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Effect of coffee pulp silage on productive and microbiological variables in New Zealand rabbits

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ABSTRACT

Objective: To determine the optimal inclusion level of coffee pulp in the diet of New Zealand rabbits based on their productive and microbiological parameters.

Methodology: Thirty (n=30) weaned male rabbits, with an average age of 30 days and an average weight of 589 g, were randomly distributed into individual cages across three treatments with inclusion levels of 0%, 25%, and 45% coffee pulp silage fermented for 60 days. The animals were fed for eight weeks with isoenergetic and isoproteic diets.

Results: Feed intake did not show significant differences, suggesting that the inclusion of 45% coffee pulp silage does not affect the diet's acceptability for rabbits. Although feed intake was not affected, daily weight gain showed significant differences among treatments. On the other hand, hot carcass yield presented significant differences between treatments, with the 0% coffee pulp inclusion showing the highest carcass yield. Regarding cecal total bacterial concentration, an increasing trend was observed in treatments with higher inclusion levels of coffee pulp silage.

Implications/Limitations: No significant limitations were observed.

Conclusions: The use of coffee pulp silage remains a viable alternative for rabbit feeding, showing excellent acceptability by the animals and promoting cecal bacterial growth.

Keywords: Tannins, Carcass, Cecum, Nutrition.

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INTRODUCTION

Over the years, various agricultural by-products have been used in animal feeding with the aim of improving productive performance and reducing feed costs. The inclusion of coffee pulp in animal diets is not recent, as its use has been documented for more than 30 years in species such as sheep (Ferreira *et al.*, 2003), poultry (Florez and Rosales, 2018), pigs (Braham and Bressani, 1978), fish (Moreau *et al.*, 2003), cattle (Vargas *et al.*, 1977),

and rabbits (Bautista *et al.*, 1999). The presence of high concentrations of tannins and caffeine in coffee pulp limits its inclusion in animal diets (Salinas *et al.*, 2015), since, by acting as enzyme inhibitors, they reduce the availability of consumed protein (Noriega *et al.*, 2008). However, these concentrations can be reduced through silage, improving its nutritional value (Ferreira *et al.*, 2000). It has been demonstrated that, at optimal levels, these compounds can provide antioxidant properties beneficial to the animal (Sroka and Cisowski, 2003). Currently, the inclusion of coffee pulp in animal diets is mainly applied to ruminants; however, it represents an excellent alternative for rabbit feeding, where inclusion levels of up to 85% have been reported (Bautista *et al.*, 1999). The dietary fiber content of coffee pulp favors the digestive function of rabbits (Zhu *et al.*, 2014), since it is known that a low intake of dietary fiber leads to a decrease in cecal bacterial biomass, associated with reduced fermentative activity and consequently lower bacterial fibrolytic activity (Bellier and Gidenne, 1996), which may result in decreased nutrient absorption and utilization. Based on the above, the optimal inclusion level of coffee pulp in the diet of New Zealand rabbits was evaluated through their productive and microbiological parameters.

MATERIALS AND METHODS

This study was conducted at the Rabbit Production Module of the Educational Program in Agricultural Engineering and Animal Science of the Benemérita Universidad Autónoma de Puebla.

Coffee Pulp Silage

The silage process of the coffee pulp was carried out in 1,100-liter airtight containers, with a fermentation period of 60 days. It is important to note that the silage was drained and dried prior to its incorporation into the diets.

Animals

Thirty (n=30) weaned male New Zealand rabbits, with an average age of 30 days and an average weight of 589±39 g, were randomly distributed into individual cages across three treatments with inclusion levels of 0% (T1), 25% (T2), and 45% (T3) coffee pulp silage (Table 1).

Feeding

The animals were fed isoenergetic and isoproteic diets for eight weeks, formulated according to the requirements for growing rabbits established by the NRC (1985), with free access to feed and water (Table 2).

Productive Variables

Feed intake (FI) was calculated as the difference between final and initial body weight divided by the number of days in the period. Daily weight gain (DWG) was measured periodically, considering the amount of feed offered and refused. Hot carcass yield (HCY) was obtained by the ratio of hot carcass weight to slaughter weight, multiplied by 100.

Table 1. Composition of the experimental diets.

