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Effect of the fermentation time on the nutritional quality of *Coffea arabica* L. pulp silage

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Abstract

Coffee pulp has been incorporated into livestock production systems through its use in silos thanks to its acceptable nutritional composition. This work was carried out at the farm La Gaviota located in the Matajira district of the municipality of Pamplonita, and its aim was to evaluate the effect that fermentation time has on the nutritional quality of coffee pulp silage. Three treatments were used: 30, 45, and 90 days of fermentation and four replicates of 20 kg. Descriptive statistics tests, one-way ANOVA with three levels, and a significance of 5 % were applied under a randomized design. Parameters such as pH, phosphorus (P), and polyphenols showed a similar behavior ($p > 0.05$) compared to the fermentation time. Meanwhile protein, neutral detergent fiber (NDF), *in vitro* digestibility (IVD) and metabolizable energy (ME) –with averages of 13.50 %, 49.85 %, 57.20 % and 4.50 Mcal/kg DM, respectively– showed the best tenors in T2 with a fermentation time of 45 days. The values of acid detergent fiber (ADF) and calcium (Ca) were higher in T4 with 90 days of fermentation. We conclude that the fermentation time has an influence on the nutritional parameters of coffee pulp silage.

Keywords: coffee pulp, fermentation, *in vitro* digestibility, ruminant feeding, silage

Efecto del tiempo de fermentación sobre la calidad nutricional del ensilaje de pulpa de *Coffea arabica* L.

Resumen

La pulpa de café ha sido incorporada en los sistemas de producción pecuarios a través de su uso en silos dada su aceptable composición nutricional. La presente investigación se desarrolló en la finca La Gaviota, localizada en la vereda Matajira del municipio de Pamplonita (Colombia), con el objetivo de evaluar el efecto que tiene el tiempo de fermentación sobre la calidad nutricional del ensilaje de pulpa de café. Se emplearon tres tratamientos: 30, 45 y 90 días de fermentación y cuatro réplicas de 20 kg. Se aplicaron pruebas de estadística descriptiva, ANOVA de un factor con tres niveles y una significancia del 5 % bajo un diseño aleatorizado. Los parámetros de pH, fósforo (P) y polifenoles presentaron un comportamiento similar ($p > 0{,}05$) respecto al tiempo de fermentación, mientras que los valores de proteína, fibra detergente neutro (FDN), digestibilidad *in vitro* (DIV) y energía metabolizable (EM) —con medias de 13,50 %, 49,85 %, 57,20 % y 4,50 Mcal/kg MS, respectivamente— presentaron los mejores tenores en el T2 con un tiempo de fermentación de 45 días. Los valores de fibra detergente ácido (FDA) y calcio (Ca) fueron superiores en el T4 con 90 días de fermentación. Se concluye que el tiempo de fermentación tiene influencia sobre los parámetros nutricionales del ensilaje de pulpa de café.

Palabras clave: alimentación de rumiantes, digestibilidad *in vitro*, ensilaje, fermentación, pulpa de café

Introduction

In ruminant breeding, forages are the basis of nutrition due to their large biomass production and low cost to obtain. However, most forages have nutritional deficiencies, especially regarding protein, and their production is affected by climatic variability, overgrazing, soil compaction, and the use of varieties that are poorly adapted to the region (Martínez et al., 2008). These factors require an almost dependent use of balanced feed, which, due to its high costs, reduces profit margins for small- and medium-sized cattle breeders in farming systems with low technology adoption (Posadas et al., 2014). Given this situation, the livestock producer is forced to explore new feeding and supplementation options. In this way, the residues and by-products of the agricultural industries (e.g., sugar cane), beer, and fruit and vegetable industries emerge as a valuable nutritional and economical alternative for production systems (Bermúdez-Loaiza et al., 2015).

