Influence of Cellulolytic Bacteria from Rumen Fluid on in Vitro Gas Production of Fermented Robusta Coffee Pulp (*Coffea canephora sp*.)

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ABSTRACT: The experiment was done to evaluate the influence of robusta coffee pulp fermented by rumen cellulolytic bacteria on in vitro gas production. The first step of this experiment was cellulolytic inoculum production by using fluid fermentation with cellulose as substrat. The inoculum produced was then used for coffee pulp fermentation. Cellulolytic bacteria was added into 200 g coffee pulp as much as 0%, 5% and 10% based on dry matter. Each treatment had three replicates. Fermentation was carried out at room temperature during 21 days anaerobically. At the end of fermentation, samples were taken out for nutrient determination including physical, chemical qualities, and in vitro gas production. Data obtained were analyzed by one way design and continued by Duncan's new multiple range test to examine the differences between mean values. The results showed that 5% and 10% cellulolytic bacteria addition decreased pH value, and crude fiber (CF) content as much as 12.89% and 16.32% compared to control (0% of cellulolytic bacteria). Whereas addition of 10% inoculum increased nitrogen free extract (NFE) content. However, cellulolytic bacteria addition up to 10% had no effect on crude protein (CP), extract ether (EE), dry matter (DM), organic matter (OM), glucose, and lactic acid content, as well as in vitro gas production. It could be concluded that cellulolytic bacteria addition in level 5% decreased CF content but did not give positive effect on in vitro gas production.

Keyswords: Cellulolytic Bacteria, Chemical Composition, Coffee Pulp, In Vitro Gas Production

INTRODUCTION

Coffee pulp is an abundant agricultural by-product derived from wet processing of coffee berries from the coffee industry. Coffee pulp is the most important by-product of the so called wet coffee processing, as it represents about 40% of the fruit on a fresh weight basis, and 29% on a dry weight basis (Gaime-Perraud *et al*, 1993). Coffee pulp is the first product obtained during processing, and it represents on a dry-weight basis about 29% of the weight of the whole berry (Ellas, 1979). This may constitute a source of severe contamination and a serious environmental problem. For this reason, efforts have been made to develop methods for its utilization as a raw material for the production of feeds. Fermented coffee pulp is a valid alternative to handling and storing the huge amounts of coffee pulp.

Limitations for the use coffee pulp in animal feeding are connected to its high contents on tannins and caffeine. However, coffee pulp contains proteins, carbohydrates and minerals that may favor its utilization in animal feeding (Mazzafera, 2002). Taking into account the average contents of about 50, 10, 2.5 and 18% for carbohydrate, protein, fat and fibres, coffee pulp appear to be a useful feed supplement for animals (Orozco *et al*., 2008). Ellas (1979) reported the dried coffee pulp has about 10% crude protein, 21% crude fibre, 8% ash, and 44% nitrogen-free extract, as well as 1.80-8.56 % tannins, and 1.3 caffeine. Due to the presence of these compounds (caffeine,

tannins and polyphenols), these organic solid residues show toxic nature and thus have not been utilized beneficially. This has also led to the problem of environmental pollution (Parani and Eyini, 2012).

Several biological treatments including the use of microorganisms such as yeast, filamentous fungi and bacteria are being applied to improve the nutritional value of coffee pulp. Although solid-state fermentation (SSF) has been used for specific biological detoxification of coffee pulp using filamentous fungi at laboratory scale, no data on the suitability of streptomycetes for this purpose has been reported. The ability of these microorganisms to colonize agro-industrial residues and to produce a wide range of enzyme activities related with lignocellulose degradation make them good candidates for biotechnological recycling of coffee pulp (Orozco *et al*., 2008). In most cases, the processes have been designed to render coffee pulp suitable for animal feeding, either in the form of silage or as a dried product (Bressani, 1979). Cabezas *et al.,* (1979) reported ensiled coffee pulp produces better performance than dehydrated pulp, due possibly to its better palatability, better digestibility, and lower content of caffeine and tannins.

In this paper, the experiments were conducted to evaluate the influence of robusta coffee pulp fermented by rumen cellulolytic bacteria on in vitro gas production. The effect of the different levels of rumen cellulolytic bacteria on chemical composition and in vitro digestibility were investigated.

MATERIALS AND METHODS

Culture conditions

Following heat sterilization (121 °C for 30 min), the enrichment medium according Omelianski (1902) cit. Skinner (1971), with cellulose as substrate, was inoculated with 10% of rumen liquor. The culture was grown at temperature 39°C, pH 7 for 7 d anaerobically under submerged culture condition. The the culture was then inoculated in growth medium according Omelianski (1902) cit. Skinner (1971), with cellulose as substrate, in the same condition with enrichment culture, and continued by inoculation for fermentation of coffee pulp. The primary bacteria in this product was cellulolytic bacteria.

Fermentation of coffee pulp

After growing for 7 d, the culture of cellulolytic bacteria was mixed to 200 g of air-dried coffee pulp, and incubated anaerobically at room temperature for 21 d. The culture was added to achieve final concentrations of 0, 5, or 10% based on DM of coffee pulp. The final water content of fermentation was 45% for all treatments by adding distilled water. At the end of the fermentation period, pH, glucose and lactic acid was determined. Then, sample was collected, dried at 55°C for 72 h, ground through a 1-mm screen Wiley mill and analyzed for chemical composition as well as for in vitro digestibility gas production.

Analysis of Fermentation Parameters

pH of fermentation. pH of coffee pulp was immediately recorded using a pH meter after fermentation process.

