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Original

Wet extrusion of Kikuyu grass (*Cenchrus clandestinus* (*Hochst ex Chiov*))

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ABSTRACT

Objectives To assess the effect of wet extrusion on the *in vitro* digestibility (pepsin-cellulase) of dry matter (IVDDM) and fiber in neutral detergent (IVDNDF) of kikuyu grass (*Cenchrus clandestinus* (Hochst ex Chiov)). **Materials and methods** Six samples of this grass harvested at 35 days of regrowth (5.0 kg/sample) were collected and cut to 2.0 cm; a subsample was taken from each sample for the formation of a mixture composed of raw grass in which the content of crude protein (CP), Neutral detergent fiber (NDF), Acid Detergent Fiber (ADF), acid detergent lignin (ADL), ashes (Ash), calcium (Ca), phosphorus (P), potassium (K), IVDDM, and IVDNDF was determined. The remaining amount of each of the six samples was wet-extrusioned into a 2.0mm outlet conical single screw extruder rotating at 1300 rpm. The same analyzes were carried out on the bagasse obtained from each sample and in the raw sample. Data were analyzed using a two-tailed Student's t-test. **Results.** The bagasse obtained by wet extrusion of kikuyu grass presented a higher content of NDF and ADF with a lower content of CP and Ash than the raw grass, while the IVDDM increased by more than 8% and the IVDNDF by more than 36%. **Conclusions.** Wet extrusion significantly improves kikuyu grass IVDDM and IVDNDF.

Keywords: Bagasse; biomass; delignification; digestibility; fiber; *in vitro* (*Source: CAB Thesaurus*).

RESUMEN

Objetivos. Evaluar el efecto de la extrusión húmeda sobre la digestibilidad *in vitro* (pepsina-celulasa) de la materia seca (DIVMS) y la fibra en detergente neutro (DIVFDN) del pasto kikuyo (*Cenchrus clandestinus* (Hochst ex Chiov)). **Materiales y métodos**. Se recolectaron seis muestras de esta gramínea cosechadas a los 35 días de rebrote (5.0 kg/muestra), que fueron cortadas a 2.0 cm; de cada muestra se tomó una submuestra con la formación de una mezcla compuesta del pasto crudo en la que se determinó el contenido de proteína cruda (PC), fibra en detergente neutro (FDN), fibra en detergente ácido (FDA), lignina en detergente ácido (LDA), cenizas (Cen), calcio (Ca), fósforo (P), potasio (K), la DIVMS y la DIVFDN; la cantidad restante de cada una de las seis muestra fue

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sometida la a extrusión húmeda en un extrusor de un solo tornillo cónico con salida de 2.0 mm que giraba a 1300 rpm. En los bagazos obtenidos de cada muestra se efectuaron los mismos análisis que en la muestra cruda. Los datos fueron analizados mediante una prueba de T de Student de dos colas. **Resultados.** Los bagazos obtenidos por extrusión húmeda del pasto kikuyo presentaron un mayor contenido de FDN y FDA con un menor contenido de PC y Cen que el pasto crudo, al tiempo que se incrementó en más del 8% la DIVMS y en más del 36% de la DIVFDN. **Conclusiones.** La extrusión húmeda mejora significativamente la DIVMS y la DIVFDN del pasto kikuyo.

Palabras clave: Bagazo; biomasa; deslignificación; digestibilidad; fibra; *in vitro* (*Fuente: CAB Thesaurus*).

INTRODUCTION

Milk production in cold zones of Colombia is carried out under grazing of different types of grasses where kikuyu grass (*Cenchrus clandestinus* (Hochst ex Chiov)) is the most important in specialized dairy systems in the department of Antioquia (1,2,3). This is a sub-tropical origin C4 grass that preserves the anatomical, physiological and chemical characteristics of this type of plants, which implies greater accumulation of cell walls and lignin than C3 grasses, resulting in a reduction in the digestibility of structural carbohydrates and, therefore, a reduction in the availability of fermentable energy (4). This is much more accentuated considering that the content of non-structural carbohydrates (NSC) in this grass is low and that of degradable protein (RDP) in rumen is high, generating an imbalance between these fractions that affects, likewise, DM digestibility in rumen (1). Recently it has been reported that the nutritional value of this grass changes very little with the age of regrowth, so grazing at an early age does not increase the digestibility of dry matter (5), for this reason, this grass fails to supply the energy demand of cows whose production level exceeds 11.5 liters/cow/day (6,3), in this sense, the use of commercial supplements with high starch content - which not only further reduce the digestibility of structural carbohydrates (7,8,9) but also increase production costs (10) - exposes animals to food dysfunctions such as ruminal acidosis and laminitis (11,12), and can generate an increase in the production of methane per animal/d due to an increase in fermented dry matter (13).

