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Cavia Porcellus: Impact of Dietary Organic Acid Supplementation on Productive Performance, pH, and Intestinal Development

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ABSTRACT

Guinea pig production has been increasing in Andean production systems in recent years due to their rapid growth, feed efficiency, and the nutritional value of their meat. The objective of this study was to evaluate the effect of supplementing the diet with organic acids (propionic, acetic, and formic) on the productive performance, gastrointestinal pH, and intestinal development of guinea pigs during the fattening phase. A total of 160 weaned male and female guinea pigs aged 3 weeks (434.62 ± 9.05 g) were used and distributed using a completely randomized design with a 4x2 factorial design; Three levels of organic acids were applied to the forage twice a week (T1: 2.25 ml; T2: 2.5 ml; T3: 2.75 ml). The animals were fed a diet based on forage and balanced feed (80:20) for 63 days. Productive variables (weight gain, feed conversion, carcass yield), digestive organ weight, stomach and cecal pH, and histological parameters (villous length and width, crypt depth) were evaluated. In T2 and T3, production parameters significantly improved (p<0.001), with greater weight increase and carcass yield.. Liver weight increased in the supplemented groups (p=0.024), and pH showed a tendency toward greater acidity in the stomach; however, no differences were found in intestinal morphology. Live weight correlated positively with liver and intestine weight and negatively with gastric pH. Supplementation with organic acids improves productive performance and promotes metabolic development without affecting the intestinal integrity of guinea pigs.

Keywords: *Cavia porcellus, Supplementation, Productive performance, Histological, Digestive organ*

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INTRODUCTION

The guinea pig (*Cavia porcellus*) is a rodent mammal native to the Andean region of South America, with a wide distribution in countries such as Peru, Ecuador, Colombia, and Bolivia (Cresi, 2019). The rearing of this species plays a crucial role in supporting the development of indigenous households in rural areas; it provides a reliable source of income and strengthens food security for a substantial proportion of the Andean population. Its production systems are particularly advantageous for families who manage small land areas, enabling localized economic growth through small-scale production (Bact *et al*., 2019).

This production system offers several advantages, including its rapid reproductive rate, high feed conversion efficiency, and adaptability to diverse management conditions. In addition, guinea pig meat is highly valued because of nutritional properties and low-fat content (Lucas *et al*., 2021). In Ecuador, the guinea pig population averages approximately 21 million animals, due to their continuous reproductive cycle; annual production reaches nearly 47 million individuals which are primarily destined for market sale and household consumption (MAG, 2022).

In recent years, guinea pig production has faced several challenges, including increasing production costs and the emergence of diseases and parasitic infestation, resulting in economic and productive losses. Nevertheless, there is a growing interest among researchers and producers in evaluating alternative strategies such as enzymes, probiotics, prebiotics, plant extracts, and acidifiers. In this context, organic acids have attracted particular attention due to their potential to reduce harmful bacterial populations, enhance intestinal absorptive capacity, and overall productive performance (Frías *et al*., 2024).

Organic acids are natural constituents of plant and animal tissues, and they function by reducing the incidence of Salmonellosis, Clostridium related infections, and parasitic diseases such as coccidiosis (Timbermont, 2009). Therefore, organic acids increase the activity of beneficial bacteria and proteases by reducing pH. On the other hand, undissociated forms of organic acids freely penetrate the bacterial cell through the semipermeable membranes of unwanted bacteria.. Once inside, they dissociate and disrupt bacterial enzymatic activity as well as nutrient transport systems (Ardoino *et al*., 2017).

Li *et al*. (2018) and de Groot, Fariñas, Cabrera-Gómez, Pallares, and Ramis (2024) reported that organic acids induce morphological modifications characterized by an increase in villus height, a reduction in crypt depth, and a subsequent increase in the villus to crypt ratio, ultimately enhancing the intestinal absorptive surface.

Currently, there is substantial evidence in pigs and poultry regarding the benefits of organic acids in animal nutrition; however, research in guinea pigs remains very limited. In this context, it becomes necessary to investigate the effects of dietary organic acid supplementation on productive performance, stomach pH, and intestinal morphology during the fattening phase in guinea pigs.

MATERIALS AND METHODS

Study area

The study was conducted in the province of Tungurahua, in the Cevallos County, located at a latitude of -1.35° and longitude of -78.61° (21′ 0″ South, 78° 37′ 0″ West). The area is characterized by a temperate climate ranging from 13°C to 16°C, an average annual precipitation of 200-500 mm, and an elevation of 2894 m above sea level.

