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# Mountain microorganisms and corn silage as probiotics in the fattening of rabbits

## Microorganismos de montaña y ensilado de maíz como probióticos en la engorda de conejos

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### ABSTRACT

There are bacteria that produce lactic acid (LAB) present in the epiphytic microflora of plants, and consortia of mountain microorganisms such as yeasts and mixed cultures that can be used as probiotics and growth promoters in animal production. To evaluate the use of mountain microorganisms in corn silage as probiotics in the fattening of rabbits for 4 weeks, 20 hybrid rabbits were used which were chosen randomly in each treatment. The preparation of the probiotics was carried out through an initial anaerobic fermentation stage and a final aerobic one. Treatment (T1) was a diet supplemented with the addition of mountain microorganisms in corn silage (MME) in the drinking water and treatment two or conventional (T2) served as a control without application of MME. The feed consumption and the feed conversion index were calculated, expressed as the mean ± the standard deviation. When performing an analysis of variance (ANOVA), it was established that during week 4 of treatment there was a significant difference ($P < 0.05$) in weight gain and feed conversion between treatments, being favorable for T1 supplemented with MME.
**Keywords**: Silage, efficient microorganisms, cuniculture, probiotics.

### RESUMEN

Existen bacterias que producen ácido láctico (BAL) presentes en la microflora epifita de los vegetales, y consorcios de microorganismos de montaña como levaduras y cultivos mixtos que pueden ser empleados como probióticos y promotores del crecimiento en la producción animal. Para evaluar el uso de microorganismos de montaña en ensilado de maíz como probióticos en la engorda de conejos durante 4 semanas, se emplearon 20 conejos híbridos. Los cuales se escogieron aleatoriamente en cada tratamiento. La preparación de los probióticos se realizó mediante una etapa de fermentación inicial anaerobia y una final aerobia. El tratamiento (T1) fue una dieta suplementada con la adición de microorganismos de montaña en ensilado de maíz (MME) en el agua de bebida y el tratamiento dos o convencional (T2) fungió como testigo sin aplicación de MME. Se calculó el consumo de alimento y el indicie de conversión alimenticia, expresados como la media ± la desviación estándar. Al realizar un análisis de varianza (ANOVA) se estableció que durante la semana 4 del tratamiento hubo diferencia significativa ($P<0.05$) en la ganancia de peso y conversión alimenticia entre los tratamientos, siendo favorable para T1 suplementados con MME.
**Palabras Clave**: Ensilaje, microorganismos eficientes, cunicultura, probióticos.

## INTRODUCTION

Mountain microorganisms (MM), also called beneficial microorganisms, are present in natural ecosystems little affected by anthropic factors, where photosynthetic bacteria, lactic acid bacteria (LAB), yeasts and actinomycetes have been identified (Campo *et al.,* 2014*;* Ramírez *et al.,* 2016) which by growing in an adequate amount of organic matter secrete beneficial substances that inhibit or control the growth of populations of pathogenic microorganisms (Cóndor *et al*., 2007).

The *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus* and *Streptococcus* genera produce Lactic Acid (LAB) and they are present in the epiphytic microflora of plants (Garcés *et al*., 2004). On the other hand, the genus *Bacillus*, from the gastrointestinal content of various animal species or their feces, is the most widely used as probiotics and growth promoters in animal production (Sánchez *et al*., 2015). *Bacillus* spp are facultative aerobic bacteria that ferment a wide range of carbohydrates and they are used to inhibit the aerobic spoilage process in silages due to their ability to produce fungicidal substances, however, they are less effective as producers of lactic and acetic acid in comparison with LAB (Garcés *et al.,* 2004; Layton *et al*., 2011).

On the other hand, silage is the fermentation process of soluble carbohydrates in forage by means of bacteria that produce lactic acid under anaerobic conditions (Garcés *et al.,* 2004). Through controlled anaerobic fermentation, the composition of the ensiled material is stable for a long time through the acidification of the material used, thus minimizing undesirable secondary fermentations such as alcoholic fermentation, produced by yeasts, which are a toxicity hazard for cattle and butyric fermentation produced by the genus *Clostridium* (Garcés *et al.,* 2004).

