

## DESARROLLO DE ALIMENTO ANIMAL PARA GANADO, UTILIZANDO BAGAZO DE CAÑA DE AZÚCAR COMO INGREDIENTE PRIMARIO, SUPLEMENTADO CON RESIDUOS PROCEDENTES DE LA PRODUCCIÓN DE TRANSGLUTAMINASA MICROBIANA

### DEVELOPMENT OF ANIMAL FEED FOR CATTLE, USING SUGARCANE BAGASSE AS THE PRIMARY INGREDIENT, SUPPLEMENTED WITH RESIDUES FROM THE PRODUCTION OF MICROBIAL TRANSGLUTAMINASE

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**Resumen**-- Se presenta el desarrollo de un alimento básico predigerido a partir de bagazo de caña de azúcar, para bovinos, suplementado proteicamente con un subproducto de la enzima transglutaminasa. Se implementó una mayor digestibilidad mediante un tratamiento alcalino con hidróxido de sodio (2.0 Normal). Se realizó el análisis de digestibilidad in vitro, se determinó contenido de proteína, minerales, humedad, fibra cruda y extracto etéreo. Para la caracterización física de las fibras se utilizaron: microscopía confocal de barrido láser. Con base en el aumento de la digestibilidad se estimó el aumento de peso en el ganado con un software (diseñado por el grupo de trabajo). De acuerdo con los resultados se pudo corroborar que el tratamiento alcalino incrementa la digestibilidad del bagazo y por lo tanto sus resultados pueden ser aplicados en tecnologías de alimentación bovina.

**Palabras Clave**-- alimento animal, bagazo, digestibilidad, transglutaminasa, ftir, microscopía confocal, caña de azúcar, digestibilidad.

**Abstract** -- The development of a basic pre-digested food from bagasse, for cattle protein supplemented with a by-product of the transglutaminase enzyme occurs. Increased digestibility was implemented by an alkaline treatment with sodium hydroxide (2.0 Normal). The tests performed in vitro digestibility, protein content and minerals, moisture, etc. For the physical characterization of fibers and comprehensive post-treatment techniques they are used: laser scanning confocal microscopy. Based on the increased digestibility was improved by weight predicted increase in cattle with a software (it is designed

for the work group) for proximity to dairy increase of the caws for 100 days. According to the results it was possible to corroborate the alkali treatment gives higher digestibility bagasse and therefore their results can be applied in technologies of cattle feed.

**Key words** – animal feed, bagasse, digestibility, transglutaminase, FTIR, Confocal Microscopy, sugar cane, digestibility.

#### INTRODUCCIÓN

In recent years the care and preservation of the environment is to become one of the main objectives of governments. In this sense the correct disposition of agro-industrial waste, organic and inorganic waste, is one of the best alternatives. One of the limitations for the use of agro-industrial wastes is complex structure.

Ligno-cellulosic substrates as bagasse and tops of sugar cane, among others crops and residues are mainly composed of cellulose, hemicellulose and lignin, these fibers had embebed in a lignin matrix, these are presented in a different proportion depending on plant species, age and organ. Lignin is recalcitrant also hampering the activity of hydrolytic enzymes [1]. In this sense, the pretreatment is needed to degraded this matrix for facilitate the vitamins and protein absorption in the cow's stomach.

In the other hand, this agroindustrial subproducts are rich in minerals and vitamins, such as potassium, iron, calcium and magnesium. This properties will be exploited by an animal food made with nutrients and fiber.

At present day, a significant amount of over 75.73 million tons of dry matter of different agro-industrial wastes from 20 crops are produced, which scarcely 10% are used, and generated in Mexico.

In this sense, the sugar cane industry produces nine by-products from which it can be fabricated a large number of useful raw materials for the production of commercial co-products from pharmaceutical or energy interest. In this work, three fibrous residues: bagasse of *Saccharum officinarum* (sugar cane), are treated by alkaline treatments with Sodium hydroxide (NaOH).

Sugarcane (*Saccharum officinarum L.*) is one of the most economically important plants and is the most important source of sugar as 70% of the sugar produced in the world is made from sugar cane. This grass generates nine products, in its agro-industrial process, within its generation volume is noteworthy bagasse, rum, and molasses bud (final molasses), since they generate more than 300 co-products such as furfural, medicines, activated carbon, chipboard, animal food, etc.

