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Value of Sugarcane Stalks in the Diet of Small Ruminants: Comparison of Protein Enrichment with *Saccharomyces cerevisiae* Produced By Solid-State Fermentation versus Protein Supplementation with Soybean Meal

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Abstract

A trial was carried out on growing lambs to compare conventional protein supplementation of sugarcane with soybean meal with *Saccharomyces cerevisiae* enrichment produced by solid-state fermentation. Yeast enrichment enabled sugarcane stalks to increase their crude protein content from 4% to 12%. No difference was obtained between these two forms of supplementation on the daily growth of 80.2 and 76.8 g per day for enriched and non-enriched sugarcane respectively.

Keywords: Sugarcane; Protein enrichment; *Saccharomyces cerevisia*; Feed; Small ruminants

Introduction

Sugar cane is mainly known as an industrial crop for sugar production, but it is also a fodder crop. The latter can be used as the main ingredient source in ruminant breeding systems or as a standing fodder reserve (cut as needed) for the dry season. Sugarcane is the world's most productive forage, with more than 50 t dry matter/ha. However, whole sugarcane is an atypical forage, as it is rich in fibre and energy, but lacking in protein and glucose precursors [1, 2]. In practice, different fractions of the sugarcane can be used after chopping whole cane (stalk + leafy top), stalk, top [3]. Protein deficiency increases when stalks are eaten without leaves. Intake increase with the proportion of stalk in the diet but animal performance (growth) decrease. Moreover, the lower intake of sugarcane compared to other grasses also limits its feed value [4, 5]. Sugarcane is most often used fresh rather than dried because, unlike other grasses, it retains its feed value as it ages.

Sugarcane supplementation strategies have mainly focused on providing protein in the form of non-conventional ingredients (tree and shrub forage leaves, rice bran, peas, etc.) or conventional ingredients (soybean meal). The ease of use of non-conventional feeds and the rising price of conventional resources may limit their use. Protein enrichment with microorganism is rarely used as a supplementation strategy, even though it has its advantages. Microorganisms have the ability to upgrade low protein feed to high protein feed via solid state fermentation systems [6]. *Saccharomyces cerevisiae* is widely accepted due to its nutritional quality and to a long history of its use in traditional fermentation [7]

The aim of this study was to investigate the enrichment of sugarcane with *Saccharomyces cerevisiae* as an alternative to protein supplementation with protein-rich feeds. Enrichment was carried out with *Saccharomyces cerevisiae*, as this yeast plays a key role in industrial activities. It is used by man to produce beverages and fermented products such as wine, beer and bread. This yeast is readily available on the market.

Materials and Methods

Experimental Site

This research was carried out at the experimental animal station of National Research Institute for Agriculture, Food and the Environment (INRAE) of the French West Indies (Guadeloupe, latitude 16.16 N, longitude 61.30 W). The experimental unit has an Accreditation to experiment (n°A971802) and involved staff trained in experimentation and animal welfare. The protocol (APAFIS#5527-2016050608133139v2) has been validated by the Ministry of National Education, Higher Education and Research under the advice of the Animal Care and Use Committee of French West Indies and Guyana (N°069). The animals were cared in accordance with the guidelines and regulations for animal experimentation of the French Ministry of Agriculture.

Feeds

Three experimental diets were evaluated (Table 1): 1) a tropical natural grassland hay based on 10 weeks regrowth age *Dichanthium* spp. supplemented with a commercial concentrate. This is a control diet, classically used in intensive farming, the aim of which was to evaluate the growth potential of the animals and to rank the experimental sugar cane diets; 2) a crushed sugarcane stalk supplemented with soybean meal; 3) a crushed sugarcane stalk enriched with Saccharomyces cerevisiae. Sugarcane-based diets were isoproteinic. The *Dichanthium* spp. was the control diet that should enable the animals to express their growth potential. The diets (roughage) were distributed ad libitum. The amounts of roughage refused have been set at between 20% and 30% of the amounts proposed.

Table 1: Diet composition

	Diets		
Ingredient	Hay	Sugarcane	SugarcaneEnriched
Hay (g)	570		
Sugarcane (g)		500	500
Sugarcane enriched (g)			
Molasses (g)		150	150
Soybean meal (g)		200	70
Commercial concentrate (g)	270		
	Composition (%)		
Organic Matter	91.9	93.2	94.8
Crude Protein	9.7	14.0	13.4
Neutral Detergent Fibre	44.1	53.5	53.5
Acid Detergent Fibre	39.0	35.3	35,3

The feed was prepared over a 10-week period. The sugarcane stalks were harvested once a week at the mature stage (12 months). They were crushed in a plant shredder equipped with blades and hammer. Particle size at the mill outlet varied between 2 and 100 mm.