Sample size

For this study, a total of 80 male and 80 female weaned guinea pigs (3 weeks of age ± 5 days) were used, with an average body weight of 434.62 g (± 9.05 g) and clinically health status. No adaptation period to the experimental diet was required. The animals that received organic acids had previously consumed them at lower doses in their regular feed. The farm operates under a semi-intensive management system (Alhussain *et al*., 2022; Alqahtani *et al*., 2022; Brekeit *et al*., 2022; Shcherbin *et al*., 2022; Khazaal *et al*., 2023; Delcea *et al*., 2024; Yılmazer & Altinok, 2024).

Organic acids (OA)

A mixture of organic acids was used, consisting of 14% propionic acid, 7.3% acetic acid, and 25% formic acid; these acids were diluted in water before administration.

Experimental design and dosages

This study used improved Type 1 guinea pigs under a completely randomized design with a 4x2 bifactorial arrangement, consisting of three treatments plus a control with two replicates per experimental unit. Organic acids were supplied in treatments T1, T2, and T3, and were applied twice per week by mixing the acid mixture into the forage **(Table 1)**.

Table 1. Formulation of the Evaluated Treatments

Treatment	Sex	Replications	Feeding	Organics Acids
T0	M	2	Forage + balanced feed	0
	F	2		
T1	M	2		2,25 ml
	F	2		
T2	M	2		2,50 ml
	F	2		
T3	M	2		2,75 ml
	F	2		

M: male; F: female

Procedure

Animal management

To ensure appropriate zoosanitary control, the corresponding biosecurity protocols were implemented in the farm according to AGROCALIDAD (2013) guidelines. Likewise, all animals received proper veterinary care following the recommendations of the guinea pig management manual (Vivas 2013), prior to the initiation of each treatment, an oral anthelmintic (ivermectin, 2 drops per animal) was administered.

Feeding was carried out in a controlled manner using a basal diet formulated according to the minimum nutritional requirements for guinea pigs recommended by FAO (Bajagai *et al*., 2016). The diet consisted of forage plus concentrate in an 80:20 proportion, and it was offered twice daily at 08:00 and 16:00 hours. The animals were housed in cages measuring 1.5x1 m2, at a stocking density of 10 guinea pigs per cage.

Animal processing and Post-mortem procedures

At the end of the study (63 days), four animals from each treatment group were subjected to a 12 hour fasting period following the methodology described by Cornejo-Espinoza *et al*. (2016) y Sánchez-Macías *et al*. (2019). After this period, the guinea pigs were euthanized in accordance with the recommendations for the euthanasia of laboratory animals (rodents) proposed by Gimeno Forner *et al*. (1990), as a substitute for the procedures established for rabbits and other rodents in the Norma Oficial Mexicana (NOM-033-SAG/ZOO-2014 2015).

Evaluated indicators

Evisceration of the guinea pigs

A laparotomy was performed to expose and isolate the gastrointestinal organs. The stomach, liver, small intestine, and large intestine were carefully detached from the mesentery. The pH of the full stomach and cecum was measured using a pH meter, following the procedure of Nakandakari *et al*. 2014). The contents of the stomach and intestines were removed, and the organs were rinsed with sterile distilled water as described by Miranda-Yuquilema *et al*. (2024). Finally, each organ was weighed using a digital electronic scale (Elerian *et al*., 2024; Lee & Ferreira, 2024; Mtenga *et al*., 2024; Rampat *et al*., 2024; Sewankambo, 2024; Xu *et al*., 2024).

Productive parameters

The animals were individually weighed each week, and the productive performance parameters evaluated included: weight gain (g), feed conversion ratio, mortality (%), and carcass yield (%).

Histological parameters

A standard histological protocol was applied to evaluate the intestinal villi. Tissue sampling was performed using forceps and scissors by collecting a 2 cm section of the duodenum located approximately 3 cm from the stomach. The segment was flushed repeatedly (3 to 5 times) with a 0.9% sodium chloride solution using a 5 mL syringe to remove residual contents. The cleaned tissue was then placed on a 2x2 cm cardboard support and fixed in 10% formalin for 24 hours.

