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Effect of probiotics administration at different levels on the productive parameters of guinea pigs for fattening (*Cavia porcellus*)

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Abstract

Background: For more than 50 years, antibiotics have been used to maintain animal welfare and improve efficiency. Recently, antibiotics were found in the muscle, liver, and kidney of guinea pig carcasses put up for sale and human consumption, which is a public health issue. Probiotics are supplements of live microorganisms that, when administered in adequate doses, could replace growth-promoting antibiotics.

Aim: This study analyzed the effect of the administration of an oral probiotic mixture on the guinea pigs productive performance (*Cavia porcellus*).

Methods: Fifty male guinea pigs, weaned at 14 days of age, were distributed in a completely randomized design of five treatments with ten repetitions for each group. The treatments were CONTROL group without probiotic; PROB 1 given 1 ml of probiotic; PROB 2 with 2 ml of probiotic; PROB 3 with 3 ml of probiotic; and antibiotic growth promoter (AGP) was given 300 ppm zinc bacitracin. The microorganisms used in the probiotic were *Enterococcus hirae*, *Lactobacillus reuteri*, *Lactobacillus frumenti*, *Lactobacillus johnsoni*, *Streptococcus thoralensis*, and *Bacillus pumilus*. Productive parameters were evaluated from weaning to 70 days of age.

Results: No statistically significant difference was found between the treatments on forage dry matter intake (DMI), concentrate DMI, or total concentrate DMI ($p > 0.05$). Similarly, no statistical difference was found between the treatments in terms of final weight or weight gain ($p > 0.05$). Regarding the feed conversion ratio (FCR), there was a significant difference between treatments ($p = 0.045$); the CONTROL group had the highest FCR, followed by the AGP group, with the best FCR observed in the PROB 3 group ($p < 0.05$). In addition, significant statistical differences were found between CONTROL and PROB 2 ($p < 0.05$). Likewise, a significant linear effect of increasing doses of the probiotic was found ($p = 0.01$), which indicated that the feed conversion was better with a higher dose.

Conclusion: The treatments evaluated in this study significantly impacted the FCR in guinea pigs for fattening. Increasing doses of probiotics had a linear effect on FCR.

Keywords: Antibiotic growth promoter, Guinea pig, Probiotics, Productive parameters.

Introduction

The use of antibiotics to maintain animal health, promote growth, and improve efficiency has been practiced for more than 50 years (Abd El-Hack *et al.*, 2020). Recently, Ampuero (2018) found antibiotics in the muscle, liver, and kidney of guinea pig carcasses put up for human consumption in three cities of Peru. Probiotics are living-microorganism supplements that if administered in adequate doses could benefit the host animal by balancing the microbial population in its gastrointestinal tract. These microorganisms compete for adhesion sites with their enteropathogenic peers (Fuller, 1989; Reid *et al.*, 2003; Tiwari *et al.*, 2012), which is complemented by the secretion of bacteriocins by probiotics and peristaltic movements of the intestine (Isolaury *et al.*, 2001; Monteagudo-Mera *et al.*, 2019). Detailed studies on the diversity of natural microbiota in the gastrointestinal tract of guinea pigs are limited; however, it is estimated that 320–376 bacterial

genera exist under equilibrium conditions with the host (Hildebrand *et al.*, 2012). The presence of these microbiotas is necessary and beneficial for animals (Turner, 2018; Adedokun and Olojede, 2019), and any imbalance favors the proliferation of harmful bacteria, affecting animal health and performance (Chaucheyras-Durand and Durand, 2010; Young, 2012; Wen and Duffy, 2017; Alayande *et al.*, 2020).

