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PROGRESS REPORT, JANUARY - JUNE 1989, AID PROJECT NO. C7-043

Title: Nutritional Upgrading of Lignocelluloses by Ozonation.

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RESEARCH ACTIVITIES AND RESULTS

Honduran copartner

In Honduras, a 20 ton silage consisting of fresh Banana plus poultry litter (PL), 1:1 on a dry matter (DM) basis, was prepared, kept covered for 3 months and then used for a preliminary feeding study with dairy cows. The chemical composition of the Banana plus PL silage is given in Table 1.

Table 1. Chemical composition of the Banana+poultry litter silage.

Component	g/100g DM
Organic matter	71.9
Fat	3.29
Crude fiber	16.3
Crude protein	15.1
Phosphorus	5.46
Calcium	1.93

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Ten Holstein cows, 3 years old in parturition period, were selected for the preliminary feeding study. Prior to the experimental period of one month, all cows had received a basal diet consisting of 5.5 Kg concentrate mixture plus fresh King

grass fed ad libitum. The experimental group consisted of five cows adapted to replace concentrate by Banana + PL silage, and of ad lib fed King grass. The control group consisted of five cows receiving the basal diet. Table 2 presents the changes in the quantities of diet components fed during the experimental period of 4 weeks, and table 3 presents the milk production of the two experimental groups of dairy cows.

Table 2. Mean quantities of diet components (kg DM/cow/day) fed to the experimental and control groups of dairy cows.

Week of experiment	Control group		Experimental group.	
	Banana+PL silage	Concentrate mix	Banana+PL silage	Concentrate mix
First	-	4.90	2.23	4.90
Second	-	4.90	4.22	3.11
Third	-	4.18	6.00	0.98
Fourth	-	4.22	6.00	0.82

Table 3. Mean quantities of milk (liters/cow/day) produced by the control and experimental groups of dairy cows.

Weeks of experiment	Control group	Experimental group
First	15.4	14.3
Second	14.4	13.7
Third	13.9	11.5
Fourth	13.7	10.9

The results of this preliminary milk production study show that 6 kg DM of silage, based on local recycled materials such as Banana

and poultry litter can replace about 3.5 kg DM of concentrate mix with slight reduction only in milk production.

It should be bear in mind that the Banana+PL silage used in this preliminary study was of low quality containing 26% ash on a DM basis, and this is probably the reason for the slight decrease in production in the third and fourth weeks. However, a new silo with better ingredients will be prepared in the coming months to assess theses results.

### Research Activities in Israel

Based on the in vitro studies of the first year, cotton stalks which had the best response to the ozone treatment, were selected to be the dicotyledonous lignocellulose feedstuff model for the metabolism and growth studies in Israel.

Cotton stalks were harvested from a commercial field and chopped to 10 mm pieces. 140 kg of the material was treated with ozone gas as described in the first year report. The final pH of the ozonated material was 2.

Metabolism study with the cotton stalks

Diets: the ozonated cotton stalks (OCS) were enriched with ammonium hydroxide solution to reach 38.4% crude protein (CP) on a dry matter basis, and pH 6 with no losses of ammonia. Two equinitrogenous dietary treatments were formulated: 1.  $\text{NH}_3$  enriched OCS which provided 30% of the ration DM plus a concentrate mixture consist of corn and minerals+vitamins mix, contributed the remainder 70% of the diet; 2. A control diet consisted of 30% untreated cotton stalks plus 70% concentrate mixture consisted of corn, soya and minerals+vitamins mix. The composition of the two dietary treatments are presented in table 4.

Table 4. The ingredients of dietary treatments provided in the metabolism study (g DM/sheep/day)

Ingredients	Dietary treatments	
	OCS	untreated cotton stalks
Ozonated cotton stalks (OCS)*	232	-
untreated cotton stalks	-	203
corn grains	401	233
Mineral+vitamins mix	41	41
Soya bean meal	-	182
Total	674	659

\* Enriched with ammonium hydroxide solution to reach 38.4% CP on a DM basis.