By January 2017, coffee production in Colombia –the country with the highest production of mild washed Arabica coffee worldwide– was 1,275,000 bags of 60 kg, which meant an increase of 12 % compared to 1,136,000 bags produced in the previous year. Between February 2016 and January 2017, the coffee harvest reached 14.4 million 60 kg bags, 1 % more than the 14.2 million produced in the same period the previous year. Concerning the coffee year (October 2016-January 2017), coffee production in Colombia was 5.6 million 60 kg bags, 7 % more than the bags harvested in the previous period (Eje 21, 2017).

Wet coffee processing is the main method for obtaining green coffee. During this process, the husk and mucilage are removed from the grain to finish its drying (Novita, 2016). The average pulp production is 2 t/ha/year (Ocampo & Álvarez-Herrera, 2017). For every million 60 kg bags of coffee almond that Colombia exports, 162,900 t of fresh pulp are generated (Rodríguez & Zambrano, 2010). Table 1 shows the residues obtained from the processing of 1 kg of coffee. The husk and mucilage constitute 56 % of the grain (Torres-Valenzuela et al., 2019). The shell, also called "pulp", weighs about 43.6 % of the fresh fruit (Rodríguez & Zambrano, 2010), contains approximately 86 % moisture, and is comprised of the epicarp and part of the mesocarp. The pulp is rich in pectins, caffeine, proteins, carbohydrates, and polyphenols, and is a potential source for the agro-industry with high added value (Murthy & Naidu, 2012).

Table 1. Residues obtained during the processing of 1 kg of coffee

Process	Residue obtained	Loss (g)
Pulping	Fresh pulp	436
Removal of mucilage	Mucilage	149
Drying	Water	171
Threshing	Parchment	42
	Silver coating	
Roasting	Volatile	22
Preparation of beverage	Grinds	104
Accumulated loss		924

Source: Rodríguez and Zambrano (2010)

There is a need –significant in times of crisis– to reduce the feeding costs of farms where the use of local resources could be an alternative (Flórez-Delgado & Rosales-Asensio, 2018), especially those that cause a negative impact to the environment due to its inadequate disposal, as is the case of coffee pulp (Yoplac et al., 2017). Conservation using the silage technique is a safe and economical option (Mayorga, 2005) to use this residue in animal feed (Pinto et al., 2014), which is composed of carbohydrates (21 % to 32 %), protein (7.5 % to 15 %) and fat (2 % to 7 %) (Esquivel & Jiménez, 2012).

During the silage process, anaerobic fermentation occurs, in which the microorganisms present lead to an increase in lactic acid and a consequent reduction in pH (Lozano et al., 2000), which in turn prevents the development of other types of microorganisms (Villa et al., 2010). The coffee pulp has anti-nutritional agents (Pujol et al., 2013) such as tannins, caffeine, and chlorogenic acid, which prevent its direct use. Tannins are molecules that make the ruminal hydrolysis of proteins impossible (Piñeiro-Vásquez et al., 2015). In ruminants, caffeine can increase diuresis and, un turn, decrease nitrogen retention (Mayorga, 2005; Mazzafera, 2002). The pulp must go through a silage process, which consists of a lactic fermentation (Rathinavelu & Graziosi, 2005) that will allow reducing anti-nutritional factors and maintaining or improving its nutritional value (Noriega et al., 2008).

In silage elaboration, the conditions must be guaranteed to maintain a pH of less than 4.2 (Aguirre-Fernández et al., 2018) to inhibit the growth of pathogenic microorganisms and preserve the nutritional value of the ensiled products (Mayorga, 2005). Previous studies have shown that with a 120-day fermentation, the best nutritional values corresponding to the percentage of protein and low presence of tannins are obtained (Noriega et al., 2008).