Bacteria constituting the intestinal microbiota and those supplied in probiotics, produce bacteriocins, organic acids, and hydrogen peroxide, which have a bactericidal action against enteropathogens (Umu *et al.*, 2016; Garcia-Gutierrez *et al.*, 2019; Alayande *et al.*, 2020). Some bacteria in the normal intestinal flora secrete enzymes such as beta-glucuronidases and bile salt hydrolases, which release bile acids with inhibitory actions on undesirable bacteria (Ferkert, 1993; Ridlon *et al.*, 2016), while others produce digestive enzymes and metabolites capable of neutralizing bacterial

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toxins, thus increasing the immunity of the intestinal mucosa (Ferkert, 1993; Coppola and Turner, 2004; Garcia-Gutierrez et al., 2019).

Alayande et al. (2020) suggested that the main microorganisms used as probiotics in animal production belong to the genera *Lactobacillus*, *Streptococcus*, *Lactococcus*, and *Bifidobacterium*. It has been stated that the requirements for a microorganism to be considered a probiotic are that it must: (i) be a normal part of the gastrointestinal microbiota of the host; (ii) not be toxic or pathogenic; (iii) be able to adhere to the intestinal epithelium of the host; (iv) be cultivable on an industrial scale; (v) be stable in commercial preparation; (vi) survive the action of digestive enzymes and rapidly colonize the host intestine; and (vii) have antagonistic action on pathogenic microorganisms (Yerlikaya, 2014; Ahasan et al., 2015).

Probiotics have been used in chickens (Park et al., 2016), pigs and piglets (Kenny et al., 2011; Dlamini et al., 2017), rabbits (Bhatt et al., 2017), cattle (Uyeno et al., 2015), and horses (Schoster et al., 2014). Several studies claim that probiotics can actively improve the growth and feed conversion ratio (FCR) in pigs and poultry, similar to those obtained with growth-promoter antibiotics (Figueiredo et al., 2010; Mehdi et al., 2018), and also actively participate in the control of pathogenic and non-pathogenic microorganisms (Londoño, 2013). However, their effect depends on the animal species, age, health status, and operating conditions, in addition to the nature of the probiotic compound and dose (Musa et al., 2009; Markowiak and Ślizewska, 2018).

Considering that the results of probiotic administration in guinea pigs are still limited and inconsistent (Torres et al., 2013; Cano et al., 2016; Valdizán et al., 2019), the present study aimed to evaluate the effect of the inclusion of different levels of probiotics as substitutes for antibiotic growth promoters (AGPs) on the productive parameters of guinea pigs.

Materials and Methods

Study place

The study was carried out during the rainy season (January–April), at the “Estación Experimental El Mantaro”, Instituto Veterinario de Investigación Tropical y de Altura of the Universidad Nacional Mayor de San Marcos, Junín, Peru (11.83° S, 75.40° W, 3320 MASL).

Animals

Fifty male guinea pigs (genetic line “Cuyes G”) were used in this study. They were 14 days old and weighed 307 ± 51 g.

Experimental design and treatments

The study used a completely randomized design in an additive model: $y_{ij} = \mu + T_i + \beta(x_{ij} - \bar{A}) + \varepsilon_{ij}$, where y_{ij} is the value of the j -th observation of the i -th treatment, μ is the general mean, T is the effect of the i -th treatment, β is the regression coefficient that relates y_{ij} to the covariate x_{ij} , x_{ij} is the weaning weight

of the j -th observation of the i -th treatment, \bar{A} is the mean weaning weight, and ε is the effect of the j -th observation of the i -th treatment (experimental error). The study evaluated five treatments, which consisted of supplementation with a probiotic (*Enterococcus hirae*, *Lactobacillus reuteri*, *Lactobacillus frumenti*, *Lactobacillus johnsoni*, *Streptococcus thoralensis*, and *Bacillus pumilus*; Torres et al., 2013; Puente et al., 2019) at different doses (1, 2, and 3 ml), a negative control (without probiotic and without antibiotic) and a diet supplemented with AGP (300 ppm Zn Bacitracin; Promozimb 10%, CUSA). Ten experimental units were used in each treatment. Each experimental unit corresponds to a randomly selected weaned male guinea pig from different litters. To determine the effect of the treatments on the animals' productive parameters, they were evaluated from weaning to 70 days of age. Once the experimental units were weaned, they were each conditioned in an individual pen $0.7 \times 0.8 \times 0.5$ m (L, W, and H, respectively), with a cement floor, wooden walls, and mesh. They continued receiving the previously assigned diet.