Experimental procedures: 4 marino rams cannulated in the rumen (simple cannula) and in the proximal duodenum and terminal ileum (T shaped cannulas) were allocated per treatment in a change over design. The animals were kept in metabolism cages equipped with automatic feeders and free water supply. The cages were located in an air conditioned animal house. Each experimental period consisted of the following activities: an adaptation period of 17 days followed by 10 days dedicated to total collection of feces and urine and sampling of rumen duodenal and ileal digesta. From the first day of this period, Cr<sub>2</sub>O<sub>3</sub> impregnated paper was given twice daily via the rumen cannula at 0800 and 2000 hours. During the last 4 days, rumen liquor, duodenal and ileal digesta were sampled 4 times per day at different intervals. The pH of rumen liquor was measured immediately after sampling. 10 ml of rumen liquor and 10 ml of rumen liquor plus 10 ml HCl 1N, from each rumen sampling were separately pooled in one composite sample per animal and stored at -18°C until analysed for VFA and NH<sub>3</sub> concentration respectively. Duodenal and ileal digesta (25ml) samples were pooled to provide one composite sample per animal and

stored at -18°C until analysed for DM, OM and total N. About 150 ml of duodenal and ileal digesta was freeze dried until analysed for Cr<sub>2</sub>O<sub>3</sub>, CW, amino acids and monosaccharide residues.

Analytical procedures: samples of food and feces were analysed for dry matter by oven drying at 105°C over night, and for organic matter by ashing in a muffle furnace at 600°C for 2 hours. Neutral detergent fiber (NDF) in feeds was determined as described by Goering and Van Soest (1970). Total N in feeds was determined by Kjeldahl method. Preliminary data regarding chemical composition of the feeds are presented in table 5.

Table 5. Chemical composition (g/100g DM) of the feeds.

	ozonated* cotton stalks	cotton stalks	corn	soya
Dry matter	53.3	88.3	87.3	87.5
Organic matter	92.3	94.6	98.4	93.2
NDF	51.0	74.0	11.2	11.4
Crude protein	38.4	5.54	9.72	46.9

\* Enriched with ammonium hydroxide solution to reach 38.4% CP on a DM basis.

## Results

Preliminary data regarding the daily intake of dry matter and organic matter of the experimental diets, the digestibilities in the entire tract and the calculated digestibility of the cotton stalks component are presented in table 6.

The ozone treatment reduced the NDF content of the cotton stalks from 74% to 51% (table 5), converting most of the lignin and part of the hemicellulose components of the cell wall into cell solubles. These changes in composition were reflected in an increase of 12 percent units in the OCS diet (table 6). The effect of ozonation on the nutritional value of cotton stalks is well illustrated in the increase of calculated OM digestion values of the cotton component: from 28.6% in the untreated cotton stalks up to 68.6% in the ozonated cotton stalks. These results demonstrates

the potential of OCS to serve as an available energy source for ruminants. However, the potential of this OCS product to supply absorbable amino acids and monosaccharides to the animal needs further clarification, and will be examined in the coming months.

Table 6. Amounts (g/day) of DM and OM present in the feed and excreted in the feces of rams fed the experimental diets.

	D R Y    M A T T E R			O R G A N I C    M A T T E R		
	Treatments			Treatments		
	OCS	cotton stalks	SEM	OCS	cotton stalks	SEM
Intake						
Cotton stalks	232	203		214	192	
Concentrates	442	456		395	413	
Total	674	659		609	605	
In feces	121.8	197.3		99.1	71.8	
Apparent Digestion (%)	81.9 <sup>a</sup>	70.1 <sup>b</sup>	0.63	83.7 <sup>a</sup>	71.8 <sup>b</sup>	0.64
Calculated digestion of cotton component (%) <sup>*</sup>	67.3 <sup>a</sup>	26.1 <sup>b</sup>	0.71	68.6 <sup>a</sup>	28.6 <sup>b</sup>	0.73

<sup>\*</sup>Calculated values, based on DM and OM digestibility values (89.6 and 91.9%, respectively) obtained from metabolic trial where rams were fed a whole concentrate diet.

### Growth study with lambs

The last months were dedicated also for preparations regarding the growth study with lambs that is being performed in present. These preparations included: production of ozonated cotton stalks, preparation of feed ingredients and experimental rations. Basicly, this growth study will consist of three groups of 5 lambs each, which will be fed ad-lib by the following dietary treatments:

1. A diet containing 40% untreated cotton stalks and poultry litter (1:1) plus 60% concentrate mix.
2. A diet containing 40% ozonated cotton stalks plus poultry litter (1:1) plus 60% concentrate mix.
3. A reference whole concentrate diet.

The adaptation of the lambs to the dietary treatments was started last week.