Sugar cane bagasse is one of the most important subproduct of sugar cane referent to production and nutritional properties, because after sugarcane is milled for juice extraction, bagasse is obtained as a residue, which corresponds to about 25% of the total weight and contains 60% to 80% of carbohydrates [2, 3]. In Mexico, for instance, 52 million tons of sugarcane were produced in 2014 (conadesuca) [4]).

Pre-treatments included mechanical, biological and chemical (acid or alkaline) agents. This pretreatments has advantages and disadvantages, among chemical pre-treatments, acids require high temperature and pressure during treatment, and involve delicate issues as equipment corrosion, and the necessity to process the residual effluent involving a huge consumption of water [5], in these sense the cost is expensive and non sustainable.

In the other hand, alkaline pre-treatments are often favored in ligno-cellulosic substrates, as they are normally performed at lower temperature and pressure than acid pre-treatments. Furthermore, alkaline pre-treatments necessitate much time of reaction, instead of minutes as acid pre-treatments. But this kind of treatment do not applies at animal food because the residues can caused future problems.

Along the time various alkali agents may be used, por example, calcium, sodium, and potassium hydroxide but NaOH has been most studied. Alkaline pre-treatments involve the saponification of the ester bonds between hemicellulose and lignin, loosening the links between lignin and structural carbohydrates [6]. This causes a swelling of pre-treated biomass, an increase of porosity and internal surface, and a concurrent decrease in the

degree of polymerization. All in all, alkaline pre-treatments are performed under severe conditions of temperature and alkali concentration [7, 8] and, in spite of the high costs incurred with this approach.

Alkali treatments were initially used to increase biomass digestibility for animal feeding. Diluted alkali solutions lead to the disruption of lignocellulosic cell walls by dissolving hemicellulose, lignin and silica, by hydrolyzing uronic and acetic acid esters and by swelling cellulose [9,10].

Lignin decomposition is usually attributed to the cleavage of the  $\alpha$ -aryl ether bonds from its polyphenolic monomers, while hemicellulose dissolution and cellulose swelling are a consequence of hydrogen bond weakening [10]. Sodium hydroxide (NaOH) presents the greatest degradation compared to other alkalis [11,12]. Rodríguez-Vázquez et al. [11] used a NaOH solution to treat the pith component of sugarcane bagasse (0.2 g of NaOH per pith gram), obtaining a maximum digestibility of 71% at 92°C. In this work was used ambient temperature.

One of the disadvantages of utilized sugar cane bagasse in animal food is that this residue do not hight conten of proteins necessary for correct grown of all animals, for this work; ruminants. In the other hand here are residues with high content of proteins, which is possible to be approved in animal food. One of this is Transglutaminase residues.

Transglutaminases (TGases; protein-glutamine  $\gamma$ -glutamyltransferase) are a family of enzymes that catalyzes anacyl transfer reaction between the  $\gamma$ -carboxamide group of a peptide-bound glutaminy residue (acyl donors) and a variety of primary amines (acyl acceptors), including the amino group of lysine [13] In food processing, this enzyme has been used to promote polymerization of proteins, texture improvement, meat restructuring, gel formation, and to increase nutritional value of proteins as well [14].

The present paper deals with alkali pretreatment and transformation of sugarcane bagasse into an added value product, emphasizing on animal food production.

## DESARROLLO

### Alkaline Pre-treatment

The pre-treatment was applied in dry milled bagasse (0.5 mm), the alkaly agent was Hydroxidum of Sodium at 2 M. The alkaline pre- treatment was performed in order to increase the digestibility of bagasse by a solution of 2 molar sodium hydroxide through the method of spraying, during 10 minutes as a retention time. Prepare a honey - urea solution for neutralization of alkaline pretreatment Bagasse (BP) that will enhance the protein content of bagasse. The product obtained in this phase, is an excellent food maintenance; however, in this technology

it is supplemented of mycelial biomass from the production of transglutaminase, maralfalfa (*Pennisetum sp*) and fresh cane, both processed in a blade mill with sieves rotating at 300 rpm Also, it is including the addition of a premix of minerals.

### Experimental Formulation

In order to evaluate options for protein enrichment, different formulations were made on the basis bagasse pretreated alkali (BP), within such formulations BP1 diet was performed as protein supplement with corn, and sorghum which are conventionally used in livestock in the northern region of Tamaulipas. The protein content of this formulation was 12.43 g / 00 g of dry sample. To formulate the foods, the NRC recommendations were followed [15]

Table 1. Grams used for preparing BP1 sample.