Glucose content. Glucose content was measured according procedure Nelson-Somogyi (Plummer, 1971).

Lactic acid content. Lactic acid content was analyzed following Baker and Summerson method (Hawk *et al.,* 1976)

Chemical composition. The samples, before and after fermentation, were analyzed for chemical composition including dry matter (DM), organic matter (OM), crude fiber (CF), crude

protein (CP), ether extract (EE), and nitrogen free extract (NFE) according to AOAC procedure (2005). These analysis were carried out for original and fermented sample of coffee pulp to determine the effect of fermentation on chemical composition and in vitro digestibility gas production.

In vitro digestibility gas production technique. Determination of in vitro digestibility gas production technique was conducted following procedure described by Menke and Steingass (1988). In vitro incubations were carried out with rumen fluid from two fistulated Ongole Cross Breed proviously fed with 40% concentrate feed (rice bran) and 60% Penicetum purpuroides at 5% body weight. The rumen liquor was collected from the beefs before they were offered the morning feed into the thermo flask that had been pre-warmed to a temperature of 39°C and was squeezed through four layers of surgical gauze into an Erlenmeyer flask and flushed with CO2 in the laboratory. One part rumen fluid was mixed with two parts buffered mineral solution (1:2 volume/volume) and maintained at 39°C. Approximately 0.300 g of air-dried fermented coffee pulp of known chemical composition that was previously ground through a 1 mm screen was carefully dropped into a 100 ml glass syringe and thereafter, 30 ml this buffered rumen fluid under continuous flushing with CO2 pipetted into incubation syringes containing the ground test substrate. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The silicon tube in the syringe was then tightened by a metal clip so as to prevent escape of gas. Incubation was carried out at 39±1°C and the volume of gas production was measured at 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h, and 72-h cumulative gas production in vitro measured following fitcurve method (Chen, 1994). Blanks were run in triplicates throughout the incubation process.

Experimental Design and Statistical Analysis

Treatments were arranged in a one way design, with the main factors being levels of rumen cellulolytic bacteria (containing 0, 5, or 10% DM basis). Fermentation experiments were separately conducted for each treatment with three replicates each treatment. Air-dried coffee pulp was utilized as substrate for solid state fermentation. The data were analyzed as a one way design. The differences of mean value were analyzed by Duncan's new multiple range test (Rosner, 1990).

RESULT AND DISCUSSION

Chemical composition of fermented coffee pulp

The chemical composition including DW, OM, CP, CF, EE, and NFE of coffee pulp was 66.00%, 87.01%, 23.27%, 42.73%, 1.46%, and 19.54%, respectively. Fermented coffee pulp had low pH value 5.91, 5.81 and 5.57 with addition of 0, 5, or 10% of rumen cellulolytic bacteria respectively. Addition 10% cellulolytic bacteria decreased pH value of fermented coffee pulp significantly ($P<0.05$). However, it did not affect lactic acid content, those were 0.12-0.15%.

Table 1. Chemical composition (% DW) and glucose content (mg/g) of fermented coffee pulp with different level of rumen cellulolytic bacteria

Chemical composition	Level of inoculum addition (%)		
	0	5	10
Dry matterns	58.73±0.04	59.90±0.01	63.19±0.01
Organic matterns	86.97±0.63	87.09±1.20	86.61±1.87
Crude proteinns	25.42±1.15	25.19±0.27	23.87±0.86
Crude fiber**	41.36^{c}±0.80	36.03^{d}±0.80	34.61^{d}±0.56

Ether extracts	2.32±0.30	2.85±0.48	2.62±0.33
NFE*	17.87[a]±2.57	23.02[ab]±1.62	25.50b±0.74
Glucose (mg/g)[ns]	0.09±0.03	0.07±0.05	0.06±0.01

[ns] not significantly different
* (P<0,05)
** (P<0,01)

Fermentation of coffee pulp using 5 or 10% rumen cellulolytic bacteria decreased CF content 12.89 or 16.32%, and increased NFE content 28.82 or 42.70% compare without inoculum (Table 1). The decrease of the CF content was due to inoculum had cellulases activity (data not shown).

In vitro digestibility gas production

As shown in Table 2, fermented coffee pulp with addition of cellulolytic bacteria did not show significant effect and resulted low cumulative gas production in vitro at 72-h incubation, it means low digestibilty of substrate, eventhough CF content of fermented coffee pulp decresed.

Table 2. Cumulative gas production in vitro (ml/300mg DW), fraction a (ml/300mg DW), b (ml/300mg DW), and c (ml/h) of fermented coffee pulp with different level of rumen cellulolytic bacteria 72-h incubation

	Level of inoculum addition (%)		
	0	5	10
Total gas productionns	12.22	12.11	13.63
a[ns]	-0.63	0.02	0.26
b[ns]	12.50	12.01	14.19
c[ns]	0.06	0.05	0.04

[ns] not significantly different

This phenomenon was due to limitations for the use coffee pulp in animal feeding are connected to its high contents on tannins and caffeine. Tannins are known to confer astringency to foodstuffs and complex proteins, affecting food digestibility and decreasing nitrogen utilization animals (Mazzafera, 2002). Getachew *et al*. (2004) reported some feeds, such as forage legumes and cottonseed, contain phenolics, alkaloids and saponins that have negative biological effects on microbes and reduce microbial growth in rumen. Tannins are naturally occurring polyphenolic compounds found in plants, which form complexes with feed and microbial proteins and can depress feed digestibility in the rumen.

CONCLUSION

The addition of 5% cellulolytic bacteria improved chemical composition of fermented coffee pulp especially decreased CF content.

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