



Wet extrusion and chemical treatment of maralfalfa grass (*Pennisetum* sp)

Ligia Johana Jaimes Cruz¹* 🖾; Héctor Jairo Correa Cardona¹ 🖾; Ángel Giraldo Mejía¹ 🖾 .

¹Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Medellín. Colombia. *Correspondencia: ljjaimesc@unal.edu.co

Received: December 2021; Accepted: July 2022; Published: September 2022.

ABSTRACT

Objectives. Test the effect of wet extrusion and the application of two alkaline compounds on the *in vitro* digestibility of dry matter (IVDDM) and neutral detergent fiber (IVDNDF) of maralfalfa grass. **Materials and methods.** 48 samples of 51 days of regrowth were chopped and assigned to eight treatments: raw, chopped, and dehydrated grass (CTRL); raw, chopped, and extruded grass (EXTR); EXTR treated with 0.45, 0.90, and 1.35% of lime (Ca(OH)₂) or urea for 21 days in micro-silos under aerobic conditions (EXTR0.45Ca, EXTR0.90Ca, EXTR1.35Ca, EXTR0.45U, EXTR0.90U, and EXTR1.35U, respectively). The content of dry matter (DM), nitrogen (N), calcium (Ca), neutral detergent fiber (NDF), acid detergent lignin (ADL) and the *in vitro* digestibility of dry matter (IVDDM) and NDF (IVDNDF) were determined for each sample. **Results.** The EXTR presented lower N content, higher NDF content, and higher IVDNDF than the CTRL. On the other hand, the EXTR1.35Ca treatment showed the highest Ca concentration and the highest IVDDM and IVDNDF, while the EXTR0.90U treatment presented the highest N concentration and an IVDNDF statistically similar to that of the EXTR1.35Ca. Conclusions. The wet extrusion of maralfalfa grass alone increases IVDNDF; however, IVDDM and IVDNDF are maximized when treated with 1.35% of Ca(OH)₂.

Keywords: Bagasse; biomass; calcium; delignification; digestibility; In vitro; nitrogen (Fuente: AGROVOC).

RESUMEN

Objetivos. Evaluar el papel de la extrusión húmeda y la aplicación de dos compuestos alcalinos sobre la digestibilidad in vitro de materia seca (DIVMS) y fibra en detergente neutro (DIVFDN). Materiales y métodos. Se picaron 48 muestras de 51 días de rebrote y se asignaron a ocho tratamientos: pasto fresco, picado y deshidratado (CTRL); pasto crudo, picado y extruido (EXTR); EXTR tratado con 0.45, 0.90 y 1.35% de cal (Ca(OH)₂) o urea durante 21 días en microsilos bajo condiciones aeróbicas (EXTR0.45Ca, EXTR0.90Ca, EXTR1.35Ca, EXTR0.45U, EXTR0.90U y EXTR1.35U, respectivamente). El contenido de materia seca (MS), nitrógeno (N), Calcio (Ca), Fibra Detergente Neutra (FDN), Lignina Detergente Ácida (LDA) y la DIVMS y DIVFDN fueron determinadas en cada muestra. **Resultados.** El EXTR presentó menor contenido de N, mayor contenido de FDN y mayor

How to cite (Vancouver).

Jaimes-Cruz LJ, Correa-Cardona HJ, Giraldo Mejía Á. EWet extrusion and chemical treatment of maralfalfa grass (Pennisetum sp). Rev MVZ Cordoba. 2022; 27(3):e2528. https://doi.org/10.21897/rmvz.2528



©The Author(s) 2021. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons. ©The Author(s) 2021. This article is distributed under the terms of the Cleave Commons Authors and a stream of sa creations under the identical terms.

DIVFDN que CTRL. Por otro lado, el tratamiento con EXTR1.35Ca presentó la mayor concentración de Ca y la mayor DIVMS y DIVFDN, mientras que el tratamiento con EXTR0.90U presentó la mayor concentración de N y una DIVFDN estadísticamente similar a la de EXTR1.35Ca. **Conclusiones.** La extrusión húmeda solo de pasto maralfalfa aumenta el IVDNDF, sin embargo, la DIVMS y DIVFDN se maximizan cuando se tratan con 1.35% de Ca(OH)₂.

Palabras clave: Bagazo; biomasa; calcio; deslignificación; digestibilidad; *In vitro*; nitrógeno (*Fuente: AGROVOC*).

INTRODUCTION

Maralfalfa grass (Pennisetum sp) is a tropical grass characterized by a low content of crude protein and non-structural carbohydrates, but it is high in structural carbohydrates and lignin (1). The latter is a polymeric structure units that combine phenylpropanoid of through ether and ester bonds to cellulose and hemicellulose (2) being able to limit the microbial fermentation or enzymatic hydrolysis of these carbohydrates, reducing their digestibility and the contribution of fermentable energy (3,4,5). In order to make up for this energy deficiency in animal production systems based on tropical grasses, the use of food supplements with a high content of non-structural carbohydrates is common, unfortunately, it has negative effects such as reduction in structural carbohydrates fermentability (6,7,8); increased probability of feeding problems such as ruminal acidosis and laminitis (9,10), which in turn affect animal welfare, animal yield and production costs; and the use of raw materials of high nutritional value for human consumption such as corn generates significant competition for said foods between humans and animal species, a use that has been criticized for decades (11). Additionally, the incorporation of these raw materials increases production costs and dependence on international markets since countries like Colombia are net importers of said foods (12). This makes this strategy counterproductive and unsustainable over time.

