



Nutritional evaluation of silage with coffee (*Coffea Arabica* L.) cherry for ruminant supplementation

Evaluación nutricional de ensilajes con cereza de café (*Coffea arabica* L.) para suplementación en rumiantes

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Abstract

The aim of this study was to evaluate nutritionally coffee cherry (*Coffea Arabica* L.) silages with different additives: efficient microorganisms (EM) and kumis, to be used in ruminant supplementation. Micro silos were prepared according to treatments: a control treatment with coffee cherry and two experimental treatments with additives (cherry + EM and cherry + commercial kumis). Five fermentation times (1, 8, 16, 21 and 31 days) were evaluated for pH and temperature, and three fermentation times (1, 16 and 31 days) were evaluated for dry matter, protein and neutral detergent fiber; organoleptic indicator evaluation (smell, color and texture) was performed at day 31. A completely randomized design with factorial arrangement was used and linear effects, quadratic and cubic were tested over time using a regression analysis. The results did not show any statistical differences between treatments ($P > .05$) for pH (4.0), temperature values showed highly significant differences ($P < .01$), with an initial temperature of 25.3°C, which stabilized over time at 23.9°C. Dry matter did not show any differences ($P > .05$) among treatments, with similar content (20.6%). Regarding protein, significant differences were found ($P < .05$), being higher for the treatment with kumis (18.1 %) which shows that the additives addition, has a positive effect on the nutritional content. Organoleptic indicators were within the parameters estimated as acceptable for animal feeding.

Keywords: Additives; feeds; fermentation; organoleptic properties; microorganisms; kumis.

Resumen

El objetivo de este estudio fue evaluar nutricionalmente ensilajes de cereza de café (*Coffea arabica* L.) con diferentes aditivos: microorganismos eficientes (ME) y kumis, para uso en la suplementación de dietas en rumiantes. Se elaboraron microsilos según los tratamientos: un tratamiento control con cereza de café, y dos experimentales con aditivos (cereza + ME, y cereza + kumis comercial). Se evaluaron cinco tiempos de fermentación (1, 8, 16, 21 y 31 días) para las variables de pH y temperatura, y tres tiempos de fermentación (1, 16 y 31 días) para materia seca, proteína y fibra de detergente neutro (FDN); valoración de indicadores organolépticos (olor, color y textura) se realizó al día 31. Se utilizó un diseño completamente al azar con arreglo factorial y se probaron efectos lineales, cuadráticos y cúbicos a través del tiempo utilizando un análisis de regresión. En los resultados no se observaron diferencias estadísticas entre tratamientos ($P > .05$) para el pH (4.0), los valores de temperatura presentaron diferencias altamente significativas ($P < .01$), registrándose una temperatura inicial de 25.3°C, la cual se estabilizó a través del tiempo en 23.9°C. La materia seca no presentó diferencias ($P > .05$) entre tratamientos, con contenido similar (20,6 %). Para proteína se encontraron diferencias significativas ($P < .05$), siendo mayor para el tratamiento con kumis (18.1 %) lo que demuestra que la adición de aditivos, tienen un efecto positivo sobre el contenido nutricional. Los indicadores organolépticos estuvieron dentro de los parámetros estimados como aceptables para alimentación animal.

Palabras clave: Aditivos; alimentación; fermentación; propiedades organolépticas; microorganismos; kumis.

Introduction

Coffee has played an important role in the history of Colombia and has been of utmost importance for its economic growth during the 20th century. Coffee can be considered the national crop and its production has increased 56 % between 2012 and 2014, from 11.1 bags per hectare in 2012 to 15.3 bags in 2014 (FNC, 2014).

Coffee production comprises three phases; one of these is coffee processing, in which fruits are transformed into parchment coffee. This is done when all the covers that wrap the grain, such as pulp and mucilage are removed (Oliveros & Sanz, 2011). These residues, when not properly treated, can become a source of pollution for the environment; this is the reason why since the middle of the last century coffee growers have tried to innovate methods to use coffee pulp as raw material for the production of organic fertilizers, biogas, peptic enzymes, alternative feed for animals such as silage, among others (Rathinavelu & Graziosi, 2005). Silage consist fundamentally in the conservation of forages and/or postharvest byproducts, that under special conditions, certain chemical and biochemical processes occur defining their quality and maintaining their mass without deterioration for a long period of time (Hiriart Le-Bert, 1998).

