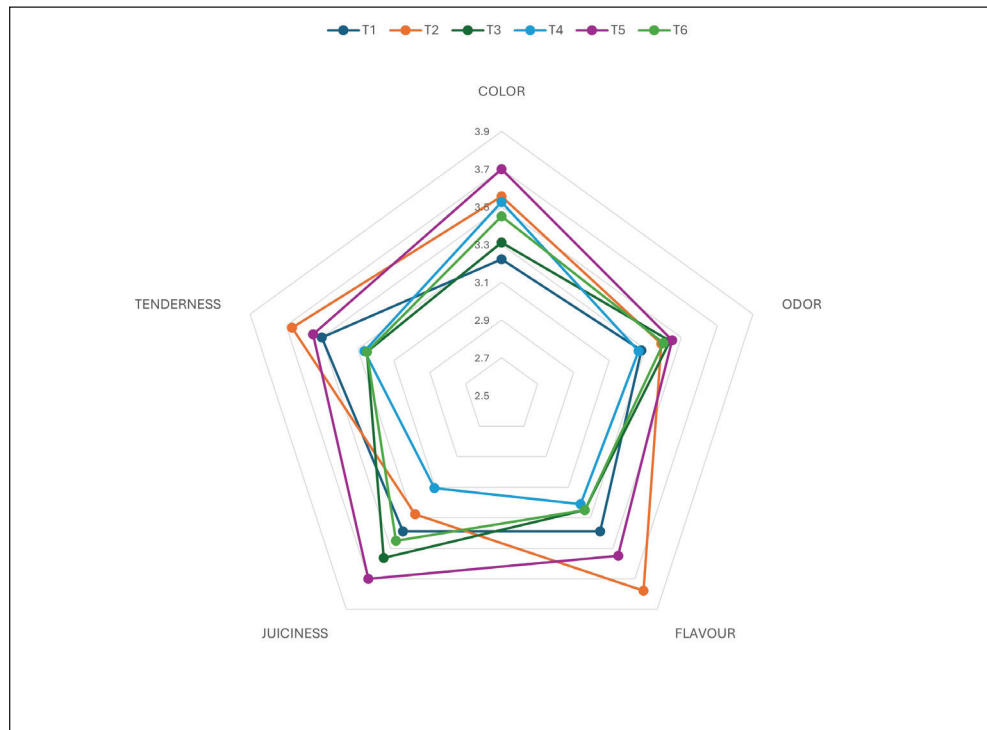


Fig. 2. Consumer acceptance evaluation (color, smell, flavor, juiciness, and texture) of guinea pig's flesh obtained from animals treated with T1: Basal diet + Probiotic 01, T2: Basal diet + Probiotic 02, T3: Basal diet + Probiotic 03, T4: Basal diet + Probiotics 01, 02, and 03, T5: Basal diet + GPA, and T6: basal diet.

Weekly feed conversion ratio

No significant differences were observed in the effect of treatments on WFCR ($p = 0.72$). However, the best mean value was found in guinea pigs from T2 (5.71 ± 1.81).

Carcass yield

The highest mean CY was observed in the control group (50.75 ± 1.50), although no significant differences were found compared with probiotic or GPA treatment ($p = 0.093$).

Economic merit

The comparison of EM among probiotics, GPA, and control treatments showed no significant differences ($p = 0.157$). Similar to CY, T6 had the highest mean EM (172.36 ± 39.98).

Organoleptic properties

Sensory analysis of meat organoleptic characteristics revealed high acceptance of probiotic treatment (Fig. 2). Evaluation of each attribute indicated that GPA addition resulted in the highest acceptance for color (3.70 ± 0.47), odor (3.45 ± 0.51), and juiciness (3.70 ± 0.47). Conversely, flavor and tenderness had the highest mean acceptance scores at T2 (3.78 ± 0.43 and 3.67 ± 0.59 , respectively). In addition, each variable evaluated showed higher averages due to the administration of some of the probiotics used compared to the control (T6). However, the differences among the treatments were not significant ($p > 0.05$).

Discussion

The addition of probiotic microorganisms to animal diets is closely related to the improvement of productive parameters in animal husbandry, such as FLW, WG, dry matter intake, and feed conversion ratio (Anee *et al.*, 2021). In this study, no significant effects were found from probiotic administration on these parameters; however, better averages were observed in each case. Similar results have been reported previously with the application of probiotics in guinea pigs, specifically regarding FLW (Canto *et al.*, 2018; Guevara-Vásquez *et al.*, 2021), WG (Ortiz, 2016; Canto, 2018; Andía and Angeles, 2021), dry matter intake (Carcelén *et al.*, 2021; Guevara-Vásquez *et al.*, 2021; Quijano *et al.*, 2023), and feed conversion ratio (Valdizán *et al.*, 2019; Quijano *et al.*, 2023). These findings highlight the complexity of optimizing probiotics in guinea pigs to improve their productive parameters.

CY did not differ between the probiotic and control treatments, with lower percentages compared to other reports. This discrepancy could be due to the exclusion of the viscera, head, and distal parts of the limbs in this study, which reduced carcass weight, whereas other studies included these parts in their analysis (Canto *et al.*, 2018; Guevara-Vásquez *et al.*, 2021; Sopla, 2024). Additionally, the use of probiotics did not significantly

increase diet costs compared with the control treatment. Because commercial media were used for probiotic preparation in this study, using lower-cost substrates could improve profitability (Berisvil *et al.*, 2021). Moreover, achieving a significant impact of probiotics on guinea pig production would enhance profitability, as reported by Quijano *et al.* (2023) with increased probiotic doses.