Another alternative to solve the energy deficiency, as it happens with the maralfalfa grass due to the high content of lignin, is to solubilize the bonds between this and the structural carbohydrates by means of chemical, microbiological or physical methods and their combinations. Extrusion stands out among the physical methods. It is a quick procedure that, by applying pressure, temperature, and shear

strength, seeks to partially break these bonds, improving the digestibility of cell walls (13). Recently (14) it was showed that the extrusion of kikuyo grass (Cenchrus clandestinus (Hochst ex Chiov)) increased in vitro digestibility of neutral detergent fiber (IVNDFD) by more than 36% and in maralfalfa grass this increase was of more than 20% (15). In other foods such as wheat bran, the combination of various thermomechanical and thermo-mechanical-chemical pretreatments such as twin screw extrusion has been used with chemical treatments such as the application of urea and calcium hydroxide $(Ca(OH)_2)$ (16), however, there are no known records of this combination in tropical grasses such as maralfalfa grass. The concentration of these alkalis has ranged between 0.3 (17) and 6.0% (18) depending on the material to be treated. In any case, it is necessary to take into account that the use of urea increases the nonprotein nitrogen content in the final product by up to 7 times (19) and in the case of lime, the ash and Ca content is increased up to 13 times (20), which can generate imbalances. Therefore, it is necessary to use relatively low concentrations of this alkalis to avoid these problems. That is way the objective of this work was to evaluate the effect of the extrusion of maralfalfa grass as well as the treatment of the extruded bagasse with urea or $Ca(OH)_2$ on the IVNDFD.

MATERIALS AND METHODS

Localization. In a property at the municipality of Támesis (Antioquia - Colombia) located at 2200 meters above sea level, 48 samples of approximately 10 kg of maralfalfa grass with 51 days of regrowth were collected. Each sample was chopped into 2 cm pieces in a grass-chopper and were randomly distributed in eight treatments (six samples/treatment).

Experimental treatments. The eight treatments were: raw and chopped grass (control: CTRL) that was dehydrated at 60oC for 24 h; grass bagasse obtained after processing the raw CTRL 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) with 1.0 mm output (EXTR); EXTR sample subjected to chemical delignification for 21 days with $Ca(OH)_2$ at 0.45, 0.90, and 1.35% of dry matter (EXTR0.45Ca, EXTR0.90Ca, EXTR1.35Ca, respectively) or urea at 0.45, 0.90, and 1.35% of dry matter (EXTR0.45U, EXTR0.90U and EXTR1.35U, respectively). The CTRL samples and bagasse obtained after the extrusion of the samples were dehydrated at 60°C for 24 h in a forced air oven previously the apply the chemical treatments.

For the chemical treatments, 225, 450 or 675 mg of Ca(OH)₂ or urea were added to 12.5 ml of water, respectively, and mixed thoroughly. Subsequently, they were mixed manually with 50 g of the dried maralfalfa grass samples (14.4% of dry matter) in 1 L plastic bottles (micro-silos) with a screw cap in order to obtain a material with 29.2% of water added on dry basis. The micro-silos were kept under aerobic conditions (with atmospheric air) and under shade for 21 days after which all the samples were dehydrated again at 60oC for 24 h and were subjected to the corresponding analyzes.

Chemycal and physical analysis. In all the samples, the dry matter (DM), nitrogen (N) and calcium (Ca) content was determined using the procedures described by the AOAC for forages (21); the neutral detergent fiber (NDF) and acid detergent lignin (ADL) contents were determined based on the procedures of Van Soest et al. (22), and the *in vitro* digestibility of dry matter (IVDMD) and NDF (IVNDFD) were determined by an enzymatic pepsin-cellulase technique described by Barchiesi et al. (23). All analyzes were carried out in the Laboratory of Chemical and Bromatological Analysis of the Universidad Nacional de Colombia at Medellín.

Statistical analysis. The results were subjected to an analysis of orthogonal contrasts using the SAS statistical package PROC GLM (24) in which CTRL vs. EXTR, CTRL vs. CHEMICAL (the treatments with urea and Ca(OH)₂), and EXTR vs. CHEMICAL treatments were compared. On the other hand, the CHEMICAL treatments were analyzed in a nested design using the SAS statistical package PROC GLM (24) under the following linear model:

$$Y_{ijk} = \mu + T_i + N(T_i) + e_{ijk}$$

 Y_{ijk} is the response variable; μ is the experimental mean; T_i is the effect of the chemical compound; $N(T_i)$ is the effect of the application level nested in the chemical compound; and e_{ijk} is the experimental error. The comparison of means were carried out using the LSMEANS statement.