The probiotic application methodology, described by Valdizán et al. (2019), consisted of three serial applications. The first application was made during the lactation period. The established doses of the probiotics were administered on days 3, 4, 5, 6, and 7 of age. The second application was made at the time of weaning, and the corresponding dose of probiotics was administered on days 14, 15, 16, 17, and 18. Finally, the third application was made in the middle of the growth period, on days 42, 43, 44, 45, and 46 of age. The treatments were delivered orally using a syringe, 1 ml of distilled water mixed with 1, 2, or 3 ml of probiotic suspension. The antibiotic growth promoter was a commercial product containing 10% zinc bacitracin. This product was administered at a rate of 3 kg per 1,000 kg of wheat bran. The treatments were as follows: CONTROL: without supplementation; PROB 1: administration of 1 ml of probiotic; PROB 2: administration of 2 ml of probiotic; PROB 3: administration of 3 ml of probiotic; and AGP: supplementation with 300 ppm of Zn Bacitracin.

Basal diet

The basal diet consisted of wheat bran and forage (a mixture of *Medicago sativa*, *Lolium multiflorum*, and *Trifolium pratense*). The forage was offered daily to the animals at a rate equivalent to 25% of their live weight, and wheat bran was offered twice a day at a rate equivalent to 5% of their live weight. Proximal analysis of the forage mixture, wheat bran, and basal diet is shown in Table 1. The amount of food offered was recorded at the beginning of each day.

Study variables

The productive parameters evaluated in the experiment were as follows: (i) forage dry matter intake (forage DMI) in g, obtained as the total difference between the dry matter offered and the residual dry matter of the

Table 1. Proximal analysis of the forage mixture of wheat bran and base diet.

Nutritional components	Forage mixture	Wheat bran	Basal diet
DE (Kcal/kg)*	3331.14	3638.36	3460.68
TDN (%)**	75.71	82.69	78.65
CF (%)	14.26	9.80	12.38
NFE (%)	53.09	67.10	59.00
CP (%)	21.33	15.10	18.70
EE (%)	2.37	3.00	2.64
Ash (%)	8.95	5.00	7.28
DM (%)	24.06	87.70	50.89

DE = digestible energy; TDN = total digestible nutrient; CF = crude fiber; NFE = nitrogen-free extract; CP = crude protein; EE = ether extract; DM = dry matter. Basal diet: 25% of live weight of forage and 5% of live weight of wheat bran as fed. The basal diet was formulated to meet the requirements of 2,800 kcal of DE, 10% of CF, and 17% of CP. (*): DE (kcal) = 4,400 × TDN (kg); (**): TDN % = (0.50 × %CF) + (0.90 × %NFE) + (0.75 × %CP) + (2.25 × 0.90 × %EE).

Table 2. Contrast scheme for the study of different responses.

Contrast	Control	PROB 1	PROB 2	PROB 3	AGP
Linear	−3	−1	1	3	0
Quadratic	1	−1	−1	1	0
Probiotic vs. AGP	0	−1	−1	−1	3

forage; (ii) concentrate dry matter intake (concentrate DMI) in g, obtained as the total difference between the dry matter offered and the residual dry matter of the concentrate; (iii) total dry matter intake (total DMI) in g, obtained as the total difference between the offered dry matter and the residual dry matter of the total mixture; (iv) final weight (FW) in g, obtained by weighing the animal at the end of the experiment; (v) weight gain (WG) in g, obtained as the difference between the final weight and the weaning weight of the animals; and (vii) FCR, obtained as the total DMI/WG ratio.

