Scientific Paper

In vitro nutritional value of the *Musa paradisiaca* L. peel, pretreated with exogenous xylanase enzyme

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Abstract

Objective: To evaluate the *in vitro* digestibility of the *Musa paradisiaca* L., pretreated with exogenous xylanase enzyme for feeding ruminants.

Materials and Methods: A complete randomized design was applied, with for treatments and three repetitions, using exogenous fibrolytic enzymes (xylanase). The treatments were: T0-Banana peel without enzymes (Control), T1-Banana peel + 2 000 IU of enzyme/kg DM, T2-Banana peel + 4 000 IU of enzyme/kg DM and T3-Banana peel + 8 000 IU of enzyme/kg DM. For conducting the experiment, 24 bottles were prepared and six bottles per treatment were used. The chemical composition of the feedstuff was characterized. In addition, the *in vitro* dry matter apparent digestibility was determined, which was measured at 24 and 48 h of incubation. Simultaneously, the gas production was recorded at different times: 3, 6, 12, 24 and 48 h.

Results: The *in vitro* ruminal digestibility, at 24 and 48 h, with the utilization of xylanase, showed highly significant differences among treatments ($p < 0,05$). The highest *in vitro* digestibility percentage at 24 h was in T2 (42,8 %), which increased until 48 h (58,2 %). In the *in vitro* gas production, during the first three hours, no significant differences were recorded among treatments; while at 6, 12, 24 and 48 h, there were differences among them ($p < 0,01$). The maximum gas production was reached at 48 h, in treatment T2, with mean value of 284,29 mL/g DM. The lowest one corresponded to T3 (229,7 mL/g DM).

Conclusions: The *in vitro* digestibility of the *M. paradisiaca* peel increased with the utilization of the fibrolytic enzyme. In turn, the best results were obtained when 4 000 IU of enzyme/kg DM of this enzyme were used.

Keywords: Chemical composition, digestibility, ruminants

Introduction

The banana peel has a high nutritional value, mainly from the energy point of view, which grants it great potential to be used in animal feeding. Among its characteristics is its high dry matter content and dry concentration of non-fibrous carbohydrates (Diniz *et al.,* 2014). According to Blasco-López *et al.* (2014), it is a potential source of antioxidant and antimicrobial substances, as well as secondary metabolites with activity, which eliminate free radicals. It also has other compounds, such as anthocyanins (delphinidin and cyanidin) and catecholamines, carotenoids (β-carotene, α-carotene), xanthophylls, sterols and triterpenes (β-sitosterols, stigmasterol, campesterol, Cycloeucalenol, cycloartenol and 24-methylene cycloartenol).

Since the 60s and at present, nutritionists work on the processes of rumen maturation, in order to reach synchronization between the energy and nitrogen metabolites in the rumen and consequently, increase the productive response of the animals (DiLorenzo *et al*., 2015; Mercadante *et al*., 2015). The byproducts with high fiber content can contribute to maintain a normal rumen pH, increase the digestion of fibrous forages and pastures and, thus, increase the dry matter intake of cattle. The effect of plant secondary metabolites on feedstuff digestibility is consequence of their action on rumen microorganisms or their interaction with the substrate and microbial enzymes (van Wyngaard *et al*., 2015).

The *in vitro* dry matter digestibility method consists in a simulation of the rumen environment, in which a reducer atmosphere is created, rich in carbon dioxide and oxygen-free, with the minerals and required pH to harbor the rumen microorganisms. An incubation of the feedstuffs is done with rumen liquid during 48 hours, with 39 ºC of body temperature in the cow and later with a neutral detergent dissolution for one hour at 100 ºC (Godoy-Espinoza, 2012).

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In general, the improvements in ruminant production with the utilization of supplementary fibrolytic enzymes are ascribed to the increase of ruminal fiber absorption. Many potential mechanisms have been proposed, which include ruminal effects and pre-feeding, as direct hydrolysis, structural changes in the fiber, increase of ruminal microbial fixation, stimulation of the rumen microbial populations and synergism with the rumen microbial enzymes (Giraldo *et al,* 2007).

Measuring digestibility is increasingly important, due to the advance that has appeared in the current feeding systems, in which nutrient availability at rumen level is calculated based on the competition between digestion rate and passage rate Espinoza-Guerra, 2016. The determination of *in vitro* gas production is valuable for the nutritionist, because it provides information about the fermentation kinetics of the forage consumed by ruminants, which depends on the passage rate and the degradation rate (Mould *et al*., 2005). The rate and reach of DM fermentation in the rumen are determinant in the nutrients utilized by ruminants (Jancík, *et al.*, 2010).

The relevance of the evaluation of the forage nutritional value contributes importantly to outline strategies to improve protein and energy ingestion of grazing animals (Cline *et al*., 2010). The current trend in cattle feeding is based on utilizing fiber-rich agricultural residues (banana peel), as feeding supplements, and on obtaining more profitable productions (Gómez, *et al.,* 2014).