Finally, a regression analysis was done between the $Ca(OH)_2$ and Urea concentration and the Ca and N concentration in the samples treated.

RESULTS

Treatment with raw grass (CTRL) presented high content of NDF and ADL but low content of N (Table 1). The Ca content, by other hand, was normal to this grass (25). According to the records in Table 1, it can be established that, except for some specific results (ADL and Ca in Contrast 1; and DM, NDF and ADL in Contrast 4), the comparisons were significant for the chemical components analyzed. Extrusion (EXTR) allowed obtaining a bagasse with higher DM and NDF contents compared CTRL but lower than N, without evidence of change in ADL and Ca.

Table 1.	Chemical	composition	of	raw	maralfalfa
	grass (Per	<i>nisetum</i> sp),	bag	jasse	product of
extrusion, and extruded bagasse subjected					
to chemical delignification					

Treatments		Chemical fraction % of DM					
		DM1	N	NDF	ADL	Са	
CTRL		14.4	1.45	72.8	7.35	0.37	
EXTR		91.3	1.07	81.9	7.15	0.28	
CHEMICAL		80.2	1.81	77.3	6.12	1.18	
Ca(OH) ₂		80.2	1.22	77.01	6.00	2.09	
Urea		80.2	2.40	77.5	6.22	0.26	
SEM ²		2.19	0.02	2.41	0.37	0.03	
	1	0.001	0.001	0.001	0.574	0.383	
Orthogonal	2	0.001	0.001	0.001	0.001	0.001	
contrasts ³	3	0.001	0.001	0.001	0.001	0.001	
	4	0.999	0.001	0.304	0.283	0.001	

 1 DM = Dry matter; N = Nitrogen; NDF = Neutral detergent fiber; ADL = acid detergent lignin; Ca = calcium.

 2 SEM = standard error of the mean

³Probability of the significance of orthogonal contrasts. The effects tested were 1) CTRL vs. EXTR; 2) CTRL vs. CHEMICALS; 3) EXTR vs. CHEMICALS; 4) Ca(OH)₂ vs. urea were evaluated.

The comparison between the CTRL treatment and the CHEMICALS (orthogonal contrast number 2) showed that there were a difference in the five chemical fractions. Except in the ADL, the content of the other fractions was higher in the samples from the chemical treatments. The application of $Ca(OH)_2$ and urea on the extruded bagasse reduced the content of DM, NDF, and ADL; however, this was not the case for N and Ca (Table 1). According to the result of the analysis of orthogonal contrast number 4, no differences were found in the average content of DM, NDF and ADL, but there were a difference on N and Ca. Finally, the contrast between urea and Ca(OH), showed that the inclusion of urea increased the content of N, while the application of $Ca(OH)_2$ increased the content of Ca. Also there were an increase of 69.2% in the content of N and 76.2% in Ca in the bagasse that received chemical treatments compared to the bagasse generated only by extrusion.

Table 2 shows the results of the chemical composition analysis of the bagasse of extruded grass subjected to the application of the chemical treatments.

Table 2. Chemical composition of bagasse from
extruded maralfalfa grass (*Pennisetum* sp)
subjected to different chemical treatments.

Treatments	Chemical fraction % of DM					
(% of DM) ¹	DM ²	N	NDF	ADL	Ca	
EXTR0.45Ca	79.9	1.15d	78.7a	5.60b	1.42c	
EXTR0.90Ca	80.0	1.15d	77.5a	6.50a	2.10b	
EXTR1.35Ca	80.0	1.38d	74.8b	5.92b	2.77a	
EXTR0.45U	79.9	1.88c	80.2a	6.23a	0.28d	
EXTR0.90U	80.2	2.52b	75.8b	6.13b	0.26d	
EXTR1.35U	80.5	2.80a ³	76.7b	6.32a	0.27d	
SEM ⁴	2.49	0.02	1.83	0.12	0.04	
P ⁵	0.907	0.001	0.001	0.002	0.001	

¹EXTR0.45Ca = grass bagasse treated with 0.45% of Ca(OH)₂; ¹EXTR0.90Ca = grass bagasse treated with 0.90% of Ca(OH)₂; ¹EXTR1.35Ca = grass bagasse treated with 1.35% of Ca(OH)₂; EXTR0.45U = grass bagasse treated with 0.45% of urea; EXTR0.90U = grass bagasse treated with 0.90% of urea; EXTR1.35U = grass bagasse treated with 1.35% of urea

 2 DM = Dry matter; N = Nitrogen; NDF = Neutral detergent fiber; ADL = acid detergent lignin; Ca = calcium

³ Means within a column with similar letter are not different according to the LSMEANS test

 $^{3}SEM = standard error of the mean$

⁴Probability of significance

Of the chemical fractions analyzed, DM was the only one that did not present a difference associated with the treatments. The independent analysis of each chemical fraction shows that the content of N increased as the level of urea incorporated increased, but there was no difference with the addition of $Ca(OH)_2$. The Ca content increased with the increase in the inclusion level of $Ca(OH)_2$ but there was no difference between samples from the addition of urea.