Likewise, different research studies through the years have demonstrated that coffee pulp can be used in animal feed; moreover, other chemical composition studies have showed that coffee pulp silage at the end of the fermentation period has higher crude protein content, lower values of nitrogen-free extracts and very low tannin values (Salazar, Acuña & García de Salcedo, 2009), which provides high nutritional value and could potentially be recommended in animal diet elaboration. Furthermore, Díaz, Rodríguez & Salinas, (2014), showed the possibility of using silage with coffee pulp as a supplement for pig feeding as digestibility reached up to 70 %. In addition, the use of additives and inoculants helps to improve fermentation in silages and their nutritional quality. Considering the aforementioned, the aim of this study was to evaluate nutritionally silages of coffee cherry (*Coffea Arabica* L.) with different additives such as efficient microorganisms (EM) and kumis to be used in ruminant supplementation diets.

Material and methods

Study site

This research was carried out in Hacienda Majavita, Universidad Libre Socorro Campus, in the laboratory of Animal Nutrition, located in Alto

de Chochos County, department of Santander, Colombia, with a latitude of 06° 28' 35.0" N, longitude 73° 14' 98.1 W, at an altitude of 1380 m.a.sl. with an average temperature of 24°C.

Treatments

Coffee cherry was obtained at Loma Linda farm, Buenavista County, which was extended in the shade for a day prior to its use to decrease moisture content, and it was then homogenized with the different additives in proportions according to each treatment (Table 1).

Table 1. Composition of treatments (micro silos) with coffee cherry and different additives

| Treatments | |
|------------|-----------------------------|
| 1 | Coffee cherry (control) |
| 2 | coffee cherry + EM (2 %) |
| 3 | coffee cherry + kumis (2 %) |

Later, it was ensiled in transparent polyethylene bags of 2.5 mm with a 1 kg capacity and compacted manually. Replicas for each treatment were stored in dark polyethylene bags.

Micro silos with cherry of coffee were elaborated taking 22.5 kg of cherry that were divided into 45 bags of 500 g according to each treatment: i) a control treatment with coffee cherry; and two experimental treatments with additives: i) cherry + efficient microorganisms (EM); ii) cherry + commercial kumis. Each treatment had three replicates.

Five fermentation times (1, 8, 16, 21 and 31 days) were evaluated for variables as pH and temperature to establish a better fermentation process control. For variables as dry matter, protein and neutral detergent fiber, evaluations were made thrice (1, 16 and 31 days). Moreover, organoleptic evaluation was carried out on day 31.

Nutritional composition evaluation of micro silos

The experimental period lasted 31 days and samples were taken thrice on days 1, 16 and 31, corresponding to the fermentation period for nutritional composition analyzes (Table 2); every sampling day dry matter (DM) content was established using the oven drying method; then, each sample was passed through a micro mill to reduce particle size for subsequent crude protein (PC) analysis using the Kjeldahl method, and neutral detergent fiber (NDF) measure applying the Van Soest method. Analyzes were carried out in the Animal Nutrition Laboratory of Universidad Libre, Socorro Campus.

Table 2. Methods to establish nutritional composition of micro silos with coffee cherry

| Variable | Method |
|-------------------|-------------------------------------------------------|
| Protein (% PC) | Kjeldhal method (AOAC, 1995) |
| Fiber (% FND) | Van Soest method (Van Soest, Robertson & Lewis, 1991) |
| Dry matter (% DM) | Oven drying method |

Physicochemical evaluation of micro silos

Regarding the physicochemical assessment pH and temperature variables were evaluated (Table 3) on days 1, 8, 16, 24 and 31, based on the methodology published by Betancourt (2001) cited by Villalba, Holguín, Acuña & Piñeros (2011).

Table 3. Evaluation of physicochemical characteristics of micro silos with coffee cherry

| | Physicochemical characteristics | | | |
|-------------|----------------------------------|-------------|--------------|---------|
| | Excellent | Good | Regular | Bad |
| pH | < 4.0 | 4.0 and 4.2 | 4.3 and 4.5 | > 4.5 |
| Temperature | Temperature between 18 and 25 °C | | 26 and 35 °C | > 35 °C |

Source: Betancourt (2001), cited by Villalba *et al.* (2011).