Recently, due to the increasing interest in the climate change, and taking into consideration the contribution of animal husbandry to the greenhouse gas emissions, the *in vitro* gas production has also been used to estimate methane (Yáñez-Ruiz *et al*., 2016).

The objective of this study was to evaluate the *in vitro* digestibility of *Musa paradisiaca* L. peel, pre-treated with exogenous xylanase enzyme for ruminant feeding.

Materials and Methods

Location. This study was conducted in the Bromatology Laboratory, of the School of Veterinary Sciences, of the Manabí Technical University, located in the Lodana Parrish, Santa Ana canton, Manabí province, Ecuador, from August, 2018, to May, 2019.

Treatment and experimental design. The *in vitro* dry matter digestibility (IVDMD) and gas production (IVGP) were evaluated through a complete randomized design, with four treatments and three repetitions, with different doses of exogenous enzymes (xylanases). Both variables were determined at 24 and 48 h. the treatments were the following:

T0-Banana peel without enzyme (control)

T1-Banana peel + 2 000 IU of enzyme/kg DM

T2-Banana peel + 4 000 IU of enzyme/kg DM

T3-Banana peel + 8 000 IU of enzyme/kg DM

Experimental procedure

Collection of the plant material. The green banana peel (BP) sample (*M. paradisiaca* var. barraganete), was obtained in a banana processing production facility. Through a simple sensorial analysis the organoleptic characteristics of smell, color, texture and maturity degree, were reviewed. Afterwards, 1 kg of the sample was weighed in an analytical scale of accuracy 0,01 g, and then it was dried. The BP was dehydrated during 72 h, at 60 ºC, in an artisanal dehydrator. Then, it was weighed again to determine the dry matter percentage and it was ground to 2 mm of diameter with a steel blade grinder (trademark Willy IKA MF 10 Basic). The ground sample was preserved in a plastic flask, duly labeled with the characteristics of its content.

Preparation of the enzymatic solutions. Exogenous fibrolytic xylanase (EC 3.2.1.4) from the commercial laboratory *Dyadic Internacional Inc*. (Jupiter, FL-USA) was used. The enzymatic activity of xylanase was determined using 1 % wood xylan (Sigma Chemical Co. St. Louis, MO); as pure substrate, with 10 mg/mL diluted in phosphate-citrate buffer 0,1 M with pH 6,6 at 39°C (Bailey *et al.,* 1992). The enzyme was diluted 1:100, and it was applied with a micropipette in the volumes that are described next, according to the evaluated doses: Xylanases, T1-2 000 IU of enzyme/kg DM, 0,5 mL + 1,5 mL of distilled water in 0,5 g DM per flask, T2-4 000 IU of enzyme/kg DM, 1 mL + 1 mL of distilled water in 0,5 g DM per flask, T3-8 000 IU of enzyme/kg DM, 2 mL + 0 mL of distilled water in 0,5 g of DM per flask.

Treatment of the BPs with the enzyme. Once the sample was weighed (0,5 g) in labeled transparent glass vials, the xylan dose was applied, according to the defined treatments. The different enzyme doses were directly diluted with the aid of a micropipette one hour after the filling process of the culture medium for the beginning of anaerobic microbial fermentation. The vials were placed in an incubator at 39 ºC to maintain an adequate temperature at the moment of filling.

In vitro essays. As microbial inoculant, the rumen liquid extracted from two cattle cannulated in the rumen was used, which was mixed and filtered through linen and gauze to eliminate the residues present. It was mixed with artificial saliva (AS) in ¼ proportion (100 mL of rumen liquor with 400 of AS). This mixture was called rumen fluid. Afterwards it was gassed with CO_2 from 10 to 20 minutes, at temperature of 39 ºC. After gassing, the rumen fluid was transferred to the glass vials which already contained substrate (0,5 g of BP), besides the enzyme applied per treatment (xylanase) in a volume of 50 mL, with the aid of a manual dosifier. After filling, the vials was gassed again with CO_2 during 10 seconds and sealed hermetically. The time at which the fermentation process started was recorded. Using a pressure transducer, the existing pressure was established as level zero, in order to calculate the *in vitro* gas production at the different incubation times (3, 6, 12, 24 and 48 h). For developing the trial, 24 bottles were prepared (6 per treatment). Three bottles per treatment were used to determine the gas production at the above-indicated times.

The *in vitro* apparent dry matter digestibility (IVDMD) was measured at 24 and 48 h of incubation. Simultaneously, the gas production at different times 3, 6, 12, 24 and 48 h, was determined, using a pressure transducer (PSI) adapted to a needle number 23 (0,6 mm), model T443A (Bailey and Mackey, Birmingham, UK) and connected to a visual device.

