Elaboration of a feed based on the solid state fermentation of sugarcane and with different levels of zeolites

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In order to evaluate the efficiency in the synthesis of microbial protein in a feed based on sugarcane with different levels of Mexican zeolite (Clinoptilolita calcica), an experiment was designed with ground stems of the Mex69-290 sugarcane cultivar, of twelve months of age. Urea (1.5 %), ammonium sulfate (0.3 %), mineral salts (0.5 %), and inoculum of lactobacilli (Vitafert) (10 %) were mixed homogeneously. Ten kilograms of the mixture were spread on the floor, with layer thickness of 10 cm. The mixture was subject to constant aeration and it was fermented under shade. A complete random design was used with factorial fit, four levels of zeolite (0, 1, 2, 3 %) and five fermentation times (0, 24, 48, 72, 96 h), with four repetitions per treatment. Nutritional variables were measured: dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), digestibility and fermentation variables, pH, NH_3, acetic, propionic, butyric, and lactic acids, and the temperature. The CP at 24 h was from 16 to 21%, according to the treatment, and it decreased at the end of the fermentation. There was increasing synthesis of microbial protein, not having effect due to the addition of zeolite. The digestibility was of 50 to 60 %, without any effect due to the zeolite addition. The DM values were of 37 and 44 %, at 72 y 96 h, respectively. At the beginning, the pH was acid, and, at the end, alkaline, due to the production of lactic acid, volatile fatty acids, and ammonium. The maximum temperature was of 45 ºC at 24 h. It was concluded that the zeolite had no effect on the production of microbial protein. The process of sugarcane fermentation in solid state increased the synthesis of microbial protein. After 48 h, the pH was alkaline, and at 24 h it reached the maximum temperature.

Key words: *lactobacilli cultura, sugarcane feed, protein synthesis, fermentation*.

Sugarcane is a perennial grass that has been traditionally used for sugar production. It is considered an important forage resource to fulfill the shortness of pastures during the drought and under adverse climates. This plant has great biomass production per surface unit (Rincón 2005). However, it has nutritional deficiencies, low content of protein and minerals, and slow fiber degradation (Martín 2004).

The fermentation processes permit increasing the nutritive value of some agricultural products and they also represent new alternatives to animal feeding. Elías *et al*. (1990) produced a protein feed named Saccharina, based on sugarcane, and obtained from solid state fermentation (SSF) (98 % of ground sugarcane stems, 1.5 % urea, and 0.5 % minerals). During the SSF process, the epiphyte microorganisms present in the sugarcane stems (Valiño *et al*. 2002) use the soluble carbohydrates as energy source to convert, through metabolic reactions, the non-protein nitrogen (NPN) present in the urea into protein (PN).

The zeolites are minerales that have clay structures and work chemically as ion exchangers. These zeolites can catch the NPN of the urea, and later release it gradually. In a way that the microorganisms present during the SSF process can use it more efficiently in the synthesis of microbial protein.

The objective of this work was to evaluate different ratios of Mexican zeolite (Clinoptilolita calcica) and different fermentation times in the synthesis of microbial protein in a feed based on sugarcane.

Materials and Methods

The work was conducted in the facilities of the Tabasco Campus, College of Postgraduates. This campus is located at the km 3.5 of the Carlos A. Molina Periphery, in Cárdenas, Tabasco, Mexico. The annual average temperature is of 25.9 º C and the average relative humidity, of 80 %, with maximum of 90 %, and minimum of 65 %.

The feed was elaborated with clean sugarcane stems (without leaves, without straw and without the tops). The cultivar of Mex69-290 sugarcane was used at twelve months of age. The stems were ground with 2 mm particle size. They were added 1.5 % urea, 0.3 % ammonium sulfate, 0.5 % mineral salts, and 10 % lactobacilli culture obtained by liquid fermentation (Vitafert). Zeolite (Clinoptilolita calcica) (ZEO-SIL, commercial brand) was added according to the treatments. All the components were mixed homogenously. Ten kilograms of the total mixture were used as experimental unit, with 10-cm layer thickness. Each experimental unit was spread on a cement floor under shade, with the aim of having good aeration. The humidity content was between 65 and 70 %.

A complete random design was used with 4 x 5 factorial fit; four zeolite levels (0, 1, 2 and 3 %), five fermentation times (0, 24, 48, 72 and 96 h), and four repetitions per treatment. The principal effects and the interactions with the GLM software of SAS were analyzed (SAS 2003). The comparison between means

was performed according to Tukey (Steel and Torrie 1980).