Organoleptic characteristics evaluation of micro silos

To evaluate organoleptic characteristics (Table 4), the Vallejo methodology was used (1995) cited by Aguilar, (2012); in this methodology, indicators were used; a numerical value was given to each indicator (color, smell and texture), obtaining at the end, the sum of organoleptic indicators (OI). The evaluation was carried out on the 31st fermentation day.

Table 4. Organoleptic indicators used for micro silo evaluation

| Indicator | Description | Score (%) | Maximum (%) |
|-----------|-------------|-----------|-------------|
| Smell | Excellent | 54 | 54 |
| | Good | 40.5 | |
| | Regular | 27 | |
| | Bad | 13.5 | |
| Color | Excellent | 24 | 24 |
| | Good | 18 | |
| | Regular | 12 | |
| | Bad | 6 | |
| Texture | Good | 22 | 22 |
| | Regular | 11 | |
| | Bad | 5.5 | |
| | Total (%) | | |

Source: Vallejo, (1995) cited by Aguilar & Giratá (2012).

Experimental design

We used a completely randomized design with factorial arrangement (3 x 5), comprised by three treatments: coffee cherry, coffee cherry + EM, coffee cherry + kumis; five measurements were carried out (1, 8, 16, 24, 31 days) for pH and temperature; for nutritional composition a factorial arrangement (3 x 3) comprised of the three treatments indicated above with three measurements (1, 16, 31 days) were carried out; additionally, the organoleptic characteristics analysis was performed in day 31. Moreover, linear, quadratic and cubic effects were tested over time using a regression analysis. To carry out analyses, the SAS statistical program (SAS/STAT version 9.1 of 2002, SAS Institute Inc., Cary, NC, USA) was used. The following statistical model was used:

Factorial statistical model

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{ijk}$$

Where:

μ = Average general

α_i = Effect of treatment i

β_j = Effect of day j

$(\alpha\beta)_{ij}$ = Effect of the interaction between treatments per day

E_{ijk} = Effect of experimental error ijk

Results

Nutritional composition of coffee cherry micro silos with different additives

Regarding dry matter there were no statistical differences ($P > .05$) between treatments (Table 5), finding similar contents for all treatments with values around 20.6 %. No effect due to application of additives (EM and kumis) on coffee cherry was found with time, observing similar values between treatments (Table 5).

Additionally, statistical differences in protein content of micro silos were found ($P < .05$) between treatments, time and interaction, finding a value of ca. 4.0 % at the beginning of the experiment. Moreover, this value increased linearly over time (Figure 1), showing a higher increase in protein content for the treatment where kumis was applied on day 31; likewise, a better protein content overall was observed for the two treatments with additives (EM and kumis, i.e. 15.9 and 18.1 %, respectively).

Table 5. Nutritional behavior of the variables dry matter, protein and fiber over time in micro silos with coffee cherry

| Micro silos | C | C+EM | C+K | Pr > F2 | | | |
|--------------------------|---------|-------|-------|---------|-----------|--------|----------|
| Variable/ Time (Days) | Average | | | SD | Treatment | Day | T*D |
| DM (%) | | | | | | | |
| Day 1 | 20.7 | 20.8 | 20.7 | 20.7a | | | |
| Day 16 | 20.5 | 20.4 | 20.5 | 20.4a | | | |
| Day 31 | 20.8 | 20.6 | 20.7 | 20.7a | | | |
| Average | 20.7a | 20.6a | 20.6a | 0.7 | 0.9797 | 0.6641 | 0.9935 |
| CP (%) | | | | | | | |
| Day 1 | 3.4 | 5.6 | 3.2 | 4.0c | | | |
| Day 16 | 14.5 | 4.7 | 12.9 | 10.7b | | | |
| Day 31 | 13.5 | 15.9 | 18.1 | 15.8a | | | |
| Average | 10.5a | 8.7b | 11.4a | 1.1 | 0.0003 | <.0001 | <.0001 L |
| NDF (%) | | | | | | | |
| Day 1 | 64.2 | 62.7 | 65.2 | 64.0b | | | |
| Day 16 | 70.3 | 65.9 | 63.7 | 66.7a | | | |
| Average | 67.2a | 64.3b | 64.4b | 1.3 | 0.0038 | 0.0012 | 0.0013 L |

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; C: silage with coffee cherry; C + EM: silage with coffee cherry and efficient microorganisms; C + K: silage with coffee cherry and kumis; T*D: interaction between treatment and days. L: Linear effect of time on the nutritional variables of micro silos. a, b and c: Rows with different letters differ significantly at $P < .05$; SD: standard deviation.