The gasses accumulated in the top were removed with a syringe until the moment in which the pressure recorded in the reader reached zero. The reported values were in mL/g DM of substrate and were estimated through the quadratic equation proposed by Mauricio *et al*. (1999):

$$Gp = 0{,}18 + 3{,}697\ Pt + 0{,}0824\ Pt^2$$

This process was repeated in all the flasks of each box. After the readings, they were manually agitated and relocated in the stove.

Determination of the chemical composition. The bromatological analyses of the BP were carried out to know the content of dry matter (DM), crude protein (CP), ashes (As) and organic matter (OM), according to the methodology proposed by the AOAC (2005). The content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined, following the technique suggested by Goering & van Soest (1970).

Statistical analysis. The data were analyzed from a variance analysis. For the mean comparison, Tukey's test ($p < 0{,}05$) was utilized. The statistical package PROC GLM (SAS Institute, 2011) was used.

Results and Discussion

The chemical composition of *M. paradisiaca* is shown in table 1. The organic matter was 91,6 %. In a work conducted by Ríos-Elizalde (2014), a value of 88,6 % is reported when studying the peel from another banana variety. This author also found ash content of 11,4 %, higher value than the ones obtained in this research.

The crude protein value (4,9 %) surpassed the one reported by Encarnación-Montero y Salinas-Alvarado (2017), when carrying out the proximal characterization and characterization of the diet fiber of the green banana meal (2,4 %). In turn, in meals of banana papocho and felipita, Espitia *et al.* (2013) referred protein content of 6,7 and 2,4 %, respectively. Hence the protein variation is directly related to the variety.

In this study, the ash value was 8,4 %, which is found directly associated to the high content of minerals, such as calcium, potassium and magnesium. According to Montoya-López *et al.* (2015), this could be due to the banana variety that is evaluated. This value is lower than the one obtained by Godoy-Espinoza (2016), when analyzing the chemical composition of the banana meal with urea in different formulations. This author found ash percentages that varied from 9,5 to 11,7 %.

Table 1. Chemical composition of the banana peel on dry basis (%).

Indicator	Mean	Standard deviation	VC, %
OM	91,6	1,76	0,05
CP	5,0	0,50	0,20
NDF	45,0	1,31	0,03
ADF	30,8	1,88	0,06
Ash	8,38	1,13	0,13

In this research, the NDF and ADF were 45,0 and 30,8 %, respectively. Similar values were obtained by Mosquera-Perea *et al.* (2013), when evaluating the NDF and ADF contents. These authors referred numbers of 46,5 and 16,5 %, respectively.

The *in vitro* digestibility is an indicator of the nutritional value of the feedstuffs (Mosquera-Perea *et al.,* 2013). When determining the *in vitro* dry matter digestibility, at 24 and 48 h of incubation, differences were found among the treatments (table 2). At 24 h, the highest digestibility values were found in T1, T2 and T3, without significant differences among them; however, they differed from T0.

Similar performance was reported by Moreno *et al.* (2007), when evaluating the effect of exogenous fibrolytic enzymes on the *in vitro* ruminal degradation of diets for dairy cows. These authors found higher digestibility when applying the enzyme (51,6 vs 50,3 % without enzyme). In their results they refer that the enzyme at 2 g/kg DM improved the IVDMD in 2,5 %, with regards to T0 (without enzymes).

The results show that at 48 h, the digestibility was higher in each of the treatments in which the exogenous fibrolytic enzymes were included, with regards to the control (T0). The highest values were obtained in T2 and T3, without significant differences between them, but they showed differences with regards to T1. This last one significantly differed from the control.

These results are similar to the ones found by Win *et al*. (2015), who fed fistulated cattle with corn silage, and observed differences in the different times that were evaluated when using fibrolytic enzymes in ruminal digestibility.

Valencia-Trujillo *et al*. (2010), when evaluating the *in vivo* digestibility in sheep, mention that the fiber content contained by pastures and forages influences the performance of ruminal fermentation. These are indicators that should be considered, when applying fibrolytic enzymes. Nevertheless, Pedraza *et al.* (2003) refer that the variations in digestion can be ascribed to the effect of such factors as species and cultivar, plant management and edaphoclimatic conditions.

Moreno *et al.* (2007) and Giraldo *et al*. (2007) mention that during the first hours the enzymes show higher activity on the substrate, and stimulate the initial phase of digestibility. These authors mention that the activity of enzymes decreases as the fermentation time advances.

The high IVDMD values have been associated with the capacity of ruminants to maintain adequate production levels, because this is an indicator of the capacity of a feedstuff to contribute nutrients to the rumen flora (Lazo-Salas *et al*., 2018).