Although the analysis of orthogonal contrasts did not show differences between the treatments with urea and Ca(OH)₂ in terms of the content of NDF and ADL (Table 1), the analysis of the nested design used to evaluate the chemical treatments, shows differences in these chemical fractions. Thus, the NDF level was lower, and not different, in the treatments with the highest $Ca(OH)_2$ concentration and with the medium and high urea concentration; in turn, the highest contents, although not different, occurred with the lowest level of urea application and with the first two levels of $Ca(OH)_2$. The content of ADL was lower with the treatments with the lowest and highest levels of application of $Ca(OH)_2$ and with the treatments with medium and high application of urea.

Table 2 also identifies that the N content increased linearly with the application of urea (y = 1.19 + 1.29X; r² = 0.92; p<0.001) but there was no incidence of $Ca(OH)_2$. The N content went from 1.07% in the crude bagasse (EXTR) (Table 1) to 2.80% in the bagasse treated with 1.35% urea, this is 2.6 times higher. Because the urea treatment reduced the NDF content (Table 2), the content of the other components increases, including N, so its concentration was exacerbated by the contribution of N made by the applied urea. Unlike the latter, the application of Ca(OH), does not translate into changes in the N content. The application of urea had no effect on the Ca content of the samples, the situation was different with the application of Ca(OH)₂; it increased linearly with the increase in its application to such an extent that with the 1.35% $Ca(OH)_2$ treatment this increase was 9.9 times greater than in raw bagasse going from 0.28 (EXTR) (Table 1) to 2.77% (y = 0.42) + 1.81x; r² = 0.93; p<0.001). Again, as with urea, there was a decrease in the NDF content due to the delignifing effect of this hydroxide, which generated the recomposition of the other chemical components of the bagasse and an increase in the Ca content.

Although the bagasse obtained by extrusion (EXTR) had a higher NDF content than the grass from the CTRL treatment and there was no difference in the ADL (Table 1), the results of Table 3 show that there was no difference in the IVDMD but that the IVNDFD increased by 33.0%.

Table 3. In vitro dry matter digestibility (IVDMD)and neutral detergent fiber (IVNDFD) of rawmaralfalfa grass (Pennisetum sp), bagasseproduct of extrusion, and extruded bagassesubjected to chemical treatment.

Treatment		IVDMD	IVNDFD
CTRL	42.9	30.3	
EXTRUDED	42.8	40.3	
CHEMICAL		48.8	43.4
Ca(OH) ₂		50.7	44.9
Urea		46.9	41.9
SEM ¹	4.55	5.63	
	1	0.949	0.001
Orthogonal contracts?	2	0.001	0.001
	3	0.001	0.006
	4	0.001	0.001

¹SEM = standard error of the mean

²Probability of the significance of orthogonal contrasts. The effects using 1) CTRL vs. EXTR; 2) CTRL vs. CHEMICALS; 3) EXTR vs. CHEMICALS; 4) Ca(OH)₂ vs. urea were evaluated.

It is clear that extrusion had a partial effect on *in vitro* digestibility by affecting only IVNDFD; but the chemical treatments increased both IVDMD and IVNDFD when compared with those of the CTRL and EXTR treatment. Together, the chemical treatments generated an average increase of 13.8% in IVDMD compared to raw grass (CTRL) and 14.0% compared to extruded grass; with respect to the IVNDFD this increase was 43.2% and 7.4%, respectively. The comparison of the two alkalizing compounds showed that the effect of Ca(OH)₂ was superior to that of urea in 8.1% and 7.2% for IVDMD and IVNDFD, respectively.

When comparing the chemical treatments, it was established that the addition of 1.35% of Ca(OH)₂ increased IVDMD and IVNDFD by 24.3% and 54.1%, respectively, compared to CTRL. Treatment with 0.9% urea generated an increase in IVNDFD statistically similar to that of treatment with 1.35% Ca(OH)₂ but, unlike this, the increase in IVDMD was only 14.7%.

Table 4. In vitro dry matter digestibility (IVDMD)and neutral detergent fiber (IVNDFD)of bagasse product of the extrusion ofmaralfalfa grass (Pennisetum sp) subjectedto delignification with three levels of calciumhydroxide (Ca(OH)₂) and urea (UREA).

Treatments (Values in% of DM)	IVDMD	IVNDFD
EXTR0.45Ca ¹	47.9b	42.8b
EXTR0.90Ca	51.0b	45.3a
EXTR0.135Ca	53.3a	46.7a
EXTR0.45U	45.2b	41.3b
EXTR0.90U	49.2b	43.4ab
EXTR0.135U	46.5b	41.0b
SEM ²	5.43	6.66
P ³	0.001	0.004

¹EXTR0.45Ca = grass bagasse treated with 0.45% of Ca(OH)₂; ¹EXTR0.90Ca = grass bagasse treated with 0.90% of Ca(OH)₂; ¹EXTR1.35Ca = grass bagasse treated with 1.35% of Ca(OH)₂; EXTR0.45U = grass bagasse treated with 0.45% of urea; EXTR0.90U = grass bagasse treated with 0.90% of urea; EXTR1.35U = grass bagasse treated with 1.35% of urea

 2 SEM = standard error of the mean

³Probability of the significance

DISCUSSION

The high content of NDF and ADL but low content of N in the CTRL is typical of this grass for the regrowth age in which it was harvested (1,25,26). Jaimes et al (15) reported an increase in the NDF content in the maralfalfa grass bagasse obtained by extrusion but with a significant reduction in the ADL content. The increase in the NDF content is a consequence of the extraction of nutrients in the grass juice, which allows obtaining a bagasse rich in cell walls (14,15) as well as chemical changes in it (27).