Extraction, dehydration, and clearing of the samples

Approximately 1 cm2 of the tubular segment previously sampled was excised and subjected to a dehydration protocol consisting of: three 10 min cycles in distilled water, three 10 min cycles in 70 % ethanol, three 10 min cycles in 90% ethanol, three 10 min cycles in 96% ethanol, and four 10 min cycles in 100% ethanol. Clearing was then performed with three 10-minute cycles in xylene, following the procedure described by Arce Olivas (2017).

Paraffin embedding and histological sectioning

The dehydrated and cleared samples were embedded in paraffin, which was maintained in a semi-liquid state in an oven at 60 °C. The molten paraffin was then poured into molds and allowed to solidify at room temperature. Histological sectioning of the paraffin blocks was carried out using a rotary microtome, obtaining 10 µm micrometric sections that produced thin paraffin-embedded tissue slices (Huamani Carrion, 2023).

Mounting on slides and staining of histological sections

After being spread out in a water bath at 40 to 50 degrees Celsius, the paraffin ribbons were gathered and placed onto glass slides that had previously been coated with a drop of glycerinated albumin. For nuclear-cytoplasmic staining, the sections underwent three sequential processes:

Deparaffinization and hydration: sections were deparaffinized in two 10 min cycles in xylene, followed by hydration in two cycles in 100% ethanol, 10 min in 96% ethanol, 10 min in 80% ethanol, and 10 min in distilled water.

Staining: the slides were immersed in hematoxylin for 3 min, rinsed in running water for 15 min, followed by two 10 min cycles in distilled water, 30 s in eosin, and a final 10 min rinse in distilled water.

Dehydration and clearing: the sections were dehydrated for 10 min in 80% ethanol, 10 min in 96% ethanol, two 10 min cycles in 100% ethanol, and finally cleared in two 10 min cycles in xylene.

Mounting and microscopic examination

After staining and drying, a drop of canada balsam was applied to the slide, and a coverslip was carefully placed on top, avoiding air-bubble formation by applying gentle pressure. the histological sections were examined under a light microscope in order to identify the anatomical structures of the intestinal tract, including villus height, villus width and crypt depth (arce olivas, 2017).

Statistical analysis

Data analysis began with an assessment of normality using the Shapiro-Wilk test. A general linear model was applied, and an analysis of variance (ANOVA) was performed for all variables. Mean comparisons were conducted using Duncan´s test at a 95% confidence level. On the other hand, Pearson correlation analyses were performed for the variables live weight, carcass weight, carcass yield, villus width, villus height, and crypt depth. All data were processed using 8the SPSS statistical package, version 24.

RESULTS AND DISCUSSION

Productive parameters

When analyzing weight gain, live weight, carcass weight, carcass yield, and feed conversion ratio of the guinea pig during the fattening period, it was determined that the treatments receiving organic acids achieved higher weights compared with the control group (p= 0.000). Likewise, weight gain varied according to the different acid dosages, with the highest values observed in T3, which received 2.75 mL of organic acid **(Table 2).**

Table 2. Effect of organic acid supplementation on productive parameters of guinea pigs during the fattening stage

Items	**Treatments**				**Sex**		***p* - value**		
	T0	T1	T2	T3	M	F	Trat	Sex	T x S
TGI (g)	395,50 c	500,5 b	535,75 a	540,25 a	503,12 a	482,87 b	0,000	0,023	0,792
LW (g)	831,00 c	931,75 b	967,25 a	980,50 a	937,25 a	918,00 a	0,000	0,083	0,970
CW (g)	511,73 d	609,28 c	666,86 b	696,77 a	625,75 a	616,57 a	0,000	0,346	0,420
CY (%)	61,58 d	65,38 c	68,94 b	71,05 a	66,48 a	67,00 a	0,000	0,350	0,090
FCR	4,36 b	3,37 a	3,19 a	3,16 a	3,42 a	3,61 b	0,000	0,034	0,931

TGI: total weight gain; LW: live weight; CW: carcass weight; CY: carcass yield; FCR: feed conversion ratio. T0: control; T1: addition of 2.25 ml of acid; T2: addition of 2.5 ml of acid; T3: addition of 2.75 ml of acid. M: male; F: female. Values the mean. Different letters indicate significant differences among groups (Duncan test).