In many cases, it is considered that the lignification content in cellulose and hemicellulose of the substrate is one of the factors that influence the digestion time of the substrate (Montenegro *et al.,* 2018). Reséndiz (2013) states that by increasing the quantity of the enzyme and fiber of the diet a barrier is formed that serves as protection against the attack of microorganisms, thus reducing digestibility.

In this study, the results of *in vitro* gas production (IVGP), at 3, 6, 12, 24 and 48 h, showed that during the first three hours no significant differences were recorded among treatments (table 3); while, at 6, 12, 24 and 48 h, there were statistical differences among them ($p < 0,01$).

Table 2. Effect of exogenous fibrolytic enzymes on the *in vitro* DM digestibility (%) in the banana peel.

Treatment	Incubation time, hours	
	24	48
T0	22,9^{b}	27,2^{c}
T1	38,2^{a}	42,2^{b}
T2	42,8^{a}	58,2^{a}
T3	39,6^{a}	48,5^{a}
SE ±	2,320	3,470
P - value	0,0001	0,0001

T0-Banana peel without enzyme (Control), T1-Banana peel + 2 000 IU of enzyme/kg DM, T2-Banana peel + 4 000 IU of enzyme/kg DM; T3-Banana peel + 8 000 IU of enzyme/kg DM
abc: Different letters in the same column significantly differ ($p < 0,05$)

Table 3. *In vitro* gas production (mL/0,5 g DM of the banana peel).

Treatment	Incubation time, hours				
	3	6	12	24	48
T0	1,6 ± 0,35	5,4 ± 1,07^{b}	42,4 ± 1,01^{b}	125,9 ± 4,41^{b}	248,5 ± 11,04^{ab}
T1	5,5 ± 3,91	7,6 ± 4,19^{b}	40,7 ± 9,03^{b}	118,5 ±5,25^{bc}	239,4 ± 34,78^{ab}
T2	2,2 ± 0,41	20,7 ± 0,68^{a}	60,7 ± 1,25^{a}	143,6 ± 4,14^{a}	284,3 ± 11,68^{a}
T3	3,6 ± 0,77	9,8 ± 0,55^{b}	14,7 ± 1,33^{c}	108,7 ± 4,34^{c}	229,7 ± 10,70^{b}
P - value	0,1532	0,001	0,0001	0,0001	0,0439

T0-Banana peel without enzyme (Control), T1-Banana peel + 2 000 IU of enzyme/kg DM, T2-Banana peel + 4 000 IU of enzyme/kg DM; T3-Banana peel + 8 000 IU of enzyme/kg DM
abc: Different letters in the column significantly differ ($p < 0,05$)

The maximum gas production (GP), reached by 48 h, was recorded in treatment T2, with mean of 284,3 mL/g DM. the lowest one was obtained in treatment T3 (229,7 mL/g DM). In this period, the fractional rate of GP was not significant between T0 and T1, which is associated to the quantity of the enzyme added in each of these treatments. Nevertheless, in the last two treatments (T2 and T3) statistical differences ($p < 0,05$) were found. Yet, in this research it was observed that the highest concentration of enzymes reduced the GP compared with the control treatment.

The results of the *in vitro* GP show that during the first six hours a similar performance appeared in each of the treatments (T0, T1 and T3), but with significant differences compared with T2 ($p < 0,001$). At 12 h, significant differences were found among the treatments ($p < 0,001$). The highest GP was obtained in T2 and the lowest, in T3. Meanwhile, at 48 h no statistical differences were found in T0, T1 and T2, but they were found with regards to T4 ($p < 0,05$).

Studies conducted by Rubanza *et al*. (2005) coincide in reporting that a high enzyme dose produces accumulation of final products in the bottle. Generally, the accumulation of these reaction products decreases the action rate of the enzyme. In some cases, they are combined with the enzyme active site and form a complex system, which inhibits the enzymatic activity.

The highest gas production could be due to the content of soluble carbohydrates, because the production of volatile fatty acids is determined by the action of microorganisms on carbohydrate metabolites (Silva-Ruilova, 2017). The report by Velázquez *et al.* (2013) corroborates this idea, when affirming that the gas volume produced and DM degradation show a positive correlation. This implies that by increasing DM degradation, gas production increases.

Conclusions

The *in vitro* digestibility of the *M. paradisiaca* peel increased with the utilization of the fibrolytic enzyme. In turn, the best results were obtained when 4 000 IU of enzyme/kg DM were used.

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Authors' contribution

- Richard Cornejo-Cornejo. Director of the research.
- José Luis Azúm-González. Technical revision and experimentation.
- Wagner Gorozabel-Muñoz. Adaptation and technical revision.
- Plinio Vargas-Zambrano. Analysis, statistical contribution and experimentation.
- Freddy Mendoza-Rivadeneira. Bibliographic review and experimentation.
- Ricardo Macías-Barbera. Collaboration of microbiological analyses.

Conflicts of interests

The authors declare that there are no conflicts among them.

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