NOTA TÉCNICA

Changes in aminic nitrogen of silages

Cambios en nitrógeno amínico de ensilados

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ABSTRACT. This paper investigated quantitative changes of aminic nitrogen as a important protein breakdown parameter in laboratory scale silages.The experiments were performed from November to April (rainy season) of 2010 year at the Agronomy Faculty of Chianga (13 km from Huambo City) in Angola. It showed that increasing aminic nitrogen below threefold value is a reasonable guide for optimal protein preservation in silages. The potenciometric formol method for aminic nitrogen determination is simple, rapid and economic. It is recommended this chemical analysis for routine control of silage quality.

Keywords: aminic nitrogen, protein degradation, laboratory silage, postharvest.

RESUMEN. Se investigaron los cambios cuantitativos de nitrógeno amínico, como un importante parámetro de la degradación de la proteína, en ensilados a escala de laboratorio. Los experimentos se desarrollaron en la época de lluvias, desde noviembre hasta abril del año 2010, en la facultad de Agronomía de Chianga, localizada a 13 kilómetros de la ciudad de Huambo en Angola. Se mostró que el incremento de nitrógeno amínico inferior a tres veces el valor inicial puede ser considerado razonable para valorar la conservación óptima de la proteína en ensilados. El método potenciométrico que utiliza formol para la determinación del contenido de nitrógeno amínico es simple, rápido y económico. Se recomienda este análisis químico de rutina para el control de calidad en ensilados.

Palabras clave: nitrógeno amínico, degradación de proteína, ensilados de laboratorio, postcosecha.

INTRODUCTION

Based on a combination of energy and digestible protein per hectare, Santana *et al.* (2010), recommended harvest age of napier grass during rainy season within an interval of 54 to 60 days of regrowth. Recycling by-products of food industry as silages is one important technological alternative to prevent environmental pollution (Revuelta, 2000; Revueltal *et al.,* 2008). Sustainability of animal agriculture requires efficient use of feed N so as to prevent its release to the environment. Therefore, there is an urgent need to explore different ways that can potentially increase the efficiency of N utilization to make the livestock industry profitable and a friendly environment operation. High protein degradability of silages decreases their usage as an efficient protein sources. Knowledge of free amino acids content during anaerobic storage is very important in understanding biological processes of protein transformation in food technology of conservation. In vitro proteolyses assays indicated that there is a potential inhibition of proteases when specific o-diphenols are supplied to grass extracts (Marita, Hatfield & Brink, 2010). Thus, the chemical composition of crude protein indicated that minimal improvement of true protein fraction in silages has a great economic effect in the animal production (Jones, 2001). Thus, a modified ninhydrin colorimetric assay was evaluated for quantification of free amino acids in silage extract (Winters *et al.,* 2002). Other alternative for this analysis is the determination of aminic nitrogen content using the formol method (Chechetkin *et al.,* 1984, USP XXIV, 2000, & Morris *et al.,* 2002). The aminic nitrogen represents nitrogen of polypeptides, free amino acids and proteins. The principle of formol method for the aminic nitrogen determination is based on the following chemical reactions:

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(I) $$H\text{-}C(H)=O + R\text{–}CH(NH_2)\text{–}COOH = R\text{–}CH(N=CH_2)\text{–}COOH + H_2O$$

(II) $$R\text{–}CH(N=CH_2)\text{–}COOH + NaOH = R\text{–}CH(N=CH_2)\text{–}COONa + H_2O$$

The success of silage-making operation can be assessed by monitoring quality changes during the ensiling process. This can be achieved by comparing the parent forage and the resulting silage. So, increasing aminic nitrogen during storage should be used as a guide to silage fermentation quality. The aim of this work is to assess the aminic nitrogen before and after ensiling as a important protein breakdown parameter.

METHODS

It was used a completely random design for making the experiments.

Local biomass: Brewers grain wastes of Nocebo Cuca Bier Plant of Huambo in Chivas town, and other plant materials such as coffee pulp (*Coffea arabica*), seven differents grasses (Table 1) and *Leucaena leucocephala* harvested at Agronomy Faculty in Chianga (13 km from Huambo City) during rainly season from November to April of 2010 year were investigated. The grasses were cutting manually at 63 days of regrowth.

Laboratory scale silages: Approximately 450 g fresh biomass was packed into 0,5 L glass flasks in order to obtain anaerobic conditions. The micro silos were opened after 90 days of anaerobic storage to evaluate the aminic nitrogen content.

Analytical Procedure: The plant material samples before and after ensiling were drying at 65 ^{0}C and finely triturated. The laboratory procedure used 25 mL of the sample obtained from 5 g dry plant material mixed with 100 mL distilled water during 1 hour of mecanical mixing. The potentiometric formol method for the aminic nitrogen determination according to the cited literature was applied. The experiments were making from November to April (rainy season) of 2010 year, at the Agronomy Faculty of Chianga (13 km from Huambo City) in Angola. Approximately 450 g fresh forage was packed into 0,5 L glass to obtain anaerobic conditions for each experimental unit of laboratory scale silage. The micro silos were opened after 90 days of fermentation.

RESULTS AND DISCUSSION

The quantitative changes in aminic nitrogen of some tropical laboratory scale silages (Table 1) have been investigated. The applicated Formol Method is simple, rapid and economic.