The bromatological variables dry matter (DM) and crude protein (CP) were measured according to AOAC (2000). The true protein (TP) was determined according to Jacobs (1965), the neutral detergent fiber (NDF), according to van Söest *et al*. (1991), and the *in situ* digestibility of dry matter, according to Mehrez *et al*. (1977). Also, the fermentation variables were evaluated: temperature by thermometer, pH through potentiometer and ammonia by the method of indophenols (McCullough 1967). The total reducers, the lactic acid and the VFA were determined according to Edwin *et al*. (1961). The Brix degrees were calculated with an Atago brixometer.

Results and Discussion

In respect to the bromatological variables, there was interaction ($P < 0.01$) in the content of CP of the feed fermented with sugarcane with different levels of zeolites and at different fermentation times. At 24 h of fermentation, there was increment in the CP content compared with the 0 hour. Nevertheless, at 48 h, the content of CP diminished in the treatments under study. The lowest values of CP were found at 96 h (figure 1). The lowest values of CP, at 48, 72, and 96 h, were probably due to the volatilization of the NPN from the urea during the fermentation, due to the highest pH values.

There was interaction in the TP content from the feed elaborated with sugarcane. The highest TP values were found at 48 h of fermentation (figure 2). According to Pandey *et al*. (2001), the TP can be an indirect form of measuring the microbial growth in the SSF processes, because the microbiota established in the system transforms the NPN from the urea into PN. Ramos *et al*. (2007), Rodríguez *et al*. (2007) and Rodríguez *et al* (2010) reported similar values of TP in the fermented sugarcane and in manzarin.

Table 1. Effect of the zeolite levels and the fermentation time on the content of DM and NDF of a feed based on sugarcane

Factors	DM, %	NDF, %
Zeolite level, %		
0	36.27	77.79^{a}
1	36.26	74.05^{ab}
2	36.66	74.46^{ab}
3	36.70	73.49^{b}
Standard error	0.14	0.51
Fermentation time, h		
0	32.42^{c}	63.16^{d}
24	33.74^{c}	69.08^{c}
48	33.54^{c}	74.66^{b}
72	37.76^{b}	78.70^{b}
96	44.89^{a}	89.13^{a}
Standard error	0.16	0.56
Zeolite level	0.673	≤0.0421
Fermentation time	≤0.0001	≤0.0001
Zeolite level * Fermentation time	0.095	0.6871

^{abcd}Different letters in the columns are different ($P < 0.0001$)

There was interaction in the dry matter digestibility, and there were not differences by the zeolite effect ($P > 0.05$) (figure 3).

In regards to the DM of the feed fermented with sugarcane, there was not effect on the zeolite levels, but on the fermentation times ($P < 0.0001$). The lowest DM values were found at 0, 24, and 48 h, without differences between them. The intermediate value was obtained at 72 h, and the highest at 96 h (table 1). Possible, there has been effect of the aeration during the SSF process of the Saccharina, because the DM values were superior to those of Elías *et al*. (1990), although these authors made the SSF in Roux bottle at the laboratory level.

In respect to the cell walls (table 1), 3 % of zeolite produced reducing effect, although it was favorable from the nutritional point of view. The fermentation time increased significantly the content of cell walls ($P < 0.01$). These values were superior to those of Ramos *et al*. (2006), with the feeds sacchapulido, sacchamaiz, sacchasorgo, and sacchacitricos. This low content can be related to the high content of starch from these feeds.

In respect to the fermentative variables, there was interaction in the concentration of lactic acid in the Saccharina. The highest concentrations were at 24 h of fermentation, with 3 % zeolite (figure 4).

There was interaction in the temperature of the Saccharina. At 24 h of fermentation it was increased compared with the 0 h, when it reached values of 45 °C. At 48 h of fermentation it diminished and later it was stabilized (figure 5). The rise in the temperature at 24 h of fermentation could be due to the accumulation of metabolic heat derived from the microbial activity. Lezcano and Martí (1997) noted that the temperature of 32-34 ºC, was the most appropriate to conduct an efficient fermentation.

The zeolite level did not provoke any effect on the pH, but the pH was increased at higher fermentation time. The highest values were at 48, 72, and 96 h (table 2). The increment in the pH was related to the rise in the ammonia concentration, regardless there was also rise in lactic acid. The values of pH at 24 h were similar to those of Ramos *et al*. (2007), when elaborating Saccharina with sorghum and molasses.