Ingredients	Treatments		
	T1	T2	T3
Coffee pulp	0	25	40
Alfalfa	20	8	0
	13	9	10
Corn stubble	20	15	20
	24	25	24
Corn kernels	10	12	0
	7	0	0
Sorghum kernels	5	5	5
	1	1	1

Table 2. Chemical analysis of the diets.

Component (%)	Treatments		
	T1	T2	T3
CP	14.17	15.24	14.70
ADF	42.00	45.35	46.46
NDF	34.23	34.00	35.00
Ash	5.52	5.60	6.33

T1: 0% control, T2: 25% coffee pulp, T3: 45% coffee pulp.
CP: Crude protein; ADF: Acid detergent fiber; NDF: Neutral detergent fiber.

Cecal Bacteria

Cecal bacterial counts were performed using the direct count technique with a Petroff-Hausser counting chamber by capillarity, with an area of 0.0025 mm^2 and a depth of 0.02 mm. Counting was carried out using an optical microscope model WF10X-18MM at 100X magnification. Total bacterial concentration was calculated using the following formula described by Ley de Coss *et al.* (2016):

$$Total\ bacteria\ \mathrm{mL}^{-1} = (mean)(dilution\ factor)(2\times10^{7})$$

Carcass Temperature and pH

Temperature and pH data were obtained at the time of slaughter for all animals. The selected region for measurement was the Biceps Femoris at 0- and 20-min post-mortem, using a Hanna potentiometer, model HI9811-5.

Statistical Analysis

A completely randomized design with three treatments was used. Data for productive variables, pH, and temperature were analyzed by analysis of variance (ANOVA). Hot carcass yield data were subjected to an arcsine transformation. Mean comparisons were performed using Tukey's test.

RESULTS AND DISCUSSION

Productive Parameters

Feed intake (Table 3) did not show significant differences ($P>0.05$), suggesting that inclusion of up to 45% coffee pulp silage does not affect diet acceptability in rabbits. This behavior is similar to that reported in other species (Salinas *et al.*, 2015; Noriega *et al.*, 2008; Bautista *et al.*, 1999; Romero *et al.*, 1995), where no significant differences in feed intake were observed with the inclusion of coffee pulp silage in the diet. On the other hand, Yoplak *et al.* (2017) evaluated inclusion levels of 5, 15, 25, and 35% coffee pulp meal in diets for Peruvian guinea pigs, finding differences between treatments, with the control and the 5% coffee pulp meal inclusion showing the best response in terms of feed intake. This behavior may be more related to diet presentation than to coffee pulp inclusion, as feed intake depends on multiple factors, including feed texture (Tarazona *et al.*, 2012), with very fine particle diets reducing voluntary feed consumption.

Although feed intake was not affected, daily weight gain showed significant differences between treatments ($P<0.05$). This effect was observed in animals fed with coffee pulp silage and may be related to the amount of tannins present in the silage, as they can bind proteins, reducing their absorption and utilization, thereby decreasing the biological availability of this nutrient (Vázquez *et al.*, 2012). This condition limits the supply of amino acids for protein synthesis, which is necessary for various physiological functions, including growth. When adequate protein intake is not achieved under chronic conditions, the body may develop protein hypercatabolism, leading to continuous depletion of protein and energy reserves, resulting in loss of fat and muscle mass (García *et al.*, 2014), reflected in a reduction in body weight.

It should not be overlooked that one of the processes to reduce tannins in coffee pulp is silage (Ferreira *et al.*, 2000). However, this process depends on the fermentation time to which the pulp is subjected during ensiling; that is, the shorter the fermentation period, the higher the tannin content.

Regarding hot carcass yield, it is directly proportional to the final body weight of the animals, showing significant differences between treatments ($P\leq0.05$), with the control group (0% inclusion) exhibiting the highest carcass yield percentage. However, all three treatments performed optimally in terms of hot carcass yield for New Zealand rabbits, according to Vásquez *et al.* (2007), who reported a hot carcass yield of 55% for this breed. Similarly, Hernández *et al.* (2015) mentioned that rabbit carcass yield ranges between 50 and 65%, depending on various factors such as diet, breed, management, visceral weight, and others.

Table 3. Productive response of rabbits fed with varying levels of coffee pulp.