Sample	Grams used for blending	Protein g/100 of sample
Alkaline pretreatment Bagase (BP)	60	NA
Sweet Corn	27.95	10
Sorghum	10.75	8.2
Salt	1.30	
Formulation BP1	NA	12.43

In BP2 formulation is used as protein supplement, Guacima (*Guazuma ulmifolia*) It is a forage, its leaves are impressive nutrient content. A sample from the Central America Containing 17% of protein, 26% fiber and 9% ash [ 16]. This foliage is consumed for cattle, horses, collared peccaries, and tapirs [17]. During periods of drought, animals consume leaves, because this a pleasant palatability and its high nutrient content.

In this formulation was obtained 12.56% of the protein dry base, comparable to the formulation made with conventional inputs (maize and sorghum), with these experiences we can assume that it is possible the used to unconventional ingredients as a protein. We can obtain diets, sustainable and economically.

A third diet (BP3) It was made having as support bagasse pretreated alkali (BP), in this diet, as protein supplement with a mycelial product of production of MTgasa enzyme, this formulation is novel and economically sustainable, as we turns waste into animal food, we approved to high protein content contained in this product. In this sense, this formulation was which protein content values of crude protein 18.8 g / 100 g sample.

### In vitro digestibility

The sample of ruminal fluid beef was donated by the National Postgraduate School of Texcoco city. The rumen fluid was obtained from a fistulated cow about two

years old weighing 500 kilograms. The two-step technique Tilley and Terry (1963) was used and first involves an incubation period of 48 hours in rumen microorganisms buffer means and, secondly, digestion with a mixture of hydrochloric acid - pepsin.

### MODELING IN DAILY WEIGHT GAIN

In order to visualize the daily weight gain of cattle, we was made in a theoretical way to model That would serve to observe the different weights That Could be expected With the three Control Points (integral bagasse , bagasse predigested Alkalineand formulation predigested BPS) Also was Compared to the Increase in weight by feeding cows With Buffel grass.

To carry out the model an initial weight of 300 kg cattle Were considered, because this food is designed to cattle finalized. Given Variables for the food is Assumed; 2% of body weight was Considered as food supply; In This context it was Assumed to varying water as a constant value ( 35 L), Which Indicates tha refer Lesur as normal consumption a range of 35- 45 L [ 18 ].

Also animal excreta, water and food were assumed. to determine the amount of non - digestible food and excreted, therefore esses and Took into account as the digestibility of food, subtracting one digestibility. to determine the amount of water excreted Considered was lost to 90 % of ingested water excreted in urine, perspiration, etc. Additionally, it subtracts an additional 2% due to water used in food remastication in order to bring the model to actual data, the health factor for Both variables Considered The equation for modeling was:

$$GPD = [y_i + (O. O2 y_{i-1})(F_{sal}) + a ] - [(O. O2)(1 - DIVMS)(F_{sal}) + (O. O2 * P1 ) + (O. O9 * a)] \text{ ec } 1$$

Where:

Y<sub>i</sub>= Initial weight of ruminant (300 for day 1 )

i = Fattening days (1 to 100)

a = Water (35 L)

DIVMS = In vitro dry matter digestibility (according to the food -evaluate)

C = ingested food calculated

P1 = Initial weight + ingested food calculated

The routine is performed in Microsoft Excel and the data were plotted in parallel Origin pro version 8.

### Scanning electron microscopy

Bagasse morphology was also analyzed by scanning electron microscopy before and after undergoing pretreatments. Sample imaging was carried out using scanning electron microscopes, models Quanta 3DFEG (SEM/FIB).It was working under vacuum conditions (10

to 130 Pa ) at 150 KV because the samples are an organic compound and the work distance of 10 mm ( Wd ).

Images were taken in the three control points, Untreated bagasse (BI), after alkaline pretreatment ( BPA ) and the final food, named bagasse predigested and supplemented (BPS).