It was concluded that the zeolite (Clinoptilolita calcica) had no effect on the production of microbial protein but the process of sugarcane fermentation in solid state increased the synthesis of microbial protein. After 48 h, it had alkaline pH, and at 24 h it reached the maximum temperature.

Table 2. Effect of the zeolite levels and the fermentation time on the pH and the concentration of ammonia and volatile fatty acids of a feed based on sugarcane.

Factors	pH	NH_3	Acetic acid	Propionic acid	Butyric acid
Zeolite level, %					
0	6.6	1.77	29.079	0.0001	3.246[b]
1	6.78	1.95	34.194	0.0001	4.932[ab]
2	6.79	1.45	32.062	0.0002	4.908[ab]
3	6.56	1.58	39.568	0.0002	7.718[a]
Standard error	0.039	0.159	2.59	0.0	0.496
Fermentation time, h					
0	4.91[c]	1.32[b]	16.95[b]	0.0001	6.997[a]
24	5.95[b]	1.71[ab]	15.11[b]	0.0001	11.282[a]
48	7.39[a]	3.00[a]	18.66[a]	0.0000	7.299[a]
72	7.78[a]	1.70[ab]	13.48[b]	0.0003	0.029[b]
96	7.39[a]	0.72[b]	9.43[b]	0.0001	0.399[b]
Standard error	0.045	0.161	2.793	0.0	0.511
Zeolite level	0.151	0.7328	0.6058	0.9176	≤0.042
Fermentation time	≤0.0001	≤0.0014	≤0.0001	0.5696	≤0.0001
Zeolite level * Fermentation time	0.097	0.997	0.9973	≤0.4827	≤0.0367

[abc]Different letters in the same column are different ($P < 0.01$)

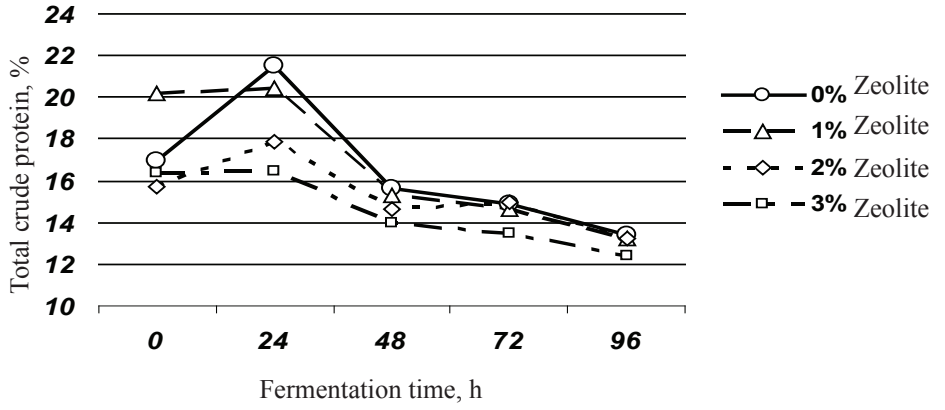

Figure 1. Effect of the zeolite levels and the fermentation time on the content of crude protein of a feed based on sugarcane

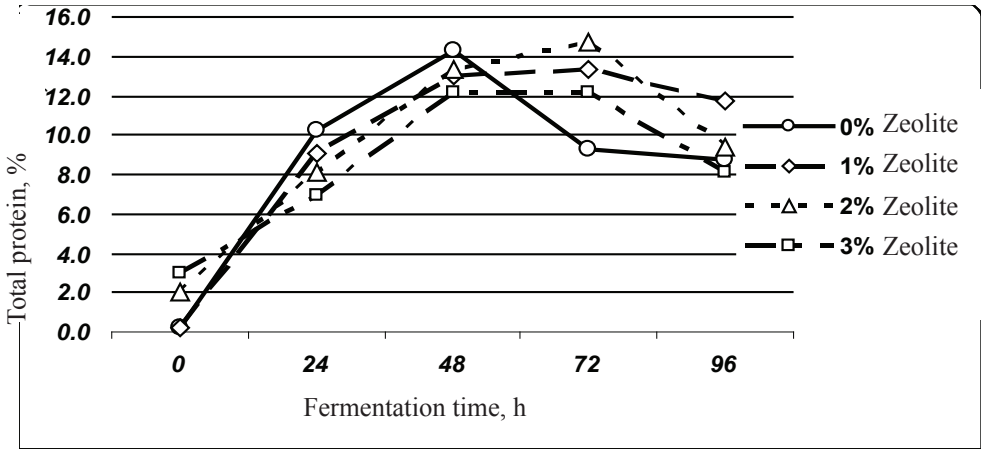

Figure 2. Effect of the zeolite levels and the fermentation time on the content of true protein from a feed based on sugarcane