The composition of bagasse treated with chemicals (Table 1), would be a consequence of the hydrolytic effect generated by both ammonia from urea (28) and $Ca(OH)_2$ (29) on the ether and ester bonds of lignin with structural carbohydrates. In general, alkaline treatments (such as urea and $Ca(OH)_2$) have the ability to remove lignin and various uronic acids from hemicellulose, which improves the access of hydrolytic enzymes that attack cellulose and hemicellulose (30). The increase in the N content of the treated biomass, by other hand, does not only depend on the level

of urea applied (31) but on the delignification method used (32).

Trach et al (33) reported that in rice husk delignification there was a reduction in NDF content by increasing the application of both urea (2.0 to 4.0%) and $Ca(OH)_2$ (3.0 to 6.0) but there was no effect of urea on the ADL content and it significantly reduced as the Ca(OH), concentration increased. These results suggest that the effect of alkaline compounds on cell wall components depends, among other factors, on the type of plant biomass treated and on the concentrations of the delignifying compounds used. In this regard, Behera et al (34) pointed out that the effect of chemical treatments conducive to improving the availability of structural carbohydrates for enzymatic hydrolysis depends on numerous factors among which the type of lignocellulosic biomass; process parameters such as time, temperature, and pressure; as well as the type of delignifying compound used stand out.

The application of $Ca(OH)_2$ linearly increased Ca concentration in bagasse until to 2.77% whit 1.35% of $Ca(OH)_2$ (Table 1). However, it is important to note that this application cannot be considered restrictive for its inclusion in ruminant feeding since it has been reported that high Ca contents in the diet of lactating cows can improve production (35,36). Some time ago it was thought that the Ca-phosphorus (P) relationship could affect the absorption of Ca and P (37), however data reviewed by NASEM (36) suggest that the ratio is not critical.

The IVNDFD (Table 3) in EXTR treatment compared with CTRL, is higher than the 24.5% reported by Jaimes et al. (15) obtained in maralfalfa grass processed in an extruder with a 1.0 mm outlet but lower than the 36.2% found by Jaimes et al. (14) in kikuyu grass extruded with an extruder with a 1.0 mm outlet. The positive effect of extrusion on IVNDFD was also reported by Elgemark (38) who explained it from the combined effects of temperature, pressure, and shear strength generated by extrusion on the physical and chemical structure of the processed biomass. For their part, Heredia et al. (39) indicated that extrusion generates a modification of the crystalline structure of cellulose with the consequent increase in its porosity and increased susceptibility of the fibrous portion of the plant biomass to enzymatic degradation.

The higher response in IVDMD and IVNDFD due to Ca(OH)₂ treatments compared with urea treatments, coincides with that reported by Sirohi and Rai (40) who evaluated the effect of various levels of application of urea and Ca(OH)₂ on the *in situ* digestibility of wheat husk organic matter and found that for similar additions the digestibility was 5.2% higher with the $Ca(OH)_2$. Various authors have evaluated the incidence of some chemical treatments on the in vitro digestibility of fibrous foods, some of these findings are found in the identified literature: Zaman & Owen (41) found that the treatment of barley husk with Ca(OH)₂ at 6% of DM increased the *in vitro* digestibility of organic matter in a greater proportion than that obtained with urea (27.5 vs. 23.1%, respectively); Sirohi & Rai (40) also compared the effect of urea and Ca(OH)₂ on digestibility in wheat husk and found that Ca(OH), at 4% improved IVDMD by 31.2% compared to the control, but with urea at the same concentration this increase was half (15.6%). In other forage materials, higher increases *in vitro* digestibility than those found in the present work have been registered, but it should be noted that applications were made at higher concentrations. Thus, Ramírez et al (42) established that in buffel grass (*Cenchrus*) ciliaris) treated with urea at 4.5% of DM there was an increase in IVDMD and IVNDFD of 58.8% and 63.0%, respectively, while Lázaro et al. (43) found increases of 69.4% and 65.6% in IVDMD and IVNDFD, respectively, in sugarcane harvest residues treated with $Ca(OH)_2$ at 9% of DM.

According to the results recorded in Table 2, it can be considered that both the addition of $Ca(OH)_2$ and urea decreased the concentration of NDF. Regarding *in vitro* digestibility, these chemical treatments also had an effect on IVDMD and IVNDFD (Table 4) but with different trends: while the addition of Ca (OH)₂ increased IVDMD from 0.45 and 0.9% to 1.35%, urea had no effect on this variable. In the case of IVNDFD a more consistent effect is observed for Ca(OH)₂ than for urea.