### Infrared Spectroscopy with Fourier transformed (FT-IR)

A FT-IR spectrum is a fingerprint of the functional group and, for that reason, it is important to know the signs region material characteristics are expressed to study. DE900 to 1200cm.1 region corresponds to the zone of absorption of sugar cane bagasse [Jagdish]. This technique is performed on a Horiba brand 50BW and VON Labram Model HR80

### DISCUSIÓN Y ANÁLISIS DE RESULTADOS

SEM analysis were realized with the objective of view the Physical changes in the native and alkali pretreated sugarcane bagasse were observed by scanning electron microscopy (SEM).

It has been made a previous characterization of bagasse before alkaline pre-treatment in order to observe changes in its structure of the fibers. Are observed in the images 1.a and 1.c parenchymal tissue (bone ) perfectly stratified sample, with spongy appearance, this tissue is the cause of the pulp and paper industry, and the agglomerates, required his removal because of its non- fibrous nature and highly absorbent , which negatively affects the quality of the final product . In the picture 1.b fiber beam is observed with its transverse dimension; also, lignin residues are observed in the form of incrustations on the fiber bundle.

The image 1.c is an Amplifications of the fiber and the pith regions. The fiber surface is formed by parallel stripes.

Meanwhile, in the 2.a micrograph it is shown one thermoplastic channel, associated with adhesion of protein. In the other hand in the figure 2.b the fiber is partially covered with residual material attribute of lignin residues. In contrast, pith is a more fragile and fragmented structure containing pits, which are small pores connecting neighboring cells on the surface of the walls.

In dice sence the results are comparable with *Aissata Ousmane Kane et al [19]* these researchers carried out a combined liquid hot water and neutral/alkaline sulfonation pretreatments of SCB at relatively low temperatures to maximize enzymatic hydrolysis yields.

They found with techniques confocal laser scanning microscopy, and solid-state <sup>13</sup> C NMR spectroscopy analyses showed that the structural desorganization of bagasse after liquid hot water pretreatment associated to

chemical modifications of lignin and its massive removal promoted by alkaline sulfonation led to efficient separation of carbohydrates and lignin, revealing underlying structural reasons of the observed efficient enzymatic hydrolysis [19]

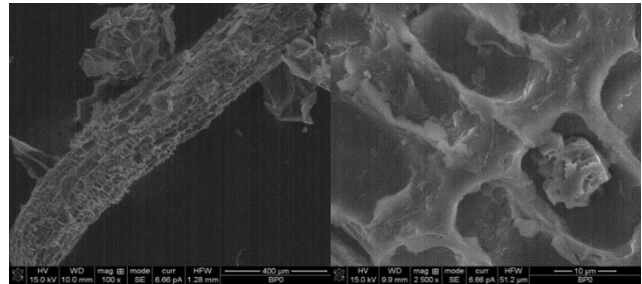


Figure 1. (a) ,(b),(c) General view of the sample showing fibers and pith (d) amplifications on the fiber and small fraction of pith.

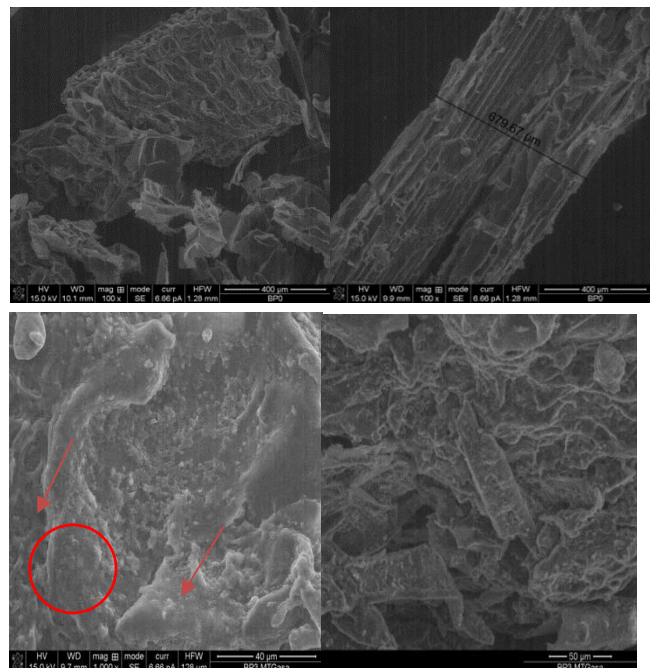


Figure 2. Alkali predigest bagasse (BPA), (a) thermoplastic channel, (b) Fiber with lignin residue. DIVMS

In the in vitro digestibility test DIIV was increased the digestibility of whole bagasse with 32 % compared to the Alkali pre - digested bagasse ( BPA ) with 60.33 % , the results are significantly higher than the “Estrella” grass ,with 51 % of digestibility.