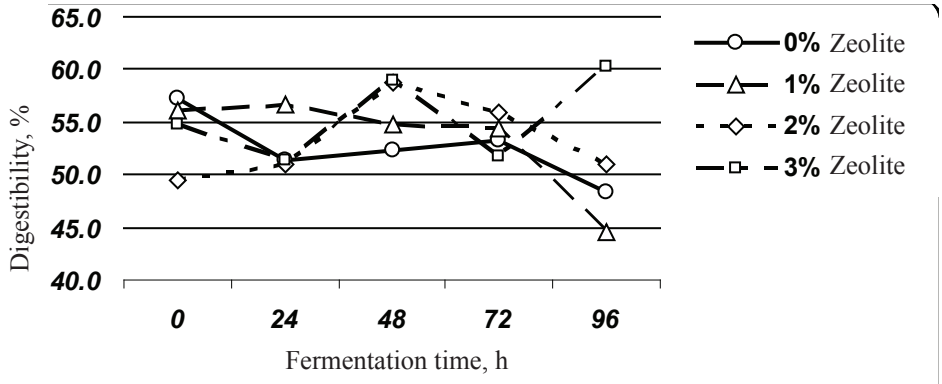

Figure 3. Effect of the zeolite levels and the fermentation time on the digestibility of a feed based on sugarcane

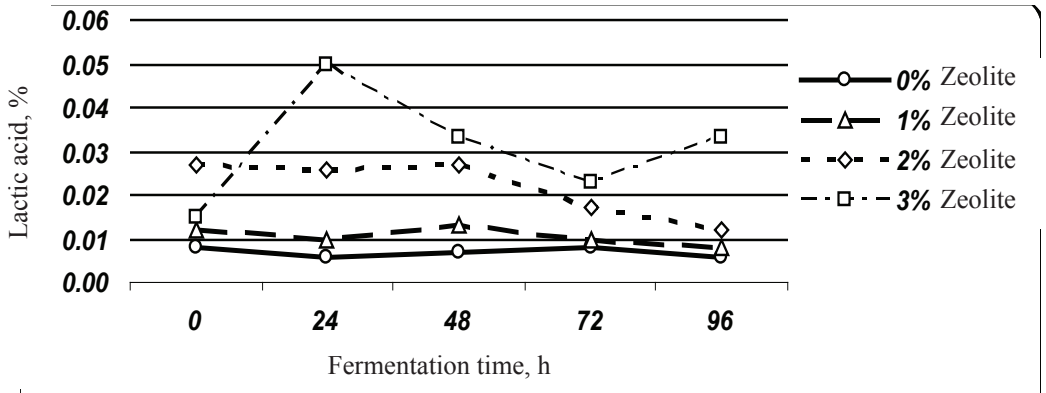

Figure 4. Effect of the zeolite levels and the fermentation time on the content of lactic acid from a feed based on sugarcane

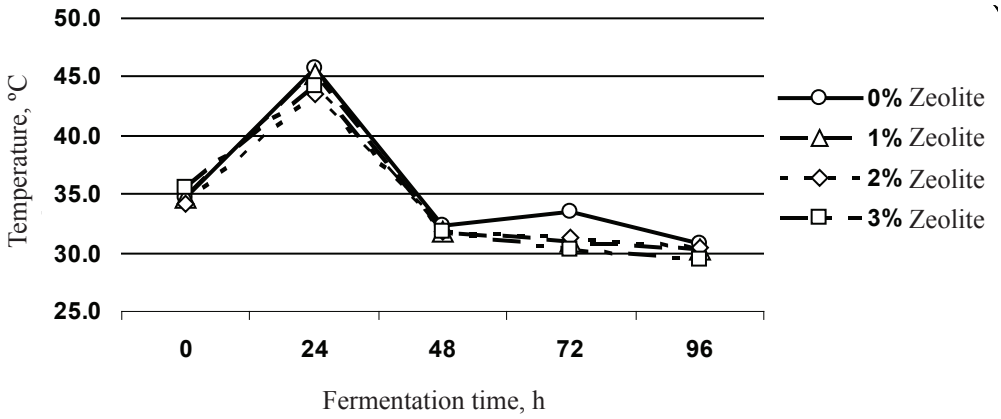

Figure 5. Effect of the zeolite levels and the fermentation time on the temperature of a feed based on sugarcane

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