TABLE 1. Variation in aminic nitrogen content of some tropical silage

No.	Biomass	% N_{aminic} (Dry Basis)[a] Forage (X) Silage (Y)	Y/X
1	*Brewer's grain residue*	0,2772 0,6997	2,52
2	*Coffee pulp var. Caturra (Coffea arabica L.)*	0,2064 0,2064	0,00
3	*Leucaena Leucocephala (var. Perú)*	0,1795 0,2535	1,40
4	*Start grass (Cynodon nlemfuensis)*	0,0634 0,2640	4,20
5	*Napier grass (Pennisetum purpureum Schum.)*	0,0739 0,3697	5,00
6	*Guatemala grass (Tripsacum laxum)*	0,0317 0,1584	5,00
7	*Guinea grass (Pannicum maximum)*	0,0422 0,2851	6,75
8	*Gordura grass (Melinis minutiflora)*	0,2218 0,2218	0 00
9	*Giant setaria (Setaria splendida)*	0,0317 0,1479	4,70
10	*Brachiaria decumbens*	0,2852 0,2852	0,00
11	CV (%)	73,02 54,72	84,48
12	Simple Lineal Equation: Y = 1,179 X + 0,153; r^2 = 0,438; ES = 0,146		

[a]The data are means of three replicates

The range of increasing of aminic nitrogen content up to below a threefold value during storage is considerated an adequated proteolytic process in the analysed silages of brewer's grain waste and *leucaena leucocephala*. The coffee pulp, guinea grass and brachiaria ducumbens grass did not variate this chemical parameter, but start grass, napier grass, guatemala grass, guinea grass and giant setaria grass showed high increasing of aminic nitrogen during storage. The pH is a key criterion to evaluate silage fermentation. So, proteolyse is inhibited more strongly by extent and rapid decline of the pH than osmolarity (Bickel *et al.,* 2006). Alfalfa showed 0,25% and 0,85% of free amino acids nitrogen content (dry basis) before and after ensiling, respectively (Kofahl, 2008). Jones (2001) reported that optimal grass conservation as silage with 15% crude protein content increasing free amino acids nitrogen on dry matter from 0,23% up to 0,63%. It is clear that the most important protein fraction for efficient ruminant utilization is the true protein component, but the increasing of the free amino acids gave good indication of the protein degradation.

Other influencing chemical parameters for protein preservation are poliphenols and tannins contents (Kofahl, 2008).

The rumen by-pass protein technology addresses the problem of inefficient use of dietary proteins by ruminants, increases nutrient use efficiency and optimizes the productive and reproductive performances. On feeding by-pass protein there is a net saving of dietary proteins and less excretion of urea and nitrogen (FAO, 2011). In conclusion, increasing aminic nitrogen below threefold value during silage fermentation can be considerated a guide for optimal protein preservation. Finally, it is recommended the use of formol method for the routine assessment of the fermentative quality according to the extent of protein degradation in silages.

REFERENCES

1. BICKEL, A., K. FRIEDEL & M. GABEL: "Factors potentiallly affecting proteolysis under in vitro conditions using Rostocker fermentationstest, first results", *Proc. Soc. Nutr. Physiol*. 15: 2006
2. CHECHETKIN, A.V., V.Y. VORONIANSKI & G.G. POKUSAY*: Prácticas de bioquímica del ganado y aves de corral,* pp.42-44, Editorial Mir, Moscú, 1984.
3. FAO: *Successes and failures with animal nutrition practices and technologies in developing countries*. Proceeding of the FAO Electronic Conference, 1-30 September 2010, Rome, Italy. Edited by Harinder P.S. Makkar. FAO Animal Production and Health Proceeding. No.11, Rome, Italy, 2011.
4. JONES, R: *Möglichkeiten zur Optimierung des Proteingehaltes in Futterpflanzen und ihre Effekte in der Milchproduktion,* Tagungsbericht der Dr. Piper Technologie- und Produktentwicklung GmbH, in Neuruppin, Deutschland, 2001.
5. KOFAHL, A: Methodische Untersuchungen zur Beurteilung der proteolytischen Aktivität, der Proteolyse and der Desmolyse bei der Silierung eiweißreicher Grünfutterleguminosen, **Inaugural–Dissertation zur Erlangung des Grades eines Doctor Agriculturae der Agrar- und Umweltwissenschaftlichen Fakultät der Universität Rostock**, Deuschland, 2008
6. MARITA, J.M., R.D. HATFIELD & G. BRINK: "In vitro Proteolytic Inhibition, Polyphenol Oxidase Activity, and Soluble O-Diphenols in Grasses and Cereals". *J. Agric. Food Chem.,* 58: 959-966, 2010
7. MORRIS, H.J., A. ALMENARES, K. ROMERO & C. VIDAL: "Validación de un método potenciométrico para la determinación de nitrógeno amínico en hidrolizados proteicos de microalgas". *Revista Cubana Farm.,* 36(1): 56–61, 2002.
8. REVUELTA, D: "Ensiling potential of coffee pulp (*Coffea arabica*)", *Newsletter Berlin Alumni Network*, Issue I, April/2000.
9. REVUELTA, D., D. MOSQUERA & F. CUBA: "Ensiling potencial of orange fruit wastes (*Citrus sinensis*)", *Revista Ciencias Técnicas Agropecuarias*, 17(2): 41-44, 2008.
10. SANTANA, A.A., A. PÉREZ AND M.E. FIGUEREDO: "Optimal harvest age for napier grass (*Pennisetum purpureum Schum.*) during rainy season based on yield and nutritional value". *Rev Mex Cienc Pecu.,* 1(3): 277–286, 2010.
11. UNITED STATES PHARMACOPOEIA XXIV: Convention U.S.P. XXIV United States Phermacopeia and natural Formulary, pp. 1438, 24 ed. Rockville: Mack Printing, USA, 2000.
12. WINTERS, A., J.D. LLOYD, R. JONES & R.J. MERRY: "Evaluation of a rapid method for estimating free amino acids in silages". *Animal Feed Science and Technology*, 99(1–4): 177–187, 2002.