For an optimal and controlled fermentation to exist, the proper ratio between lactic acid bacteria and soluble carbohydrates is necessary. Different additives can be used to induce and optimize the fermentation process, such as molasses, citrus pulp or crushed corn, which provide a source of soluble sugars that the bacteria use to produce lactic acid, thus stabilizing the medium (Valencia *et al.,* 2011). The silage process does not improve the quality of the forage, it only preserves its nutritional value, such as the energetic and protein components through fermentation processes and keeping it stable for a long time (Villa and Hurtado, 2016).

The use of probiotics contributes to the intestinal microbial balance, stimulating the immune system of the animal, producing organic acids, bacteriocins and enzymes that favor the absorption of nutrients, improving the productive parameters (Gutiérrez *et al*., 2014).

Many studies have been published about probiotics and their different effects, in the case of rabbit production. However, the guarantee of success in rabbits is given by the expected productive results. Rabbits are characterized by their ease of handling, rapid reproduction and obtaining a quality animal protein, which places rabbit farming in a place that favors small and medium-scale production. Rabbit meat represents great advantages, with an adequate balance of fatty acids, protein, vitamins and minerals, low in cholesterol and sodium (Para *et al.,* 2015).

According to data from the National Institute of Statistics, Geography and Informatics (INEGI) the states with the highest production of rabbits are Hidalgo, Puebla, Tlaxcala, States of Mexico and Guanajuato. The development of rabbit farming in Mexico is limited by the lack of official support, coupled with an inadequate management of health and nutrition, in addition to the little promotion of the benefits of rabbit meat, reflected in a low per capita consumption, between 38 and 134 g during 2008 and 2009 (Armada, 2016) (Armada 2016), despite the great advantages from the nutritional point of view (Coreno *et al.,* 2017). The objective of the present work was to evaluate the use of mountain microorganisms in corn silage as probiotics in the fattening of rabbits.

## MATERIAL AND METHODS

The present study was carried out at the Salvatierra Headquarters of the University of Guanajuato, located in Salvatierra, Guanajuato, Mexico (20° 12'45.51 "N, 100° 52'30.09" W, at 1,749 m a.s.l) (Google Earth, s.f). The experiment was carried out with hybrid rabbits of the California breed of 30 days of age and similar weight. Each one was housed in individual cages provided with a trough and trough. The experiment lasted four weeks.

### Preparation of corn silage with mountain microorganisms

The mountain microorganisms in corn silage (MME) were obtained from a process carried out in three stages, the first two in a solid anaerobic way and the third in a liquid and aerobic way. During stage one, mountain microorganisms (MM) were collected using litter from an ecological site with little anthropic affectation located near the City of Salvatierra Guanajuato and 10% corn flour, 5% molasses was added, reserving it for 30 days in a plastic container with a capacity of 20 liters and the lid was sealed so that oxygen did not enter.

During the second stage, the product resulting from the previous process was taken and mixed with the same amount of corn silage, in addition to 10% corn flour and 5% molasses, which was stored for 30 days, anaerobically. Finally, the third stage consisted of taking 500 g of the product resulting from stage 2 and it was wrapped in a blanket and placed in a 20-liter container that contained non-chlorinated water added with 1% molasses and provided for 72 hours aeration to later be stored in plastic containers.

### Total microorganism count

To determine the number of total microorganisms present in the liquid phase of the MMEs at 24, 48 and 72 h, 100 µL samples were taken and serial dilutions were made. Subsequently, in Petri dishes containing solid medium potato dextrose agar, 100 µL of the 1: 100,000 and 1: 1,000,000 dilutions were inoculated (each of the aforementioned procedures was performed in triplicate). The inoculated boxes were incubated in a Terbaf$^{MR}$ brand culture stove at 30 °C for 48 h, after the incubation time, the colonies in each box were counted and the colony-forming units per milliliter were calculated (CFU/mL).