The results of this work confirm that there is a technological possibility to substantially improve the potential of the fermentable energy inputs of tropical grasses whose NDF and lignin contents are high and limit production with ruminants. Oba and Allen (44) estimated that an increase in a percentage unit in the *in vitro* digestibility of NDF would be reflected in an

increase of 0.17 kg/d in the consumption of dry matter from the pasture and of 0.25 kg/d in milk production. Based on these premises and under the assumption that the use of extruded maralfalfa grass treated with Ca(OH), as a supplement would generate a substitution effect close to 1.0 when dealing with fibrous materials (45), it can be estimated that the replacement of 1.0 kg of DM of raw maralfalfa grass by 1.0 kg of DM of bagasse of the same grass obtained by extrusion would generate an increase of 0.27 L/Cow/d in milk production. If this replacement is made with 1.0 kg of DM of maralfalfa grass bagasse extruded and treated with 1.35% Ca(OH)₂, the increase would be 0.32 L/Cow/d.

In conclusion the wet extrusion process of maralfalfa grass, as used in this experiment, generates a bagasse with a higher NDF content and a higher IVNDFD but with a lower CP content. The treatment of this bagasse with $Ca(OH)_2$ at 1.35% further increases the

IVNDFD and also increases the Ca content while reducing that of NDF and ADL. The increase in IVNDFD in this experiment suggests that there is a technological possibility to increase the potential for milk consumption and production in animals fed with tropical grasses such as maralfalfa grass.

Conflicts of interest

The authors declare no conflict of interest

Funding

This experiment was partially financed with resources from the COLCIENCIAS 110180864120 research project entitled "Development of technological processes for delignification of sugarcane (Saccharum officinarum) and maralfalfa grass (Pennisetum sp.) for industrial use".

REFERENCES

- Correa HJ. Calidad nutricional del pasto maralfalfa (Pennisetum sp) cosechado a dos edades de rebrote. Liv Res Rural Dev. 2006; 18:Article84 <u>http://www.lrrd.org/lrrd18/6/ corr18084.htm</u>
- Chavez M, Domine M. Lignina, estructura y aplicaciones: métodos de despolimerización para la obtención de derivados aromáticos de interés industrial. Av Cien Ing. 2013; 4(4):15-46 <u>https://dialnet.unirioja.es/</u> servlet/articulo?codigo=4710101
- Chandra RP, Bura R, Mabee W, Berlin A, Pan X, Saddler JN. Substrate pretreatment: the key of effective enzymatic hydrolysis of lignocellulosics? Adv Bioechem Eng Biotechnol. 2007; 108:67–93. <u>https://doi.org/10.1007/10_2007_064</u>
- Lee M. A global comparison of the nutritive values of forage plants grown in contrasting environments. J Plant Res. 2018; 131: 641–654. <u>https://doi.org/10.1007/s10265-018-1024-y</u>

- Raffrenato E, Fievisohn R, Cotanch KW, Grant RJ, Chase LE, Van Amburgh ME. Effect of lignin linkages with other plant cell wall components on in vitro and in vivo neutral detergent fiber digestibility and rate of digestion of grass forages. J Dairy Sci. 2017; 100:8119–8131 <u>https://doi.org/10.3168/</u> jds.2016-12364
- de Visser H, Klop A, Van der Koelen CJ, Van Vuuren AM. Starch Supplementation of Grass Harvested at Two Stages of Maturity Prior to Ensiling: Intake, Digestion, and Degradability by Dairy Cows. J Dairy Sci. 1998; 81(8):2221-2227 <u>https://doi. org/10.3168/jds.S0022-0302(98)75801-1</u>
- Ferraretto LF, Shave, RD. Effects of wholeplant corn silage hybrid type on intake, digestion, ruminal fermentation, and lactation performance by dairy cows through a meta-analysis. J Dairy Sci. 2015; 98(4):2662-75 <u>https://doi.org/10.3168/ jds.2014-9045</u>