It is common to find livestock production systems combined with agricultural activities that use products, by-products, and crop residues as raw materials for ruminant feeding (Flórez-Delgado & Rosales-Asensio, 2018). These systems allow agricultural producers to reduce food costs (Castaño & Cardona, 2014) and give sustainable management (economic and environmental) to their production system (Bampidis & Robinson, 2006). In this context, the current research focused on the elaboration of coffee pulp silage

(CPS) as a food source for ruminants aiming at evaluating its nutritional quality and the presence of other substances such as caffeine and polyphenols in 0, 30, 45 and 90 days of fermentation.

Materials and methods

Location

The research was carried out on the farm La Gaviota located in the Matajira district in the municipality of Pamplonita (Colombia), at the coordinates 07°32'34" N and 72°37'36" W, with an altitude of 1,300 m a.s.l. It has an extension of 4 ha, an average temperature of 20°C, irregular topography, and an annual rainfall of 1,400 mm.

Experimental procedure

The coffee pulp used to make the silage belonged to the Colombia variety in a fresh state and was obtained from the neighboring farm, the experimental farm Villa Marina that belongs to Universidad de Pamplona. Polyethylene silo bags (7 caliber) were used (Fernández, 2015) with a capacity of 50 kg. The bag was filled with small layers of compacted coffee pulp while removing as much air as possible until the capacity of the bag was complete (Triana et al., 2014). Once sealed, the bags were stored in a place protected from the sun and rodents to guarantee the fermentation process. An initial and three fermentation times (30, 45, and 90 days) were considered (Noriega et al., 2008), as well as four replicates per fermentation time.

After the total fermentation time was achieved, the bags were uncapped, and their contents were homogenized; subsequently, a sample was taken per replicate. Then, the following tests were performed: pH, dry matter (DM) through the gravimetric method (Association of Official Analytical Chemists [AOAC], 1996), protein by the Kjeldahl method (AOAC, 1996), FDA and FDN contents with the Van Soest et al. (1991) method, ME through combustion in a calorimetric pump (Posada et al., 2012), calcium and phosphorus by direct calcination, and *in vitro* digestibility (IVD) through the Tilley and Terry method (Nieto et al., 2005). The presence of other substances such as caffeine using the Dionex Ultimate 3000 HPLC system and total polyphenols with the Folin-Ciocalteu reagent were also registered.

Experimental design

A randomized design was used under the following mathematical model:

$$Y_{ij}=\mu+\tau_i+\varepsilon_{ij}$$

Where Y_{ij}: nutritional response of the coffee pulp silage (CPS) to the treatment; τ_i: effect due to the treatment, and ε_{ij}: experimental error. An analysis of variance of the fermentation time factor was carried out at three levels (30, 45, and 90 days of fermentation) and with a significance of 5 %. Assumptions of normality and homogeneity of variances were applied to evaluate the effect of treatments on these nutritional variables.

Results and discussion

The nutritional parameter values of this silage are within the normal ranges for the raw materials that are preserved by this technique. Table 2 shows the results of the analysis of variance applied to the nutritional composition of the silage in three fermentation periods, and the presence of other substances such as caffeine and polyphenols.

Table 2. Nutritional composition and fermentative parameters of coffee (*Coffea arabica*) pulp silage preserved at 30, 45 and 90 days of fermentation