Probiotics also affect the organoleptic properties of meat by modifying its odor and flavor via lipid oxidation. In addition, they promote an increase in muscle fiber density, enhancing meat tenderness (Liu *et al.*, 2022). Meat color can also change because of the modulation of myoglobin oxidation or reduction (Nie *et al.*, 2022). In this study, probiotic treatments did not negatively affect meat quality; on the contrary, they had high acceptance, similar to the control group (T6). These results align with the findings of Pedemonte-Córdova and Peña-Arias (2018) and Enriquez (2019), who reported that the tenderness, color, flavor, juiciness, and odor of meat do not significantly change with the use of native probiotics or a symbiotic composed of *Lactobacillus reuteri*, *Enterococcus hirae*, *Bacillus pumilus*, *Lactobacillus frumenti*, *Streptococcus thoraltensis*, and *Lactobacillus johnsonii* with inulin.

Among the bacteria used as probiotics in this study, the genus *Exiguobacterium* has been reported as part of the intestinal microbiota of guinea pigs (Goicochea-Vargas *et al.*, 2024) and other rodents such as mice (Bercik *et al.*, 2011; Han *et al.*, 2021). However, its use as a probiotic has only been reported in aquaculture animals (Hadi *et al.*, 2014; Kim *et al.*, 2022; Zhang *et al.*, 2024). On the other hand, the genus *Enterococcus* has been successfully used as a probiotic in guinea pigs (Flores-Gutiérrez, 2014; Carcelén *et al.*, 2021; Guevara-Vásquez *et al.*, 2022), but the *E. gallinarum* strain has only been used in aquaculture. *Lactococcus lactis* is also widely recognized for its high safety and use in the food industry, with positive effects on productive parameters in weaned piglets (Yu *et al.*, 2021), calves (Nayel *et al.*, 2019), and aquaculture animals such as flounder (Nguyen *et al.*, 2018). In all cases, the probiotic potential of these microorganisms has been demonstrated both *in vitro* and *in vivo* (De Chiara *et al.*, 2024; Goicochea-Vargas *et al.*, 2024; Xie *et al.*, 2024; Totewad and Gyananath, 2024). However, the limited effect observed in this study may be because these bacteria had not previously been tested as probiotics in guinea pigs, requiring further optimization to achieve successful results.

Additionally, the low efficiency of these bacteria on productive parameters may result from various factors related to the microorganisms or treated animals. The quantity of microorganisms applied may have been insufficient, as approximately 10^7 CFU/ml was used, compared to more successful studies that used 10^8 CFU/ml or higher (Hadi *et al.*, 2014; Nayel *et al.*, 2019; Zhang *et al.*, 2024). Moreover, the resistance of these strains to low pH was tested up to 4.0 (Goicochea-

Vargas *et al.*, 2024), which may have been inadequate to ensure high survival in the stomach of this rodent, known to have a much lower pH (Ramón, 2017).

Another important factor is the stress caused by animal handling during probiotic administration. Guinea pigs are highly sensitive to stress induced by external factors, such as frequent or improper handling by unfamiliar individuals (Grégoire *et al.*, 2010). In this study, probiotics were administered using a syringe, which may have caused considerable stress. Such stress in small animals can temporarily reduce feed intake rates and lead to permanent weight reduction (Harris *et al.*, 1998; Jeong *et al.*, 2013). Furthermore, due to the interaction between the gastrointestinal tract and the central nervous system, stress exposure may disrupt the microbial balance, reducing lactic acid bacteria populations such as the *Lactococcus* genus (Shevchenko *et al.*, 2023; Tanelian *et al.*, 2023), potentially explaining the diminished probiotic effects on productive parameters.

Furthermore, the probiotic preparation involved mixing these microorganisms with lactic acid at a low concentration (0.07%). The addition of lactic acid was based on safety limits of up to approximately 2% of the diet (Azimonti *et al.*, 2022) to prevent animal harm. Lactic acid has an inhibitory effect on pathogenic bacteria that cause diseases in guinea pigs, such as *E. coli* and *Salmonella sp.* (Wang *et al.*, 2015). Because the lactic acid solution was supplied in all treatments, its beneficial effects on productive parameters (Rychen *et al.*, 2017) may have minimized differences between probiotic and control treatments.

Conclusion

The oral administration of the native probiotic bacteria *Enterococcus gallinarum*, *Exiguobacterium sp.*, and *Lactococcus lactis* did not significantly affect the productivity or meat quality of guinea pigs.

Acknowledgment

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Conflict of interest

The authors declare no conflict of interest.

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Author contributions

JGV, MSA, FAP, and WRJ participated in the study design and conception. JGV, MSA, JCA, and AHB performed the tests and collected the required data. JGV, MSA, FAC, MR, WRJ, and JCA analyzed the collected data, and JGV, MSA, EMP, WRJ, MS, and EM wrote the manuscript. All authors have revised and approved the final manuscript.

Data availability

All data supporting the findings of this study are available in the manuscript.

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