Statistical analysis

To evaluate the probiotic and AGP levels' results concerning the productive parameters (forage DMI, concentrate DMI, total DMI, FW, WG, and FCR), an analysis of covariance (ANCOVA) was used. Given the increasing nature of probiotics' doses, linear and quadratic polynomial contrasts were used to evaluate their effects (Table 2). Similarly, a contrast between the control and AGP groups was used to determine the differences between them. If statistical differences were found in the ANCOVA, the estimated marginal means were examined with pairwise comparisons to establish the difference between the groups, and subsequently, the optimal level of the probiotic. Statistical calculations were carried out with the help of the IBM Statistical Package for the Social Sciences Statistics 25 package. A significance level of 0.05 was used for all analyses.

Ethical approval

This study was approved by the Graduate School of the Faculty of Veterinary Medicine at the Universidad

Nacional Mayor de San Marcos, Peru. Sampling procedures were carried out in accordance with the animal welfare protocols.

Results

Table 3 details the results obtained in this work. Regarding the consumption of dry matter, no significant statistical differences were found between the treatments with forage DMI, concentrate DMI, and total DMI ($p = 0.465$, $p = 0.259$, and $p = 0.399$, respectively). The probiotics levels did not present a linear or quadratic response in relation to these treatments ($p > 0.05$). Regarding the guinea pigs' final weight, there was no evidence of a significant difference between treatments ($p = 0.556$). There was also no statistical difference regarding WG during the fattening period ($p = 0.556$). Similarly, no linear or quadratic effect of increasing the probiotic levels was observed on the final weight or on WG during the fattening period in guinea pigs. Finally, there were statistical differences between treatments regarding FCR ($p = 0.045$), revealing that the group that received the CONTROL treatment had the highest FCR, followed by the AGP group. On the contrary, the best FCR occurred in the PROB 3 group ($p < 0.05$). In addition, significant statistical differences were found between CONTROL and PROB 2 ($p < 0.05$). A significant linear effect of increasing doses of the probiotics was found ($p = 0.010$), which indicates that the higher the dose of probiotic supplementation, the lower the FCR. Additionally, a significant effect of the probiotic application was found ($p = 0.032$).

Table 3. Effect of probiotic levels on forage DMI, concentrate DMI, and total DMI in guinea pigs and on FW, WG, and FCR of guinea pigs for fattening.

	Treatment					Responses		
	Control	PROB 1	PROB 2	PROB 3	AGP	Linear	Quadratic	Probiotic versus AGP
	<i>p</i> -value							
Forage DMI (g)	1,795.00	1,752.98	1,753.95	1,822.83	1,875.51	0.341	0.802	0.529
Concentrate DMI (g)	1,270.89	1,152.73	1,256.80	1,163.93	1,154.50	0.729	0.310	0.119
Total DMI (g)	3,065.89	2,905.72	3,010.75	2,986.77	3,030.00	0.618	0.251	0.362
FW (g)	985.11	971.83	1,001.81	1,001.60	982.90	0.241	0.663	0.609
WG (g)	677.67	664.39	694.37	694.16	675.47	0.241	0.663	0.609
FCR	4.53c	4.38 ^{abc}	4.35ab	4.30a	4.49bc	0.010*	0.388	0.032*

Different letters (a,b,c) indicate significant differences between treatments ($p < 0.05$). *Values less than 0.05 are statistically significant.

Discussion

The results of this study differ from those presented by Saldarriaga (2018), where three groups were considered (control, probiotic with six strains of bacteria, and a growth-promoting antibiotic). No significant difference was found in that study between the treatments regarding dry matter consumption, WG, or FCR ($p > 0.05$). However, our results are similar to those of Saravia (2018), where four doses of probiotic (control, 1, 2, and 3 g) were administered in a mixture of amino acids and vitamins; a significant difference was found between food consumption and the FCR of these groups ($p < 0.05$). The higher the concentration of probiotics, the lower the food consumption.

