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# EFFECT OF UREA ADDITION ON THE FERMENTATIVE CHARACTERISTICS AND MICROBIAL POPULATION CHANGES OF SILAGE MADE FROM CATTLE MANURE AND FINAL MOLASSES

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The effect of urea addition or urea plus minerals on the microbial population and fermentation characteristics of silage made from manure and final molasses was studied in laboratory silos. Manure from grazing cows was collected from the floor of a shaded pen and mixed in the following proportions: A) 40% manure and 60% final molasses; B) 40% manure, 58% final molasses and 2% urea and C) 40% manure, 57.5% final molasses, 2% urea and 0.5% minerals. The experiment lasted 30 days. A significant effect ($P < 0.001$) of pH resulting from urea addition was observed. The highest pH values in these treatments were: 6.47 and 6.44 at 30 days for B and C, respectively. Neither salmonellae nor coliforms were detected. Urea treatments resulted in a lower population of lactobacilli, streptococci and yeasts, the lactobacilli showing greatest reduction. These effects were more marked by mineral addition. There was a trend towards a reduction in population of all the physiological groups and increasing total VFA production after 8 days of storage.

Key words: cattle manure, silage, molasses, urea, minerals, pH, microflora

Manure ensiling has been claimed as one of the most economic and hygienic methods of treatment, allowing its use in animal feeding (Smith 1973; Runkle et al 1975; Smith and Wheeler 1979; Arndt et al 1979) and diminishing the risk of environmental pollution.

During the ensiling process, acid-producing bacteria found in manure attack the readily-fermentable carbohydrates forming lactic acid and volatile fatty acids (VFA), (Moore and Anthony 1970; Hardy and Elías 1974). As a consequence of fermentation, there is a decrease in pH and anaerobic conditions are established thereby eliminating the activities of the undesirable aerobic and spore-forming microorganisms (Whittenbury 1968).

In Cuba, some experiments using mixtures of manure and final molasses for ensiling have been carried out (Hardy and Elías 1974). Results obtained showed that animals readily accepted the product. Urea addition to different silage mixtures improves chemical composition (Hardy et al 1978), and the amino acid content in particular (Betancourt et al 1978).

The objective of the present work was to study the possible changes occurring in some physiological groups of microorganisms from the microflora of these silages, as well as on the fermentative indices when manure and final molasses mixtures are ensiled with urea or urea plus minerals.

## Materials and Methods

*Preparation of mixtures:* Manure samples were collected with a shovel in the morning, from the shaded pens where cows rest before milking. Afterwards they were taken to the laboratory where they were mixed with final molasses in the following proportions: A) 40% manure and 60% final molasses; B) 40% manure, 58% final molasses and 2% urea and C) 40% manure, 57.5% final molasses, 2% urea and 0.5% of a mineral mixture. Twelve laboratory silos were prepared from each mixture using glass jars with screw-on lids provided with safety valves and stored at ambient temperature (20 - 25°C). Three silos per treatment were destructively sampled for analysis at 0, 4, 8, 15 and 30 days after sealing .

*Chemical analyses:* The pH of silage was obtained with 10 g samples blended for 1 minute in 90 ml of distilled water, using a potentiometer with a glass electrode. In the resulting liquid, VFA's were also determined by steam distilate with 0.1 N NaOH using methyl red as the indicator . (Pennington 1952).

*Microbiological analyses:* The counts of the groups of micro-organisms present was carried out at the same intervals as the chemical analyses . Each silo was aseptically opened and the upper parts of the mixtures were removed. 10 g from the centre of the silo were taken. Afterwards 90 ml of a 0.1% sterile peptone solution were added and blended for 3 min at high speed. This constituted the dilution $10^{-1}$ from which the remaining serial dilutions were prepared. The groups of micro-organisms studied and the media used are presented in Table 1.

*Table 1:*

*Culture media and incubation times used for the enumeration of specific groups within the microflora of silage made from manure and final molasses*

| Medium | Specific groups | Incubation time, hr |
|---|---|---|
| Tryptone-glucose-yeast[a] agar (plate count) | Total faculative aerobic bacteria | 48 |
| Non rumen fluid[b] NRF | Total strictly anaerobic bacteria | 72 |
| Rogosa-Agar[a] | Lactobacilli | 72 |
| Azide blood agar[c] | Streptococci | 72 |
| Brilliant green agar[c] | Salmonellae | 24 |
| Buffered yeast agar[c] | Yeasts | 72 |
| Violet Red bile agar[c] | Coliforms | 18 |
| Cellulose agar[b] | Cellulolytic | 72 |

[a] Prepared from the constituents according to the Oxoid Manual (1967)

[b] Elfas (1971)

[c] Oxoid prepared medium

Strictly anaerobic bacteria were cultured under oxygen - free $CO_2$ according to the technique of Hungate (1966a) in roll tubes. Other micro-organisms were cultered in roll tubes without CO2 . All media were incubated at 37°C.