Variable	T1	T2	T3
Feed intake (g day^{-1})	97.45^{a}	96.98^{a}	108.95^{a}
Daily weight gain (g day^{-1})	29.25^{a}	10.77^{b}	8.73^{b}
Carcass yield (%)	72.02^{a}	61.15^{ab}	59.58^{b}

T1: 0% coffee pulp, T2: 25% coffee pulp, T3: 45% coffee pulp.
^{a,b,c} Different letters in the same row indicate significant differences (P 0.05).

Temperature and pH

Regarding carcass temperature, significant differences were observed between treatments ($P \leq 0.05$), with a marked decrease at 20 minutes post-mortem for each treatment (Table 4). These values are consistent with Flores (2009), who reported temperatures of 38.0±5.8 °C at 0 minutes and 23.0±7.2 °C at 20 minutes post-mortem using the stunning by neck dislocation method, which was the same method applied in this study. The relationship between pH and temperature is very close, and a primary response of this relationship is meat shortening, an irreversible effect that negatively affects meat quality. This occurs when cooling drops below 10 °C before rigor mortis, causing contractions of muscle tissues (sarcomeres) and increasing meat toughness.

pH is one of the main variables for determining meat quality, as color, water-holding capacity, and tenderness depend directly on this parameter (Cornejo *et al.*, 2016). The results obtained in this study regarding pH did not show significant differences ($P \geq 0.05$) at 0 and 20 min post-mortem, with a minimal decrease compared to expected values. It should be noted that normal pH values for rabbit meat range from 5.71 to 6.0 (Ouhayoun and Dalle, 1993).

This pH response is influenced by several factors, such as the amount of glycogen present in the muscle during stunning, the type of muscle analyzed (in glycolytic muscles, pH can drop to 5.6, while in oxidative muscles it ranges up to 6.4) (Cabanes, 1996), sex, management, species (Mota *et al.*, 2012), breed (Smitzis *et al.*, 2014), and certain hormones present in the bloodstream. Hernández *et al.* (2015) analyzed carcass quality in New Zealand and California breeds, reporting a sharp decrease in pH in New Zealand rabbit carcasses during the first 20 minutes post-mortem, stabilizing after 12 hours with a final pH of 6.1.

Total Cecal Bacteria Count

Total cecal bacterial concentration showed significant differences between treatments ($P \leq 0.05$), as shown in Table 5. Although the diet analyses revealed similar fiber contents (ADF and NDF), a growing trend was observed in treatments containing coffee pulp silage. This suggests that, even when fiber percentages were similar, the nature of the fiber allowed greater cecal fermentation and, consequently, an increase in bacterial development, as these microorganisms are responsible for degrading approximately 75% of the fiber consumed by the animal (Hernández and Cobos, 2001).

Table 4. Post-mortem carcass variables of rabbits fed with varying levels of coffee pulp.

Variable	Time (min)	T 1	T 2	T 3
Temperature (°C)	0	34.15[a]	31.50[b]	29.31[c]
	20	28.20[a]	23.85[b]	24.90[c]
pH	0	7.83[a]	7.65[a]	7.50[a]
	20	7.64[a]	7.46[a]	7.19[a]

T1: 0% coffee pulp, T2: 25% coffee pulp, T3: 45% coffee pulp.
[a,b,c] Different letters in the same row indicate significant differences ($P \leq 0.05$).

Table 5. Total cecal bacteria results.

Treatments	Total bacteria in cecal appendix
T1	1.7×10^{11a}
T 2	1.9×10^{11b}
T 3	2.2×10^{11c}

T1: 0% coffee pulp, T2: 25% coffee pulp, T3: 45% coffee pulp. [a,b,c] Different letters in the same row indicate significant differences ($P\leq0.05$).

This is important because it has been demonstrated that cecal bacteria directly participate in immunoglobulin synthesis, thereby enhancing the animal's immune system and contributing to the presence of beneficial bacteria in the digestive tract. Additionally, increased lignin content in rabbit diets has been associated with a lower incidence of digestive transit problems (Caîsin *et al.*, 2020).

CONCLUSIONS

The use of coffee pulp silage remains a viable alternative in rabbit feeding, showing excellent acceptability by the animals and promoting cecal bacterial growth. However, further research is needed on optimal inclusion levels and ensiling times, as inclusion levels of 25% and 45% of coffee pulp silage fermented for 60 days negatively affected carcass yield and daily weight gain. It is worth noting the potential for studying the quality of fiber provided by coffee pulp silage and its role in managing intestinal microbiota, which could help elucidate the prebiotic effects it may have on the animal.

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