Various studies have reported no significant effect of the addition of probiotics on WG, including Guevara and Carcelén (2014), who found no difference in the WG of guinea pigs between the control and the treatments with yeast and *Lactobacillus* spp. Torres et al. (2013) used the same probiotic but with different doses and did not observe a significant response in total DM consumption. Ortiz (2016) used four groups which included the control group and three probiotic groups, and evaluated the daily gain, total weight, and FCR in guinea pigs. No significant difference were noted between the groups ($p > 0.05$).

However, there have also been studies conducted on guinea pigs where a significant effect of probiotics on WG has been observed. Among these studies, we found that Huamán (2018) found a significant difference ($p < 0.05$) between treatment with and without a probiotic concerning WG in guinea pigs; the WG of guinea pigs that received a diet with a probiotic (1 ml) was superior to the WG of the animals that received no probiotic. In this study, the probiotic chosen was a biomodulator composed of five types of bacteria.

The probiotic effect on the FCR in this study could be due to the probiotic benefits to the host's health. Studies on bacterial probiotics in other monogastric animals have shown increased levels of additional organic acids, such as lactic and acetic acids. Accordingly, different

strains of bacterial probiotics assist in decreasing the pH of the gut. This allows the microbiome to become more favorable in its environment to some resident microorganisms, reducing pathogen colonization (Abd El-Hack et al., 2020). Also, the use of probiotics can reduce pathogen translocation across the intestinal mucosa by enhancing intestinal barrier integrity and maintaining immune tolerance (Lee and Bak, 2011).

Similarly, several studies in chickens have documented the preventive and protective role of probiotics against *Salmonella enterica* and *Escherichia coli*, which are pathogens that negatively affect the health of guinea pigs (Carey et al., 2008; Maragkoudakis et al., 2009). In addition, the use of probiotics in the diet changes the bacterial populations in the small intestine. It affects the mucin dynamics and intestinal epithelium. These alterations may influence gut health, function, and may also affect nutrient uptake (Abd El-Hack et al., 2020).

The possible mechanisms of probiotic action begin with the competitive exclusion (CE) of pathogenic microorganisms, the production of antimicrobial substances, competition for growth factors and nutrients, the enhancement of adhesion to the intestinal mucosa, the improvement of epithelial barrier function, and the improvement of IgA secretion. Bacteria are naturally competitive, and therefore, they attempt to eliminate pathogenic bacteria that could negatively affect the intestinal tract (Abd El-Hack et al., 2020). This is often referred to as CE, bacterial antagonism, or bacterial interference (Fuller, 1989). Additionally, the probiotic benefits to host health include the manufacture of small molecules with systemic effects and enzymes' production (Sanders et al., 2019).

In general, other studies have shown inconsistent results about probiotic supplementation in guinea pigs. However, some positive results have been reported for treatment with probiotics. For example, Molina (2008) found that the use of *Lactobacillus acidophilus* and *Bacillus subtilis* affects the productive parameters in guinea pigs which received treatment when compared to the control group. The results of our study on feed

conversion indicate that there is a linear effect of increasing doses of the probiotic. This response was statistically different between the control and higher doses of probiotics (PROB 2 and PROB 3) group. Within the framework of these results, we cannot ensure that higher doses than those used in the study benefit guinea pigs for fattening. However, there remains a possibility that the usage of higher doses than those evaluated may have a beneficial effect on the productive parameters of guinea pigs.

In conclusion, the evaluated treatments had a significant impact on the FCR in guinea pigs for fattening. A linear effect of increasing doses of probiotics on the FCR was found, indicating that the higher the probiotic supplementation level, better the FCR.

Authors' contributions

All authors conceived the study, wrote the paper, and participated in monitoring the results. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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