Similary, regarding sex, differences were found for total weight gain and feed conversion ratio **(Table 2)**, but not for the remaining variables, with males showing the highest values. Likewise, no significant interactions were observed for any of the variables evaluated (Al-Abbad *et al*., 2022; Kitama *et al*., 2022; Atrushi *et al*., 2023; Cissé *et al*., 2024; Ganea *et al*., 2024). On the other hand, it was observed that guinea pigs supplemented with organic acid achieved an average daily weight gain of 9.38 g, with males showing greater gains. A similar trend was observed in the control treatments; however, the daily weight gain was lower (AlAwwad *et al*., 2022; Dehaghi *et al*., 2022; Huong *et al*., 2022; Singh *et al*., 2022; Bahrawi & Ali, 2023; Budko *et al*., 2023; Jeung & Chang, 2023; Ranganadhareddy, 2023; Miranda *et al*., 2024; Zhou & Dewey, 2024).

Gastrointestinal organ weights

When evaluating the effects of dietary organic acid supplementation on the weight of the gastrointestinal organs in guinea pigs, significant differences were found for liver weight, with animals receiving organic acids showing increased liver weights compared with the control group **(Table 3)**.

Table 3. Gastrointestinal organ weights of guinea pigs supplemented with different levels of organic acids

OG	**Treatments**				**Sex**		***p* -value**		
	T0	**T1**	**T2**	**T3**	**M**	**F**	**Trat**	**Sex**	**T x S**

SIW (g)	20,75 a	18,25 a	19 a	21,5 a	19,5 a	20,25 a	0,579	0,688	0,981
LIW (g)	20 b	22,75 ab	23 ab	25 a	21,87 a	23,5 a	0,101	0,215	0,293
SW (g)	7 a	9,5 a	9,5 a	9,5 a	9,25 a	8,5 a	0,272	0,475	0,874
CecW (g)	14 a	16,25 a	13,75 a	15,25 a	15,37 a	14,25 a	0,704	0,52	0,752
LW (g)	28,25 b	34,75 ab	35 ab	44,25 a	36,37 a	34,75 a	0,024	0,58	0,255
pH_St	4,41 a	3,66 a	4,07 a	3,27 a	4,02 a	3,68 a	0,345	0,454	0,851
pH_Cec	6,63 a	6,62 a	6,34 a	6,51 a	6,55 a	6,5 a	0,296	0,703	0,56

OG: gastrointestinal organs; T0: control; T1: addition of 2.25 ml of acid; T2: addition of 2.5 ml of acid; T3: addition of 2.75 ml of acid. M: male; F: female. SIW: small intestine weight. LIW: large intestine weight. SW: stomach weight. CecW: cecum weight. LW: liver weight. pH_St: stomach pH. pH_Cec: cecum pH. Values represent the mean. Different letters indicate significant differences among groups (Duncan test).

For the remaining organs, no significant differences were observed in their weights, and no difference were detected between sexes. However, numerical trends indicated higher organ weights in animals supplemented with organic acids, suggesting a potential effect on the development of the guinea pig gastrointestinal tract.

Similarly, no significant differences were found among treatments for stomach or cecal pH. However, stomach acidity increased in rats given organic acids, with the impact being most noticeable in T3 (3.27). In the cecum, lower acidity was observed in T2, although overall differences among treatments were minimal.

Histological parameters

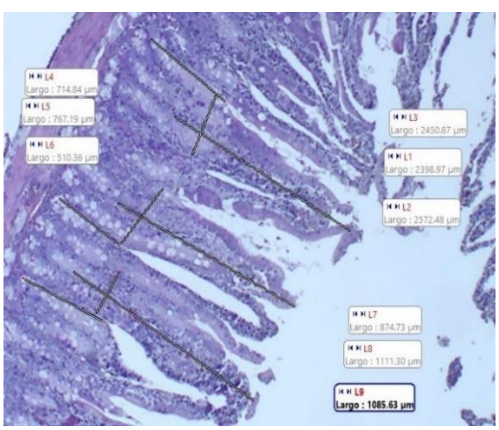
When comparing the histological parameters, no significant differences were observed in response to the addition of organic acids across treatments for villus height, villus width or crypt depth **(Table 4)**. Similarly, no differences were detected between sexes.

Table 4. Effect of organic acid supplementation on the histological parameters of the small intestine of guinea pigs.