Half of the production (400 kg of raw product) was dried in a greenhouse for 3 to 5 days, turning several times a day to avoid fermentation. The other half of the crushed sugarcane was enriched in protein by yeast culture in aerobiosis in 4 bioreactors (concrete tanks treated with food-grade paint), each with a capacity of 100 kg. Enrichment consisted in sequentially adding sugarcane (100 kg) and urea (350 g) into an inoculum (2.5 L warm water (37°C), 1 g of commercial baker's yeast under active dry yeast form, Saccharomyces cerevisiae, 7,5 g molasses). The feed was stirred regularly by five-minute rotation cycles of the concrete mixer (electric programming). Each rotation cycle was followed by a 10-minute rest period.

Animals and Trials

Two trials were conducted: a fattening trial involving 30 female Blackbelly lambs aged 4 months was followed by a digestibility trial involving 18 of the female lambs used in the fattening trial. The animals came from INRAE's experimental farm. The mean body weight of the rams was 18.9 (±1.59) kg at the beginning of the experiment.

The fattening trial lasted seven weeks consisting of 21 days of diet adaptation followed by 28 days growth measurement. Lambs were kept in individual floor cages during the growth trial. Rams were fed twice a day, at 10-h intervals (07.00 and 17.00 h). Lambs had free access to water and salt blocks. Salt block composition was as follows (g/kg): Ca (60.0), P (20.0), Mg (10.0), Na (280.0), Zn (17.5), Mn (5.5), Fe (1.5), I (0.03), Co (0.03) and Se (0.01). The amounts of feed offered, feed refused and water intake were weighed daily.

During the digestibility trial, the animals were maintained in metabolism cages. The amounts of feed offered, feed refused, faeces and urine excreted were weighed daily for 5 consecutive days. Urine was collected in 10-L drums containing sulphuric acid (2.5 mL of 10% H2SO4 per 100 mL urine). Daily samples of feed offered, feed refused, faeces and urine were taken. They were then pooled to form representative samples for the 5 days of measurement, in preparation for laboratory analysis.

Measurements and Calculations

During the fattening trial, lambs were individually weighed at the beginning and end of growth measurement. Daily feed intake was estimated as the difference between feed offered and feed refused. Daily growth was estimated by weight gain divided by the number of measurement days (28 days). Consumption index (kg) recorded during this period were estimated as: feed intake/ weight gain (kg). Energy and protein values were estimated using INRAE feed value tables. For the particular case of enriched sugarcane, we assumed that the protein gain was due exclusively to the growth of yeast composed of 50% protein.

During the digestibility trial, the lamb intake and apparent digestibility were determined by daily weighing of the amounts of diet offered and refused, and of faeces over five consecutive days. N retention was estimate as: (N intake) - (N urinary loss + N faecal loss)

Chemical Analyses and Analytical Procedures

The dry matter contents of forage, refusals and faeces were determined by drying in a forced-draught oven at 60 °C until constant weight was attained. The samples were used for chemical analyses. Before, they were milled through a 1-mm screen (Reich hammer mill, Haan, Germany) prior to analysis. Organic matter (OM) and N analyses were performed according to AOAC (1990) methods 923.3 and 992.15, respectively. Lyophilised faecal samples were analysed for ash and N, and N was analysed in fresh samples of urine using the same methods as used for diets. Crude protein (CP) was estimated as N × 6.25. Cell wall components (neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL)) in diet and faecal samples were determined as described by [8] using a sequential procedure 200.04 (NDF) and 973.18 (ADF + ADL) [9].

Statistical Analysis

Statistical analyses were performed on mean intake (feed, water), Average Daily Growth (ADG) and consumption index and recorded during the growth trial.

Concerning the digestibility trial, means were calculated over 5 days of measurement for intake, total tract digestibility and N balance.

Statistical analyses were performed using the MIXED procedure of SAS 9.2 release (SAS 2008). Diet was designated as fixed effects; animal was a random effect. Significance was declared at probability levels of 5%.