The in vitro digestibility trials put on show a correct action by alkaline pre - treatment, hence is observed increase of 28 % digestibility in the bagasse after application of the pre - treatment. The food sample BPS meanwhile shows a 72 % digestibility (Table 2), in these sence the results of this research are comparable with other publications, *Zambrano et al* they elaborated four diets based on corn silage, corn grain, glycerol, soybean

cake and *sacha inchi* cake were evaluated as protein substituents for soybean cake, in four proportions (0,5,10 and 15%), obtaining in vitro digestibility results of 69.06%, 67.23%, 65.92% and 66.09% respectively. In the case of the final food BPS ( 72.30 % DIVMS), greater digestibility was obtained than diets made with *sacha inchi* [20].

Table 2. Results for the three sampling points of DIVMS.

Sample	%DIVMS
Raw bagasse (BI)	32.04 ± 1
Alkaline predigested bagasse (BPA)	60.33 ± 0.83
Bagasse predigested and supplemented (BPS)	72.30 ± 0.37
Buffel grass	56% ± 1

### MODELING IN DAILY WEIGHT GAIN

Four routines of weight gain was developed, described in Figures 3 the increase in weight is apparent, for the final formulation fed individuals were made; as compared to those fed integrated bagasse difference in weight gain by a period of 100 days is 351 kilos, in the other hand in the same conditions the routine of animals fed with “grass bufel” weight 439.6 kilos, which is equivalent to a gain of 200.5 kg for beef cattle fed with BPS formulation.

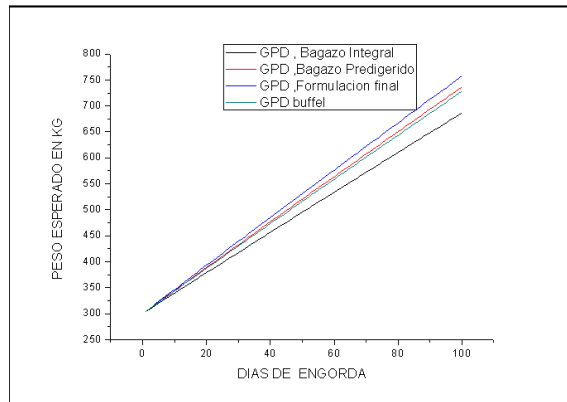


Figure 3. Graphic of modeling in daily weight gain.

The weight gain results simulated by the program are comparable with the experiments carried out by Pachon in 2005 where an experiment was proposed in which multi-nutritional blocks were made based on sugar cane bagasse. The experiment was carried out on a farm of a producer and 57 zebus steers were used with ages between 11 and 24 months and weights between 124 and 463 kilograms [21]. The animals were divided into two groups that were kept on unmanaged pastures. The experimental group was additionally offered multinutritional blocks. The animals were examined to check their physiological state, in order to determine possible health problems that could affect the trial. Weighings were carried out to measure weight gains in the two groups. The results showed average daily weight gains similar to  $Pr < 5\%$ , where those of the group fed

with the multinutritional blocks were slightly higher (0.848 vs. 0.769 kg animal/day).

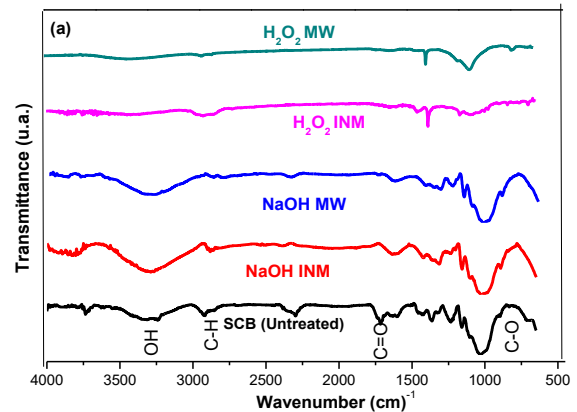


Figure 4. Spectrum of Infrared Spectroscopy with Fourier transformed.