### Treatments

The treatments were applied to 20 30-day-old California hybrid rabbits, which were randomly distributed into two groups. The rabbits were fed a diet supplemented with $10^7$ CFU/mL of MME in drinking water and commercial food (T1). Treatment two (T2), which served as control, consisted of a conventional diet without probiotic supplement (MME) in drinking water and commercial food. In both treatments, food and water were provided ad libitum. The drinking water that they did not consume was changed daily. Food consumption was calculated with the following formula:

$$Fc = (Fo - Fr)$$

Where:
Fc = Food consumed
Fo = Food offered
Fr = Food rejected
The weekly weight increase of the rabbits was recorded by weighing at the beginning of the test and during four weeks, calculating the difference between the current live weight and the live weight of the previous week.
The food conversion index (CI) was determined by the following formula:

$$CI = (FCW / WWG)$$

Where:
CI = feed conversion index
FCW = Food consumed weekly (kg)
WWG = Weekly weight gain (Kg)

The data obtained on weight gain and feed conversion were expressed as the mean ± standard deviation, and an analysis of variance (ANOVA) was performed using the Statgraphics Centurión program (Statgraphics.Net, 2021).

## RESULTS AND DISCUSSION

The number of total microorganisms present in the liquid phase of MME expressed in CFU/mL are shown in table 1. The results show an increase in the number of microorganisms as time passes, obtaining a total count of 9 X $10^7$ CFU/mL over a 72 hour period.

**Table 1. Total microorganisms present in the liquid phase**

| Time (hours) | UFC/ mL |
|---|---|
| 24 | 3 X$10^6$ ± $2.0^a$ |
| 48 | 4 X $10^7$ ± $2.8^b$ |
| 72 | 9 X $10^7$ ± $2.5^c$ |

Guo *et al.* (2017), when supplementing with $10^5$, $10^6$ and $10^7$ CFU/g of *Bacillus subtilis* the diet of rabbits, they observed a better performance in weight with the supplementation of $10^6$ CFU/g. On the other hand, Phuoc and Jamikorn (2017) supplemented the diet of rabbits with 1X $10^7$ CFU/g of *B. subtilis*, 1X $10^7$ CFU/g of *L. acidophilus* or a combination of them at a concentration of 0.5 X $10^7$ CFU/g, suggesting that dietary supplementation with these microorganisms has probiotic benefits in rabbits. In the present work, a total number of 9 x $10^7$ CFU/mL was obtained, a quantity of microorganisms similar to the aforementioned works and that may have a probiotic potential when supplied in drinking water for rabbits.

The results of weight gain and feed conversion are presented in table 2. Statistically, no significant differences are observed between the two treatments with respect to weight gain. However, it can be noted that, in relation to feed conversion, there are significant differences between treatments, with T1 being the treatment with the highest feed conversion 5.65 ± 0.6. Gutiérrez *et al*. (2014), fed pigs with native probiotic microorganisms, reporting that there was no statistically significant difference in weight gain and in relation to feed conversion and that the treatment that had the best conversion profile was to which no probiotic microorganisms were added.

According to Villa and Hurtado (2016) rabbits fed with silages obtain the highest weight gain, than animals fed with only fresh forages, within the lactic acid bacteria present in the silage and the MM are the genera *Lactobacillus*, *Streptococcus* and *Bifidobacterium*, which produce organic acids, bacteriocins, preservatives, vitamins, sweeteners, flavorings, flavors, antioxidants, among others (Parra, 2010) that contribute to improving animal production, in relation to parameters of quantity and/or composition of milk, body condition, live weight gain and reproductive development (Phipps *et al*. 2000).

**Table 2. Average weight gain and feed conversion during the evaluation time (4 weeks).**

| Treatment | Average weight gain (kg) | Feed conversion |
|---|---|---|
| T1 | 0.163±0.03[a] | 5.65±0.6[a] |
| T2 | 0.198±0.04[a] | 3.76±0.6[b] |

In Figures 1 and 2, the variability of the treatments can be observed. In figure 1 it can be seen that the maximum weight gain in T1 was 0.211 kg and the minimum was 0.135 kg, in relation to treatment 2, the maximum weight gain throughout the evaluation time was 0.262 kg and the minimum was 0.167 kg. Figure 2 shows the variability of feed conversion in rabbits treated with and without mountain microorganisms. The maximum feed conversion was 6.22 and the minimum was 4.8 for treatment 1, while for treatment 2 the maximum feed conversion was 4.2 and the minimum was 2.9.