The negative effect that lignin generates on the fermentability of cell walls structural carbohydrates (cellulose and hemicellulose) constitutes a limiting factor not only in the field of ruminant nutrition, but also in obtaining second-generation fuels (14) as well as in the extraction of derivatives of lignocellulosic materials that involve the fermentation or hydrolysis of cellulose and hemicellulose (15). To mitigate this limitation, chemical, biological and physical methods have been developed for several decades leading to the reduction of the covalent bonds between lignin and these structural carbohydrates (delignification) (16). Among the physical methods, extrusion has emerged as a fast, continuous flow pre-treatment. It is a process in which the plant material is introduced through a conical metal sleeve and is propelled by an endless screw towards the narrowest end of the sleeve, which ends in a die with an outlet which size can be modified. As the material advances through the sleeve, pressure, temperature and shear force effects are generated on the processed material cell walls (17). This process has been evaluated in lignocellulosic materials used in the production of fuel alcohol, with few reports on the use of this technology in forages used in animal feed. Therefore, there are no reports on the effect of this technology on the digestibility of cell walls in grasses such as kikuyu grass. That is why the objective of this experiment was to evaluate the effect of wet extrusion of kikuyu grass on the digestibility of dry matter and cell walls.

MATERIALS AND METHODS

Location. Samples were collected at the Paysandú Experimental Station, located in the Santa Elena district (Medellín) (6° 12’29.2 “N 75° 29›58.4» W) at an altitude of 2400 masl (bh-PM).

Sampling and processing. Six samples of kikuyu grass with 35 days of regrowth were collected from a homogeneously managed lot (approximately 5.0 kg/sample), which were immediately cut to 2.0 cm. The harvest age selected corresponds to one of the most used in Antioquia (18).

Experimental treatments. A sub-sample of 200 g was taken from each one of the six samples, these were mixed and formed the raw grass sample. The remaining amount of the six samples (4.8 kg/sample) was wet extruded in a conical single screw extruder with a processing capacity of approximately 60 kg of green matter/h (220 V motor; 5.0 HP; 1300 rpm) and with 2.0 mm output; bagasse from the extrusion process was retained.

Chemical and physical analysis. In the sample composed of raw grass and in the six bagasses, the content of crude protein (CP), ash, calcium (Ca), phosphorus (P) and potassium (K) (19) was determined, as well as neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (20). The percentage of each chemical fraction of raw grass recovered in the bagasse after wet extrusion was estimated. Likewise, all samples were subjected to an *in vitro* digestibility of dry matter (IVDDM) and NDF (IVDNDF) test by the sequential enzymatic method using pepsin-cellulase (21).

A particle size analysis was carried out for the sample composed of raw kikuyu grass and for the bagasse obtained by extrusion, sieves # 4, 10, 14 and 20 were used for this (Pinzuar Ltda., Colombia), according to manual procedure (22).

Statistical analysis. Data obtained from the extruded bagasse were subjected to statistical analysis against the results obtained from the sample composed of raw grass using the two-tailed Student's t-test for quality control (23,24).

RESULTS

Table 1 shows the chemical composition of raw grass and bagasse. The chemical composition of bagasse showed significant differences with respect to raw grass except in the case of ADL. Thus, bagasse presented higher NDF and ADF contents, and lower in the other fractions.

The recovery of the chemical fractions in bagasse was highly variable (Table 2). This was very high in the fibrous fractions (NDF, ADF and ADL) followed by CP, and was lower in the ashes. However, among the minerals, there were differences, resulting in higher recovery in Ca and less in P and K.

Table 1. Comparison of the chemical composition of raw kikuyu grass and bagasse obtained by extrusion (%DM).

Fraction	Raw	Bagasse	MSE	P
CP*	14.9	12.8	0.403	0.001
NDF	68.6	77.3	1.54	0.001
ADF	34.7	38.0	0.14	0.001
ADL	4.67	5.22	0.382	0.154
Ash	10.1	5.56	0.065	0.001
Ca	0.415	0.310	0.0005	0.001
P	0.545	0.302	0.0002	0.001
K	2.67	1.05	0.012	0.001

* CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; Ca: calcium; P: phosphorus; K: potassium; MSE: Mean Squared Error; P: probability

Table 2. Recovery of chemical fractions in the bagasse of raw kikuyu grass obtained by extrusion.