Parameter	Unit	Fermentation period (days)				SE
		0	30	45	90	
DM	%	15.64 ± 0.21^{a}	16.00 ± 0.18^{a}	15.42 ± 0.17^{b}	15.90 ± 0.08^{a}	0.001
Protein	%	8.96 ± 0.32^{a}	13.50 ± 0.79^{b}	12.42 ± 0.17^{c}	12.82 ± 0.09^{bc}	0.030
ADF	%	28.34 ± 0.28^{a}	39.02 ± 0.29^{b}	37.97 ± 0.22^{c}	40.75 ± 0.64^{d}	0.000
NDF	%	43.79 ± 0.64^{a}	47.05 ± 0.56^{b}	49.85 ± 0.52^{c}	47.07 ± 1.00^{b}	0.001
Energy	Mcal/kg	4.15 ± 0.03^{a}	4.36 ± 0.06^{b}	4.50 ± 0.01^{c}	4.46 ± 0.01^{c}	0.004
Ashes	%	5.87 ± 0.25^{a}	6.70 ± 0.18^{b}	6.97 ± 0.15^{b}	6.17 ± 0.09^{c}	0.000
Ca	%	0.32 ± 0.02	0.47 ± 0.05^{b}	0.40 ± 0.01^{c}	0.60 ± 0.01^{d}	0.000
P	%	0.19 ± 0.09	0.22 ± 0.05^{a}	0.20 ± 0.01^{a}	0.27 ± 0.05^{a}	0.075
IVD	%	51.67 ± 0.33^{a}	52.47 ± 0.69^{a}	57.20 ± 1.12^{b}	54.55 ± 0.92^{c}	0.000
pH		4.51 ± 0.05	3.82 ± 0.09^{b}	3.87 ± 0.05^{b}	3.85 ± 0.05b	0.622
Caffeine	%	6.53 ± 0.16^{a}	6.39 ± 0.27^{a}	6.60 ± 0.05^{a}	7.34 ± 0.11^{b}	0.000
Polyphenols	%	5.18 ± 0.38^{a}	7.71 ± 0.47^{b}	7.45 ± 0.42^{b}	7.59 ± 0.48^{b}	0.740

Note: the means with different letters in superscript showed statistically significant difference ($p < 0.05$).
DM: dry matter; ADF: acid detergent fiber; NDF: neutral detergent fiber; Ca: calcium; P: phosphorous; IVD: *in vitro* digestibility.

Source: Elaborated by the authors

Ojeda and Cáceres (2002) state that due to the effects of cellular respiration, which occurs at the same time as fermentation and the concentration of soluble carbohydrates (Ítavo et al., 2000), the loss of humidity increases and, consequently, DM levels increase. In the same way, López-Herrera and Briceño-Arguedas (2017) point out that this fermentation process is very intense in the first days and causes moisture loss until stabilization is reached and the DM content is maintained (Londoño et al., 2016). On the other hand, Benítez and Poveda (2011) mention that low DM contents occur because pectin absorbs water through the cellular respiration process, and because soluble carbohydrates are fermented.

The average DM in T2 was 16 %; although this value does not exceed the minimum content of 20 % suggested by Aguirre et al. (2017) to categorize good quality silage, it is in an optimal range that, together with the acid pH, allows controlling the presence of *Clostridium*, reduces losses by effluents and guarantees a fermentation process, which in turn, increases voluntary consumption by ruminants (Villa & Hurtado, 2016). Similar reports were obtained by Encalada et al. (2017) with 18 % DM and fermentation time of

45 days, while Aguirre et al. (2017) reported 26 % of DM, i.e., higher than the 15.4 % obtained by Bautista et al. (2005).

Regarding protein, there was a statistically significant difference between the treatments; T2 protein values were the most representative with an average of 13.5 %. This protein content is possibly due to the bacterial growth produced during fermentation and the silage time (Encalada et al., 2017). Puertas-Mejía et al. (2012) affirm that about 0.9 % of the wet weight is comprised of pulp proteins of this fruit, which are part of the substrates necessary for the growth and development of microorganisms. Navarro-Ortiz and Roa-Vega (2018) indicate that forages, raw materials, and any other food intended for animal feed are classi fied as good quality when their protein content exceeds 11.0 %, which means that coffee pulp silage can be considered as a nutritional alternative due to its high protein content. The results reported by Londoño et al. (2016) are slightly higher (14.83 %). For their part, Noriega et al. (2008) and Benítez (2016) found 30.52 % and 27.16 %, respectively, values much higher than those recorded in the current research and lower than those reported by Encalada et al. (2017) (12.56 %).