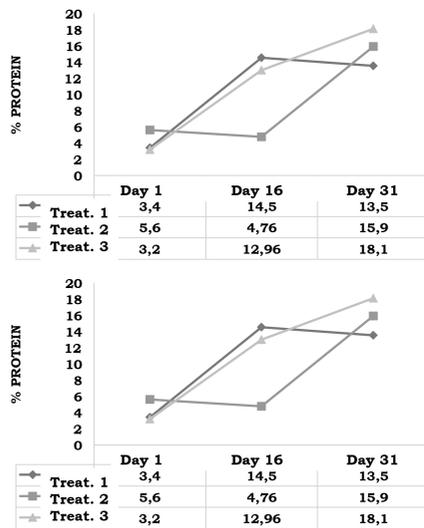


Figure 1. Protein content behavior in silages over time

Neutral detergent fiber (Table 5) showed highly significant differences ($P < .01$) between treatments and time; similarly, significance was observed for their interaction ($P < .01$), its content increased the first 16 days (Figure 2) for EM treatment. In the control treatment, a higher NDF value was observed in day 16 (70.3 %), in relation to other treatments with additives (EM and kumis, 65.9 and 63.7 %, respectively).

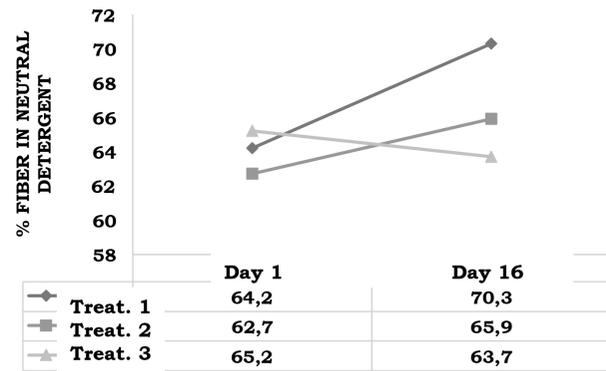


Figure 2. Neutral detergent fiber behavior in silages over time

Physicochemical characterization of coffee cherry micro silos with different additives

No statistical differences were observed between treatments ($P > .05$) for pH (Table 6), neither for the time nor for its interaction with treatments; however, at the beginning of the fermentation process low values for the control (coffee cherry) and EM treatments were observed (3.9 and 3.4, respectively); moreover, values increased in the fermentation process times, obtaining in the end average values of 4.08 for pH that were within the expected range (Table 3).

Furthermore, values for temperature showed highly significant differences ($P < .01$) among treatments. In relation to time, it had a tendency to decrease linearly until it stabilized around 23.9°C for all treatments. Values registered showed variations between 25°C at the beginning and 23.9°C at the end of the period.

Table 6. pH and temperature behavior over time in micro silos

| Micro silos | C | C+EM | C+K | Pr > F2 | | | |
|--------------------------|---------|-------|-------|---------|-----------|--------|----------|
| Variable/ Time (Days) | Average | | | SD | Treatment | Day | T*D |
| pH | | | | | | | |
| Day 1 | 3.94 | 3.45 | 4.3 | 3.89a | | | |
| Day 8 | 4.06 | 4.04 | 4.0 | 4.03a | | | |
| Day 16 | 4.20 | 4.13 | 4.13 | 4.15a | | | |
| Day 24 | 4.12 | 4.08 | 4.07 | 4.08a | | | |
| Day 31 | 4.11 | 4.07 | 4.08 | 4.08a | | | |
| Average | 4.08a | 3.95a | 4.11a | 0.2 | 0.1377 | 0.2132 | 0.0610 |
| Temperature (°C) | | | | | | | |
| Day 1 | 25.3 | 25.4 | 25.1 | 25.3d | | | |
| Day 8 | 26.7 | 26.8 | 26.8 | 26.8a | | | |
| Day 16 | 25.8 | 26.1 | 25.7 | 25.8b | | | |
| Day 24 | 25.4 | 25.7 | 25.6 | 25.6c | | | |
| Day 31 | 23.9 | 24.2 | 23.7 | 23.9e | | | |
| Average | 25.4b | 25.6a | 25.4b | 0.1 | 0.0005 | <.0001 | 0.2048 L |