Fraction	Recovery %	
	Average	DE
CP*	74.0	2.73
NDF	97.1	2.02
ADF	94.5	3.35
ADL	97.0	17.0
Ash	47.4	1.25
Ca	64.4	2.32
P	47.7	1.75
K	33.9	1.94

* CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL; acid detergent lignin; Ca: calcium; P: phosphorus; K: potassium; MSE: Mean Squared Error; p: probability

The effect of wet extrusion of kikuyu grass on IVDDM and IVDNDF is presented in Table 3. It was observed that the extrusion process increased IVDDM by about 8% and IVDNDF by more than 36% compared to raw grass, suggesting a very positive effect of the extrusion process on the digestibility of cell wall components.

Table 3. *In vitro* digestibility of dry matter (IVDDM) and neutral detergent fiber (IVDNDF) of raw and extruded kikuyu grass.

Fraction	Raw	Bagasse	MSE*	P
IVDDM	41.0	42.2	2.44	0.005
IVDNDF	29.3	39.9	3.54	0.001

* MSE: Mean Squared Error; p: probability

Table 4 presents the particle size distribution of the raw and cut kikuyu grass, as well as the extruded one. As can be seen, the extrusion process did not modify the percentage of particles retained in sieves number 4 and 10, but it reduced the proportion of particles in sieves number 14 and 20.

Table 4. Particle size distribution of raw kikuyu grass and extruded bagasse.

Sieve #	Raw	Bagasse	SD*	P
4 (4.75 mm)	58.5	58.2	2.22	0.797
10 (2.0 mm)	28.5	30.6	1.78	0.175
14 (1.4 mm)	2.81	0.48	0.049	0.000
20 (850 mm)	5.15	2.01	0.259	0.002
Bottom	4.99	8.72	2.24	0.102

* SD: Standard deviation; p: probability

DISCUSSION

Because wet extrusion is a process that separates the cell content from that of the cell walls, changes in the chemical composition of bagasse versus raw grass were expected. Although the CP content was reduced in bagasse, it nevertheless continued to be nutritionally important and sufficient to support a milk production level of approximately 14.0 l/d in a 550 kg live weight cow (25). This meant that bagasse even though it presented a different chemical composition than raw grass, its milk production potential continued to be important, with the advantage that it allows it to be conserved or to be pelletized, which facilitates its storage and management.

The recovery of the different chemical fractions in bagasse depends on their location between the content or the cell walls. As it has been observed with the sugarcane bagasse (26), the chemical fractions associated with cell walls (NDF, ADF and ADL) were the most recovered from the kikuyu grass bagasse (Table 2). Among minerals, it was observed that the recovery of Ca was greater, possibly due to the fact that it is closely linked to the cell walls (27, 28), while K had the lowest recovery percentage in bagasse because it is a highly soluble ion in the liquid fraction of plant tissues (27).

The extrusion process improved DIVMD and IVDNDF (Table 3). It has been reported that semi-dry extrusion generates a significant increase in the digestibility of IVDDM and IVDNDF of *Pheleum pratense* L. grass, probably due to the fact that said process affects its physical and chemical structure (29). Other authors have also found increases in the fermentability of other forage grasses after processing using different types of extruders, expressed in the increase in the yield of different monosaccharides due to the degradation of cellulose and hemicellulose (glucose, xylose, arabinose) (30.31).

The proportion of particles of the extruded bagasse retained in sieves numbers 4 and 10 was greater than 88% and exceeded the recommended minimum of 1.18 mm (32). This suggests that the consumption of significant amounts of kikuyu grass bagasse obtained by extrusion according to the procedure described here would not be associated with risks of ruminal acidosis. This effect, however, needs to be evaluated in vivo to verify the relationship of the particle size distribution in kikuyu grass bagasse with ruminal pH.

In conclusion, the tested extrusion process allowed obtaining the bagasse from kikuyu grass whose content of fibrous fractions was higher than in raw grass, with the retention of a significant portion of the CP with a high potential to be used in ruminants supplementation. This process increased the IVDDM by more than 8% and the IVDNDF by more than 36%, with a reduction in the need to use starch-rich supplements in ruminants feeding by improving the supply of fermentable energy in the rumen.

Conflict of Interest

The authors of this manuscript declare that the publication of this study does not conflict with our professional activities.

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