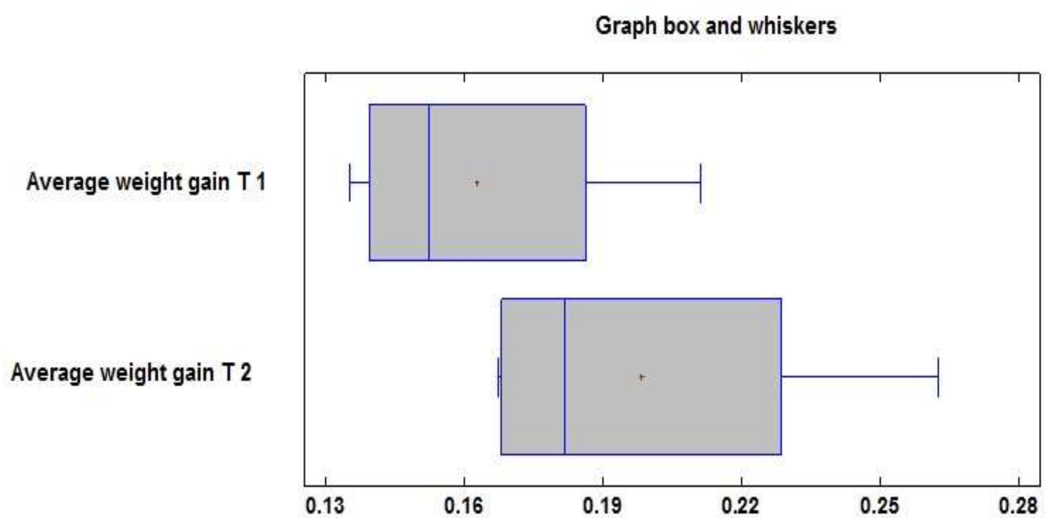


**Figure 1. Distribution of average weights in rabbits treated with and without mountain microorganisms**

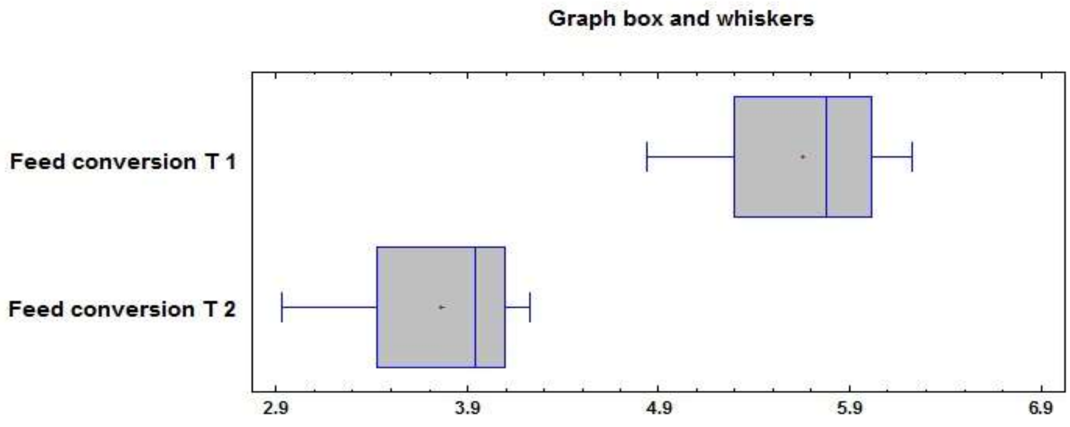


**Figure 2. Distribution of feed conversion averages in rabbits treated with and without mountain microorganisms**

## CONCLUSION

The mountain microorganisms obtained from ecosystems little affected in an anthropogenic way are a source of lactic acid producing bacteria, which can improve the nutritional value of the silage and be used as probiotics in drinking water, increasing the performance in the rabbit fattening process, in order to stimulate the development of rabbit breeding techniques in Mexico, and to suggest methods for characterizing lactic acid producing microorganisms.

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