FTIR analysis is used as important indicator of the chemical composition of the raw materials, (Figure 4). In the bagasse without treatment IR spectrum, is shown the typical fingerprint of cellulose, hemicellulose, and lignin compounds. The band centered at  $3270\text{ cm}^{-1}$  corresponded to O-H stretching vibrations of the absorptions of the alcoholic OH groups of polysaccharides and phenolic OH of lignin, which are forming hydrogen bonds inter- and intramolecular mainly vibrations valence in area of  $3400\text{-}330\text{ cm}^{-1}$  and dub vibrations of  $1000\text{-}1200\text{ cm}^{-1}$ . These signals are observed diminished in bagasse pre-digested (BPA), as far as I know you can assume a decrease of registered links lignin, and at  $2850\text{ cm}^{-1}$  appeared the C-H stretching vibration. It was observed that the signal at  $2260\text{ cm}^{-1}$  represents alkyne groups. The band at  $1750\text{ cm}^{-1}$  corresponded to the C=O stretching vibration band of polysaccharides, hemicellulose or ester linkages in lignin, hemicellulose, pectin. , Also , they appreciate absorption characteristics of C = O groups in the region the  $1730\text{ cm}^{-1}$  , primarily of ester groups of the lignin. The proximate composition of bagasse and the different feeding formulation is reported in Table 3. As result, the Alkaline pre-treatment bagasse (BP) contains low ligning as a raw bagasse and higher protein, in this sense, this bagasse will be applied as a maintenance food for cows. The protein content of the formulation BP1 (traditional supplements) was  $12.43\text{ g} / 100\text{ g}$  of dry sample. In the other hand the formulation supplemented with *Guazima ulmifolia* content  $12.56\text{ g}$  of protein /  $100\text{ g}$  of dry sample; However, the best formulation was BP3 with  $18.8\text{ g}$  protein /  $100\text{ g}$  of dry sample. Furthermore proximate composition of raw bagasse before mixing with protein supplements in diet 1 ( BP1 ) resulted low in protein. In fact, the diet 2 (BP2) was found to contain similar content of proteins.

Gomes et al. (2015), who evaluated the biodregation of in natura bagasse and hydrolyzed bagasse with sodium hydroxide by ruminal microorganisms of sheep and goats found that the digestibility in vitro increased 73.5% by alkaline treatment [22]. Although data relating to bromatological composition have not been subjected to statistical analysis, It appears that the content of neutral detergent fiber, acid detergent fiber and hemicellulose were drastically reduced by treating the bagasse with sodium hydroxide, which somehow justifies the increased digestibility of dry matter. For another hand, it appears that the crude protein content does not presented a high numerical difference, which, for on the other hand, it was statistically verified by Pires et al. (2006) [23]. Ahmadi et al. (2015), however, verified that alkaline hydrolysis (sodium hydroxide+quicklime) caused degradation of the protein fraction, beingthe protein content reduced by 28.1%, from 1.64% in fresh bagasse to 1.28% in hydrolyzed bagasse [24].

Table 3. Chemical composition of bagasse, vinasse and silages used in the feeding experiment (% on DM basis).

Item	Bagasse	BP	BP3
Dry matter	92.5	91.2	
Crude protein	4.2	6.1	18.8
Ether extract	1.7	1.8	
Ash	4	7.8	
FC	80.7	63.89	25.36
FDA	63.89	50.22	
Acid detergent lignin (ADL)	25.36	13.23	
Potassium (K)		1.31	2
Sodium (Na)		1.48	1.16
Magnesium (Mg)		0.12	0.26
Calcium (Ca)		0.52	1.15
Phosphorus (P)		0.11	0.10
Gross energy (kcal/kg)			2.2

## CONCLUSIONES

The alkaline pre- treatment based on a solution of NaOH at 12.5 % , with a retention time of 10 minutes, allowing the pre- digestion of the fibrous component of sugarcane

bagasse , showing the morphological changes and ruptures by MCBL in fiber texture , likewise, compositional analysis in a decrease of 12% lignin by delignification of NaOH is observed , the information being supplemented with significant percentage increase in digestibility DIIV.

The inclusion of non-conventional inputs such as grass “Maralfalfa” and MTGasa -subproduct of transglutaminase, represents a contribution to the development of a balanced food rich in protein (like flour), which in turn could help alleviate the impact of adverse weather conditions in the agricultural sector. It is expected in future research to test the food with increased digestibility in cattle, in order to compare it with the expected weight increase calculated by the program.

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