- Quang dV, Ba NX, Doyle PT, Hai DV, Lane PA, Malau-Aduli AE, Van NH, Parsons D. Effect of concentrate supplementation on nutrient digestibility and growth of Brahman crossbred cattle fed a basal diet of grass and rice straw. J Anim Sci Tech. 2015; 57:35 <u>https://doi.org/10.1186/s40781-015-0068-y</u>
- Nocek JE. Bovine Acidosis: Implications on Laminitis. J Dairy Sci. 1997; 80:1005– 1028 <u>https://doi.org/10.3168/jds.S0022-0302(97)76026-0</u>
- Shabani E, Ceroni V. Subacute ruminal acidosis (SARA) in different groups of age and lactation in cows for milk production. Anglist J (IJLLIS). 2013; 2(4):230–234 <u>https://www.anglisticum.org.mk/index.</u> php/IJLLIS/article/view/1290/1789
- 11. di Paola A, Rulli MC, Santini M. Human food vs. animal feed debate. A thorough analysis of environmental footprints. Land Use Pol. 2017; 67:652-659 <u>https://doi. org/10.1016/j.landusepol.2017.06.017</u>
- 12. Consejo Nacional de Política Económica y Social. Política Nacional Para Mejorar La Competitividad Del Sector Lácteo Colombiano. Departamento Nacional de Planeación: Colombia; 2010 <u>https://</u> www.minagricultura.gov.co/ministerio/ <u>direcciones/Documents/d.angie/conpes%20</u> <u>3675.pdf</u>
- Duque A, Manzanares P, Ballesteros M. Extrusion as a pretreatment for lignocellulosic biomass: Fundamentals and applications. Ren Energy. 2017; 114(Part B):1427-1441 <u>https://doi.org/10.1016/j.</u> renene.2017.06.050
- 14. Jaimes LJ, Mendoza EO, Menjivar CA, Montoya EV, Giraldo Á, Correa HJ. Extrusión húmeda del pasto Kikuyo (Cenchrus clandestinus). Rev MVZ Córdoba. 2021; 26(1):e1964 <u>https://doi.org/10.21897/</u> rmvz.1964
- 15. Jaimes LJ, Menjivar CA, Montoya EV, Mendoza EO, Correa HJ, Girañdo Á, Ruíz ÁA. Hidrólisis enzimática del pasto maralfalfa (Pennisetum sp) sometido a extrusión húmeda. Rev ion. 2021; 34(1): 111-120 https://doi.org/10.18273/revion.v34n1-2021009

- 16. Vandenbossche V, Doumeng C, Rigal L. Thermomechanical and thermo-mechanochemical pretreatment of wheat straw using a twin-screw extruder. Biores. 2014; 9(1):1519-1538 <u>https://doi.org/10.15376/ biores.9.1.1519-1538</u>
- Zaman M, Owen E. The effect of calcium hydroxide and urea treatment of barley straw on chemical composition and digestibility in vitro. Anim Feed Sci Tech. 1995; 51:165-171 <u>https://doi.org/10.1016/0377-8401(94)00669-Z</u>
- Sirohi SK, Rai SN. Synergistic Effect of Urea and Lime Treatment of Wheat Straw on Chemical Composition In Sacco and In Vitro Digestibility. Asian-Austral J Anim Sci. 1999; 12:1049–53 <u>https://doi.org/10.5713/</u> ajas.1999.1049
- 19. Wanapat M, Sundstol F, Garmo TH. A comparison of alkali treatment methods to improve the nutritive value of straw. I. Digestibility and metabolizability. Anim Feed Sci Tech. 1985; 12:295–309 <u>https://doi.org/10.1016/0377-8401(85)90006-9</u>
- 20. Djajanegara A, Molina BT, Doyle PT. The utilization of untreated and calcium hydroxide treated wheat straw by sheep. Anim Feed Sci Tech. 1985; 12:141-150 <u>https://doi.org/10.1016/0377-8401(85)90060-4</u>
- Association of Official Analytical Chemist

 AOAC. Methods of Analysis. 20 ed.
 Washington D.C. AOAC Int. 2016.
- 22. Van Soest P, Robertson JB, Lewis BA. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J Dairy Sci. 1991; 74:3583–3597 <u>https://doi. org/10.3168/jds.S0022-0302(91)78551-2</u>
- 23. Barchiesi C, Alomar D, Miranda H. Pepsin-Cellulase Digestibility of Pasture Silages: Effects of Pasture Type, Maturity Stage, and Variations in the Enzymatic Method. Chilean J Agric Res. 2011; 71(2):249-257 <u>https://doi. org/10.4067/S0718-5839201100020001</u>
- 24. Statistical Analysis Software (SAS). Statistics (Version 8). CaryNC: the Institute. 1998.