Results and Discussion

Protein Enrichment Results

The enriched sugarcane used during the experiment had an average Crude Protein content of 12% compared with an initial value of 4.2 % for unenriched cane. Protein enrichment has enabled sugarcane stalks to become a high-value tropical grass, based on their protein content [10]. The final CP content of the sugar cane stalk is probably close to that of true protein, as soluble nitrogen is removed by washing the feed before dosing. This protein gain corresponds to a 300% increase in the initial value which corresponds to the highest values achieved by this solid-phase enrichment technique. In a study on protein enrichment of potato peels using *Saccharomyces cerevisiae* via solid-state fermentation process, Maxwell et al. [11] recorded an increase from 12.5% to 21.9% and 12.5% to 18.42% with ammonium sulphate and urea supplementation respectively. In a trial of protein enrichment of sugarcane by-products (sugar cane trash blended with salts and diluted molasses) using solid-state cultures of *Aspergillus terreus* [12], satisfactory protein content (9-10% dry matter) and protein yield (23% of spent sugars) have been recorded. Compared to conventional rich protein-crops used as feed, the production of microbial biomass has several advantages. Microorganisms have a high

protein content on a dry mass basis (30–80%) and good nutritional value for these proteins. The productivity of microorganisms is high due to their high growth rate and their short doubling time (algae and molds, 2–6 h; bacteria and yeasts, 0.33–2 h) [13]. Protein content varies with microorganism. Among those used for solid-state fermentation, microalgae have the highest protein content (60–70% dry matter), followed by bacteria (30–80% dry matter), yeasts (30–50% dry matter) and protists (10–20% dry matter) [14].

Growth Trial

The main results of the fattening trial are summarized in Table 2. The recorded intakes were significantly lower with the control diet and higher with the unenriched sugarcane diet ($P < 0.05$). In the case of the Diet control, the average hay intake was very low at 30 g/$LW^{0.75}$/day. This intake is very low compared with the 45 to 50 g expected for such a late age of forage regrowth[10]. This result could be partly explained by the depressive effect of concentrate intake on forage consumption [15]. In addition, the very high fill value of the forage limited its intake [16]. Sugarcane intakes were similar for sugar-based diets, an average of 51 and 54 g for unenriched and enriched sugarcane respectively. These values are within the order of magnitude recorded for this forage, known for its high fill value [2].

Table 2: Effect of diet on growth performance (LSmeans[1])

	Diet				
Trait	Hay	Sugarcane	Enriched Sugarcane	RSD	Significant effects[2]
Number of animals	10	10	10		
Initial BW (kg)	20.2[a]	18.9[a]	17.6[a]	2.9	
Final BW (kg)	23.0[a]	21.1[ab]	19.7[b]	2.6	D[t]
DM intake (g/d)	589.6[a]	855.3[b]	663.3[a]	65.4	D***
DM intake (g/$LW^{0.75}$)	59.1[a]	91.8[b]	74.2[c]	10.7	D***
Water intake (g/d)	1686.8[a]	1395.2[a]	1756.3[a]	799.8	
ADG (g/d)	98.8[a]	80.2[a]	76.8[a]	26.1	
IC	6.3[a]	11.8[b]	9.6[ab]	4.0	D**
Energy (UF)/ day	0.52	0.66	0.55		Untested
Protein (PDI)/day	57.6	78.8	58.2		Untested

[1]LSmeans followed by different letters significantly differed within a row ($P < 0.05$)

[2]t$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

The relatively low intake of the control diet explains the relatively low growth of the animals compared with their potential, which is around 150 g/day [15]. The low intake of the control diet could be explained by the poor quality of the hay, contrary to the protocol's programming. Climatic constraints leading in a late grass harvest were at the origin of this discrepancy. The nutritional value of the control diet was nevertheless good, given the low consumption index recorded.

The daily growth rates recorded are at the lower end of the range of variation (80 to 180 g) to those observed with sugarcane-based diets consumed by sheep with growth potentials similar to those used in this experiment [5]. However, the daily growths recorded are in line with those permitted by the energy and protein intakes of the diets (INRA, 2018). The comparison of the 2 sugarcane diets shows that substituting yeast for soybean meal did not penalize animal growth or feed conversion.