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; C: silage with coffee cherry; C + EM: silage with coffee cherry and efficient microorganisms; C + K: silage with coffee cherry and kumis; T*D: interaction between treatment and days. L: Linear effect of time on the physicochemical variables of micro silos. a, b and c: Rows with different letters differ significantly at $P < .05$; SD: standard deviation

Organoleptic indicators evaluation of micro silos

Regarding organoleptic indicators (OI), the sum did not show statistical differences ($P > .05$), finding very similar values for all treatments with a percentage of ca. 80 % (Table 7). For the control (40.5 %) and for the EM additive (39.6 %) treatments, the smell indicator was good with a slight vinegar odor for both. However, there were no significant differences ($P > .05$). Similarly, for texture in all treatments no statistical differences were found ($P > .05$), showing defined contours and separation easiness (22 %). On the contrary, for color there were statistical differences ($P < .05$) among the control treatment with EM, regarding the treatment with kumis, which showed an excellent coloration (24 %).

Table 7. Organoleptic indicators in micro silos with coffee cherry and different additives

| Micro silos | C | C+EM | C+K | SD | Pr > F |
|----------------|-------|-------|-------|------|------------------|
| Average | | | | | Treatment |
| Color (%) | | | | | |
| average | 18.0b | 18.0b | 24.0a | 0 | <.0001 |
| Odor (%) | | | | | |
| average | 40.5a | 40.5a | 39.6a | 14.3 | 0.9966 |
| Texture (%) | | | | | |
| average | 22.0a | 22.0a | 18.3a | 3.6 | 0.4212 |
| OI (%) | | | | | |
| average | 80.5a | 80.5a | 82a | 18 | 0.9931 |

OI: organoleptic indicator; C: silage with coffee cherry; C + EM: silage with coffee cherry and efficient microorganisms; C + K: silage with coffee cherry and kumis; a, b and c: Rows with different letters differ significantly at $P < .05$; SD: standard deviation

Discussion

Regarding dry matter content, values were similar between treatments with higher values (20.6 %) than those found by Ramírez *et al.* (2002), cited by Villalba *et al.* (2011), who obtained for coffee cherry silages values from 13.5 to 14.3 %. This behavior may be due to the fact that the production of lactic acid by lactic acid bacteria (LAB) was possibly high in the micro silos with an average pH of 4.0. LAB are classified as homofermentators that produce more than 85 % lactic acid from hexoses, as well as heterofermentators that degrade both hexoses and pentoses (Elferink, Driehuis, Gottschal & Spoelstra 2000).

Likewise, McDonald *et al.*, (1991) cited by Bezabih & Tamir (2014), mention that “the lactic acid produced increases in the concentration of hydrogen ions and undissociated acids to a level in which undesirable organisms are inhibited”. This was probably one of the factors that could have influenced dry matter content which

was constant over time, contrary to what was published by Villalba *et al.*, (2011). In addition, the additives did not influence its content as they remained similar. According to Kung & Muck, (1997), cited by Contreras & Muck (2006), “the addition of microbial-hydrogen fermentative inoculants helps to lower pH faster, inhibiting other bacteria and conserving the plant protein. Moreover, a rapid decrease in pH and a low pH at the end of the process is able to inhibit *Clostridia* bacteria that produce butyric acid. Normally, less acetic acid, butyric acid, and ethanol is produced during homofermentation, which improves dry matter recovery by 2 % to 3%. “It is known that LAB carry out functions that increases not only the nutritional value, but also the improvement of the fermentation process in silage; furthermore, it also reduces dry matter losses due to inefficient sugar fermentation (Merensalmi & Virkki 1991, cited by Bezabih & Tamir, 2014).