The ADF and NDF remained within the normal ranges with percentages of 39.24 % and 47.99 %, respectively, which agrees with the results of Noriega et al. (2008), who found that the fiber content in the pulp of this ensiled fruit increases with the fermentation time and the sealing time of the silo. According to Quiroz-Cardoso et al. (2015), the low content of acid detergent fiber in food increases its consumption. Furusho et al. (2000) recorded lower results with 27.8 % of NDF and 45.40 % of ADF, similar values to those obtained by Aguirre et al. (2018).

The pH values showed a similar behavior between the treatments, showing a mean of 3.8. This is due to the production of acidophilic lactobacilli that grow in the first 72 hours of sealing the silo and are responsible for the production of lactic acid, which lowers the pH. Benítez (2016) considers that the decrease in pH is due to the consumption of substrate, and Mayorga (2005) suggests that all conditions must be met in the silage process to maintain an acid pH and avoid variations in the nutritional properties of the ensiled product. The pH value obtained in this research (3.80) indicates that the anaerobic fermentation process had all the appropriate conditions for its development and achieved fermentation stability for being lower than 4.2 (Garcés et al., 2004). This value is very similar to those reported by Pinto-Ruiz et al. (2017) (3.90) and Encalada et al. (2017), who included additives in the silage process.

The ash content showed a different behavior ($p < 0.05$); T3 obtained the best value with 6.97 %, which corresponds to the results of Aguirre et al. (2018) and Benítez (2016), who affirm that the ash content increases with the fermentation process and time. Noriega (2008) and Benítez (2016) obtained higher values compared to those reported in the current study, with 12.46 % and 9.90 %, respectively. Pulido et al. (2016) point out that the percentage of ash indicates the mineral content of food. In the current study, the Ca and P values at 90 days of fermentation were 0.60 % and 0.27 %, respectively. Furusho et al. (2000) obtained values of 0.76 % and 0.52 % in these two minerals, respectively. The pulp of this fruit must have adequate ash contents that provide suitable levels of minerals to enrich animal diets (Noriega et al., 2008), especially for those species with a very high demand for minerals, such as milk-producing cattle.

The results obtained in the IVD of this silage show differences between the treatments, with values ranging between 52 % and 57 %. Navarro-Ortiz and Roa-Vega (2018) consider that food is of good quality if it has values for IVD higher than 70 %, NDG lower than 50 % and protein content higher than 15 %; in contrast, a low-quality food has an IVD lower than 50 %, an NDF of more than 65 %, and protein content of less than 8 %. In the current study, a maximum digestibility value of 57 % was obtained after 45 days of fermentation, making this type of silage a digestible nutritional alternative for ruminants and with a high energy content (4.5 Mcal/kg), much higher than the energy of 2.38 Mcal/kg reported by Furusho et al. (2000). Encalada et al. (2017) found a range between 50.27 % and 56.92 % of IVD, which is consistent with the results of our research. The caffeine content was 6.39 %, i.e., much higher than the 0.87 % obtained by Ferreira et al. (2001) in a fermentation time of 45 days. Furthermore, a polyphenol content of 7.45 % was also identified. All the results mentioned above allow this feed to be incorporated as a partial replacement for commercial balanced feed in the diet of milk-producing ruminants in a range of 20 % to 40 %, and of fattening animals in a range of 20 % to 30 %, without adverse effects to the health, well-being, and productivity (Flórez-Delgado & Rosales-Asensio, 2018).

Conclusions

The 45-day fermentation showed the best values in protein, NDF, IVD, and gross energy variables, with averages of 13.05 %, 49.85 %, 57.20 %, and 4.50 Mcal/kg, respectively. Fermentation at 90 days only influences the ADF and Ca content. Parameters such as pH, P, and polyphenols did not show statistically significant differences; that is, the fermentation time does not affect these nutritional parameters in this silage.

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Disclaimers

The author made all the significant contributions to the document and agreed to its publication; he also declares that there are no conflicts of interest in this study.

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