- 25. Mendoza-Grimón V, Fernández-Vera JR, Hernández-Moreno JM, Palacios-Díaz MP. Mineral balance and absorption from soil of Pennisetum sp at different stages. Int J Environ Agric Res. 2016; 2(10):29-35. <u>https://accedacris.ulpgc.</u> es/bitstream/10553/70505/2/Mineral balance_absorption.pdf
- 26. Clavero T, Razz R. Valor nutritivo del pasto maralfalfa (Pennisetum purpureum x Pennisetum glaucum) en condiciones de defoliación. Rev Fac Agron Univ Zulia. 2009; 26(1):78-87 <u>http://ve.scielo. org/scielo.php?script=sci_arttext&pid =S0378-78182009000100005</u>
- Zhan X, Wang D, Bean SR, Mo X, Sun XS, Boyle D. Ethanol production from supercriticalfluid-extrusion cooked sorghum. Ind Crops Prod. 2006; 23(3):304–310 <u>https://doi. org/10.1016/j.indcrop.2005.09.001</u>
- 28. Kim TH, Kim JS, Sunwoo C, Lee YY. Pretreatment of corn stover by aqueous ammonia. Biores Tech. 2003; 90:39– 47 <u>https://doi.org/10.1016/S0960-8524(03)00097-X</u>
- 29. Kim S, Holtzapple MT. Lime pretreatment and enzymatic hydrolysis of corn stover. Biores Tech. 2005; 96:1994-2006 <u>https:// doi.org/10.1016/j.biortech.2005.01.014</u>
- 30. Chang VS, Holtzapple M. Fundamental factors affecting biomass enzymatic reactivity. Appl Biochem Biotechnol. 2000; 84:5-37 <u>https:// doi.org/10.1385/ABAB:84-86:1-9:5</u>
- 31. Ventura M, Barrios A, Morales I, Toro C, Barreto K, Noguera F. Efecto de la "amonificación seca" sobre el valor nutricional de la soca de sorgo (Sorghum bicolor). Rev Cien. 2002; 12(Supl 2):513-516 <u>https:// produccioncientificaluz.org/index.php/ cientifica/article/view/14915/14892</u>
- 32. Jiménez R, San Martín F, Huamán H, Ara M, Arbaiza T, Huamán A. Efectos del tamaño de partícula y tipo de amonificaciónconservación sobre la digestibilidad y consumo del rastrojo de maíz en ovinos. Rev Inv Vet Perú. 2010; 21(1):19-25 <u>http://www.scielo.org.pe/pdf/rivep/v21n1/</u> <u>a03v21n1.pdf</u>

- Trach NX, Mo M, Dan CX. Effects of treatment of rice straw with lime and/or urea on its chemical composition, in-vitro gas production and in-sacco degradation characteristics. Liv Res Rural Dev. 2001; 13: Article35 <u>http://www.lrrd.org/lrrd13/4/</u> <u>trac134a.htm</u>
- Behera S, Arora R, Nandhagopal N, Kumar S. Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. Renew Sust Energ Rev. 2014; 36:91–106 https://doi.org/10.1016/j.rser.2014.04.047
- 35. Kincaid RL, Hillers JK, Cronrath JD. Calcium and Phosphorus Supplementation of Rations for Lactating Cows. J Dairy Sci. 1981; 64(5):754-758 <u>https://doi.org/10.3168/</u> jds.S0022-0302(81)82644-6
- 36. National Research Council (NRC). The nutrient requirement of dairy cattle. Seventh ed.; National Academy Press, Washington D.C., 2001.
- Beitz DC, Burkhart DJ, Jacobson NL. Effects of Calcium to Phosphorus Ratio in the Diet of Dairy Cows on Incidence of Parturient Paresis. J Dairy Sci. 1974; 57(1):49-55 <u>https://doi.org/10.3168/jds.S0022-0302(74)84830-7</u>
- Elgemark E. Intensively processed silage using Bio-extruder. Animal Science Degree, Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management: Uppsala; 2019 <u>https://stud.epsilon.slu.se/14511/7/</u> Elgemark E_190405.pdf
- Heredia E, Pérez E, Montoya M, Serna SO. Effects of Extrusion Pretreatment Parameters on Sweet Sorghum Bagasse Enzymatic Hydrolysis and Its Subsequent Conversion into Bioethanol. BioMed Res Int. 2015; 2015:325905 <u>https://doi.org/10.1155/2015/325905</u>
- Sirohi SK, Rai SN. Synergistic effect of lime and urea treatment of wheat straw on chemical composition, in-sacco and in-vitro digestibility. Asian-Aust J Ani Sci. 1999; 12:1049-1053 <u>https://doi.org/10.5713/</u> <u>ajas.1999.1049</u>

- Zaman MS, Owen F. The effect of calcium hydroxide and urea treatment of barley straw on chemical composition and digestibility invitro. Anim Feed Sci Tech. 1995; 51:165-171. <u>https://doi.org/10.1016/0377-8401(94)00669-Z</u>
- 42. Ramirez GR, Aguilera JC, Garcia G, Nunez AM. Effect of Urea Treatment on Chemical Composition and Digestion of Cenchrus ciliaris and Cynodon dactylon Hays and Zea mays Residues. Anim Vet Adv. 2007; 6(8):1036-1041 <u>http://docsdrive.com/pdfs/</u> medwelljournals/javaa/2007/1036-1041.pdf
- Lázaro C, Aranda E.M, Ramos JA, Vargas LM, Hernandez O. Efecto del hidróxido de calcio y conservación en el valor nutritivo de alimentos a base de residuos de caña de azúcar. Agro Produc. 2018; 7:2 <u>https://www. revista-agroproductividad.org/index.php/</u> agroproductividad/article/view/517/397

- 44. Oba M, Allen MS. Evaluation of the importance of the digestibility of neutral detergent fibre from forage: effects on dry matter intake and milk yield of dairy cows. J Dairy Sci. 1999; 82:589–596 <u>https://doi. org/10.3168/jds.S0022-0302(99)75271-9</u>
- 45. Bargo F, Muller LD, Kolver ES, Delahoy JE. 2003. Invited Review: Production and digestion of supplemented dairy cows on pasture. J Dairy Sci. 2003; 86:1-42 <u>https://doi.org/10.3168/jds.S0022-0302(03)73581-4</u>