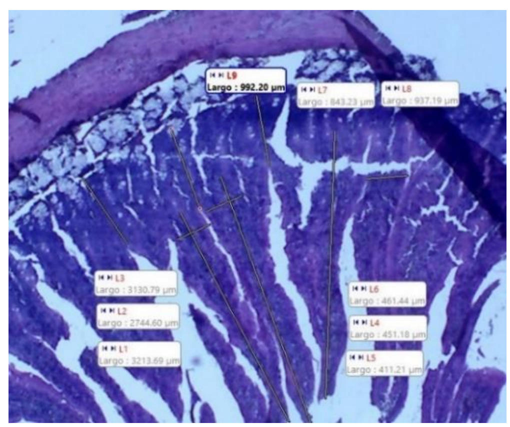
Items (µm)	**Treatments**				**Sex**		***p* -value**		
	T0	T1	T2	T3	M	F	Tra	Sex	TxS
Length	2824,4**a**	2228,1 **a**	3306,9**a**	3287,1**a**	3100.7 **a**	2722.6**a**	0,1	0.30	0.7
Width	458,9**a**	791.6**a**	589,5**a**	618**a**	629. **a**	599.4**a**	0,3	0.81	0.3
Depth	778,5**a**	604,5**a**	761,7**a**	604,5**a**	792.7**a**	663.6**a**	0,5	0.21	0.2

T0: control; T1: addition of 2.25 ml of acid; T2: addition of 2.5 ml of acid; T3: addition of 2.75 ml of acid. M: male; F: female. Values represent the mean. Different letters indicate significant differences among groups (Duncan test).

However, numerical trends were observed among the treatments, with the greatest villus height recorded in T2 (3306.93 µm), followed by T3 (3287,15 µm), while the lowest values were found in the control group and T1 **(Table 4)**. Similarly, for villus width, animals supplemented with organic acids exhibited higher values, particularly in T1 (791.62 µm) and T3 (618 µm). These results indicate that organic acids promote modifications in the intestinal structures evaluated. The measurement of the different structures are presented in **Figure 1**,



a)



b)

Figure 1. Histological sections of the duodenum showing measurements of villus height, villus width and crypt depth in guinea pigs supplemented with organic acids. L1, L2 and L3: villus height. L4, L5 and L6: villus width. L7, L8 and L9: depth of the crypts of Lieberkühn

Correlation between productive and histological parameters

When assessing the strength of the correlation between productive performance, pH and intestinal development, a significant association ($p<0.05$) was observed among the evaluated variables. These findings suggest that villus width, small intestine weight, liver weight and stomach pH directly influence the productive performance of the animals **(Figure 2).**

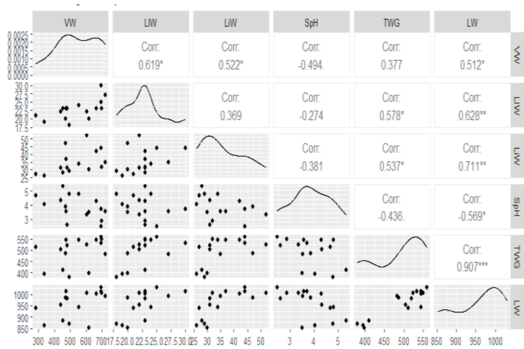


Figure 2. Pairwise correlation matrix and scatterplot relationships between villus width (VW), large intestine weight (LIW), liver weight (LiW), stomach pH (SpH), total weight gain (TWG), and live weight (LW) in guinea pigs.
* Correlation is significant at the 0,05 level; **Correlation is significant at the 0,01 level.

Live weight was the variable showing the strongest associations among all variables evaluated, presenting a positive correlation with total weight gain (r= 0.907; p<0.01), liver weight (r= 0.711; p<0.01), and large intestine weight (r= 0.628; p<0.01) and villus width (r=0.512; p<0.05). Likewise, a significant negative correlation was found between live weight and stomach pH (r= -0.569; p<0.05).

Total weight gain was positively correlated with large intestine weight (r= 0.578; p<0.05) and liver weight (r= 0.537; p<0.05). Similarly, liver weight showed a positive correlation with villus width (r= 0.543; p<0.05). In contrast, stomach pH was significantly negatively correlated with live weight (r= -0.569; p<0.05), with no correlations detected with the histological parameters.

CONCLUSION

Dietary supplementation with organic acids represent a nutritional strategy that support metabolic development and enhances productive performance in guinea pigs, as reflected by increased live weight, carcass weight and feed efficiency

The physiological and anatomical modificatios induced by organic acid supplementation further confirm their role as modulators of the gastrointestinal tract, promoting more efficient nutrient digestion and absorption

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