This trial was conducted with crushed sugarcane stalks rather than whole sugarcane. Cattle trials indicate that intake and growth increase with the proportion of top in the cane. In contrast, feed conversion became worse as the proportion of tops in the ration increased [3]. The choice to work with stalks was motivated by the desire to increase the amount of sugar available for microbial growth. In addition to the disadvantages outlined above, this scenario has the drawback of excluding part of the biomass produced (20%). It would therefore be relevant to study the protein enrichment of whole cane and its valorisation by ruminants.

Total Tract Digestibility Trial

The main results of the digestibility trial are presented in Table 3 and 4. The digestibility values of the dry and organic matter in the diets are relatively high for this type of diet due to the significant presence of concentrate. Hay, unenriched sugarcane and enriched sugarcane rations contained respectively 34%, 42% and 30% concentrate (commercial concentrate, soybean meal and molasses, soybean meal and molasses), on a dry matter intake basis. The low digestibility of the hay, due to its late regrowth age, was partially compensated for by the concentrate [10, 15].

There were no differences, or only slight ones, between the different diets for the digestion of dry matter, organic matter, NDF and ADF. The digestibility of sugarcane-based diets is comparable to that generally observed for this type of diet [17]. DM digestibility of whole cane or cane stalks fluctuates around 60% [2]. The evaluation of energy and protein intake with digestibility data is similar to that obtained with table-based predictions and in line with observed growth. The nitrogen balance (Table 4), with in particular the lowest quantity of nitrogen retained with the control diet, is consistent with the lowest growth observed.

Table 3: Effect of diet on digestibility parameters (LSmeans[1])

	Diet				
Trait	Hay	Sugarcane	Enriched Sugarcane	RSD	Significant effects[2]
Number of animals	6	6	6		
Intake					
Dry Matter (g/LW$^{0.75}$)	74.6^{a}	82.3^{b}	75.2^{a}	6.3	D^{t}
Dry Matter (g/d)	853.7^{a}	825.3^{a}	715.6^{b}	42.1	D***
Organic Matter (g/d)	784.3^{a}	769.4^{a}	678.5^{b}	38.9	D***
Crude Protein (g/d)	83.1^{a}	115.8^{b}	95.8^{c}	3.6	D***
Neutral Detergent Fibre (g/d)	486.5^{a}	364.3^{b}	383.2^{b}	30.5	D***
Acid Detergent Fibre (g/d)	255.3^{a}	321.8^{b}	252.5^{a}	21.8	D***
Energy (UF)/ day	0.47	0.48	0.40		Untested
Protein (PDI)/day	60.3	67.0	55.0		Untested
Total tract digestibility					
Dry Matter	64.8^{a}	70.5^{b}	68.2^{ab}	3.9	D*
Organic Matter	68.2^{a}	71.8^{a}	69.4^{a}	3.7	
Crude Protein	61.0^{a}	69.6^{b}	64.9^{c}	3.1	D**
Neutral Detergent Fibre	64.6^{a}	59.9^{a}	63.0^{a}	3.8	

Acid Detergent Fibre	61.1[a]	70.9[b]	62.8[a]	4.2	D**

[1]LSmeans followed by different letters significantly differed within a row (P < 0.05)

[2]tP < 0.10; *P < 0.05; **P < 0.01; ***P < 0.001.

Table 4: Effect of diet on Nitrogen balance (LSmeans[1])

	Diet				
Trait	Hay	Sugarcane	Enriched Sugarcane	RSD	Significant effects[2]
Number of animals	6	6	6		
Nitrogen intake (g/d)	13.3[a]	18.5[b]	15.3[c]	0.6	D***
Faecal nitrogen (g/d)	5.2[a]	5.6[a]	5.4[a]	0.7	
Urinary nitrogen (g/d)	1.7[a]	3.9[b]	2.1[a]	0.8	D***
Nitrogen retained (g/d)	6.4[a]	8.9[b]	7.9[c]	1.2	D***
Nitrogen retained (% intake)	0.48[a]	0.48[a]	0.51[a]	0.005	

[1]LSmeans followed by different letters significantly differed within a row (P < 0.05)

[2]tP < 0.10; *P < 0.05; **P < 0.01; ***P < 0.001.

Conclusions

Sugarcane protein enrichment with yeast, Sacharomyces cerevisiae, resulted in a 3-fold increase in the protein content of sugarcane stalks. The feed value of diets based on enriched sugar cane was similar to that obtained with soybean meal. Further trials could be carried out to test the enrichment of whole cane sugarcane, more commonly used for livestock feed.

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