Regarding protein, superior values were found compared to the ones published by Villalba *et al.* (2011), while Bermúdez, Santos & Poveda (2013), also observed a progressive increase in protein content during the fermentation period in days 1 and 21 (12.5 and 15.5%, respectively). This may be due to the synthesis produced by bacteria in the silage, generating an increase in levels of soluble and degraded protein, according to Noriega, Silva, & García (2008; 2009). Wilkinson & Phipps (1978), cited by Lanuza (1990), point out that true protein can be degraded to non-protein nitrogen, decreasing from 75 % to 52 %; Ammonium nitrogen can rise from 0 % to 6 % and other non-protein nitrogen compounds rise from 25 % to 42 %.

Subsequently, additives incorporated into the micro silos contributed to obtain a higher protein content in treatments. According to Valeriano, Pinto, Ávila, Evangelista, Tavares & Schwan (2009), “this increase during silage occurs mainly as a result of the use of soluble carbohydrates, causing a percentage increase in raw protein content”.

Regarding neutral detergent fiber content (70.3 %) behavior, the value was similar to the one found by Villalba *et al.* (2011) for coffee cherry silages with a content of 61.5 % in day 21. This may be due to fermentation of structural carbohydrates (Reyes, Montañez, Rodríguez, Ruiz, Salcedo, Avellaneda & Quintana, 2012).

Moreover, Kung Jr and Stanley (1982), cited by Valeriano *et al.* (2009) mentions that “percentage increases of the fibrous fraction in silage material increases in relation to the original material can be seen, as a result of water-soluble constituent losses”, together with effluents produced during

fermentation (Bolsen, 1995, cited by Valeriano *et al.*, 2009) or by gas losses.

Likewise, pH values obtained in this research were lower than those reported for coffee pulp silage (3.8) by Ramírez *et al.* (2002) and Cárdenas (2003), cited by Villalba *et al.* (2011), in mixed silages. According to Díaz, Rodríguez & Salinas (2014), in “this process it is important to promote an environment with a pH of up to 4.2, which inhibits the growth of pathogens and retains nutritional characteristics of the silage product.” According to Merensalmi and Virkki (1991), cited by Bezabih & Tamir (2014), pH required for silage stability at 150 and 250 g.DM⁻¹.kg⁻¹ is 4.10 and 4.35, respectively. Furthermore, pH is an indicator of silage quality, and in this research, the value found was approximately 4.08, a factor that was closely related to dry matter content of the micro silos (206 g.DM⁻¹.kg⁻¹ that represents 20.6 %). Likewise, the temperature values found showed values within the range estimated as good (between 18 and 25 °C) according to Betancourt (2001), cited by Villalba *et al.* (2011). It is important to note that the suitable temperature for lactic acid bacteria growth provided in the additives for the treatments is between 25 and 40 °C (Elferink *et al.*, 2000).

Additionally, when evaluating organoleptic indicators (OI) these showed good results for the three parameters (color, texture and smell). Villalba *et al.* (2011), reported a regular smell in coffee cherry silages, and regarding color, they mentioned that their silage quality was good, regardless of the raw materials used in their treatments.

According to Ojeda *et al.* (1991), cited by Tobia & Vargas (2000), “the parameters considered in order of importance are: smell, color and texture. Silage materials that score values of 36, 16 and 22, respectively for odor, color and texture, can be considered acceptable for animal consumption.” Average values found in this research were 40.2, 20 and 20.7, respectively.

Conclusion

This study shows that coffee cherry as a byproduct of coffee processing, and can be used in the elaboration of silages. Treatments in general showed pH (4.0) and temperature (25° C) values within the normal range, which contributed to maintaining stable dry matter content which is closely related to these variables; therefore, good nutritional quality was obtained as these are quality indicators. Protein content on average was 15.8 % on day 31 for all treatments, being the treatment with kumis the one with the highest protein value (18.1 %); this shows that inoculum addition has a positive effect on protein

content and a reduction in neutral detergent fiber content. Given these concerns, organoleptic characteristics were within the parameters considered as acceptable for feeding animals. Therefore, the most appropriate treatment to be used in future ruminant feeding diets according to the results found of this research is cherry coffee with kumis.

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