*Design and statistical analysis:* A factorial 3 x 5 arrangement in random blocks with 3 replications was used. For mean comparison between treatments, Duncan's (1955) multiple range test was employed.

## Results

*pH and production of organic acid:* Changes in pH and VFA formation during the 30 days of the experiment are shown in Table 2.

*Table 2:*
*Changes in pH and total VFA in manure:molasses silages prepared with or without urea and minerals*

| Treatment | Days | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 4 | 8 | 15 | 30 | |
| | | | pH | | | |
| Manure/molasses | 5.32 | 5.48 | 5.20 | 4.98 | 4.33 | 4.99[a] |
| Manure/molasses /2% urea | 6.03 | 7.20 | 6.63 | 6.70 | 6.27 | 6.47[b] |
| Manure/molasses/urea/ minerals | 5.60 | 5.95 | 6.70 | 6.68 | 6.13 | 6.44[b] |
| | | | SE ± 0.19*** | | | |
| | | | Total VFA (%DM) | | | |
| Manure/molasses | 1.47 | 1.29 | 1.73 | 2.27 | 1.83 | 1.88 |
| Manure/molasses/2% urea | 1.34 | 1.30 | 1.82 | 2.36 | 2.31 | 2.00 |
| Manure/molasses/urea/ minerals | 1.40 | 1.70 | 1.63 | 2.52 | 1.96 | 1.91 |
| | | | SE ± 0.16 | | | |

[ab]Values with different superscripts in the same column differ significantly at $P < 0.05$
***$P < 0.001$

The mixture without the urea sustained a slight increment in pH four days after ensiling, progressively decreasing thereafter until reaching a value of 4.33 after 30 days. The addition of urea significantly increased pH ($P < 0.001$). This increment was marked in the mixtures where urea was added alone, reaching a value of 7.20 four days after ensilage and showing ammonia odour. From then on, pH began to decline slowly until a value of 6.27 was attained after 30 days.

The addition of minerals plus urea to the mixtures did not raise pH values as much as when urea alone was added, but were still higher by 2 pH units than the control without urea. The highest values were obtained in this treatment 8 days after ensilage, the pH decreasing slowly thereafter until day 30 (6.13).

Total VFA production was low in all silages during the period analysed, irrespective of whether urea alone or urea plus minerals was added. Peak acid production was reached at 15 days, decreasing thereafter.

*Changes in microbial populations:* Changes occurring in the different groups studied in the three mixtures are presented in Figures 1 - 6. Coliforms or salmonellae were not detected at the dilutions employed ($10^{-2}$ - $10^{-5}$).

The number of facultative aerobic bacteria found was in the range of 30 x $10^6$ to 80 x $10^6$ organisms/g of fresh matter of silages(Figure 1). The control treatment (without urea) showed a slight increase after the fourth day, decreasing thereafter until reaching the lowest counts at 30

*Figure 1:*

*Changes in population of total facultative bacteria in silages made from mixtures of manure and molasses with or without the addition of urea*

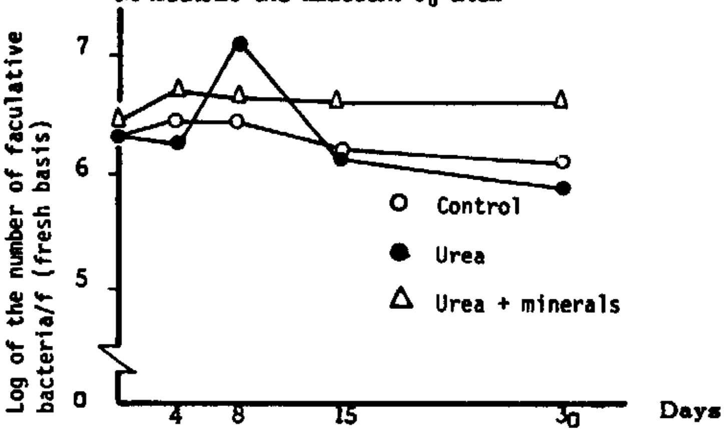



*Figure 2:*

*Changes in the population of total anearobic bacteria in silages made from mixtures of manure and molasses with or without the addition of urea*

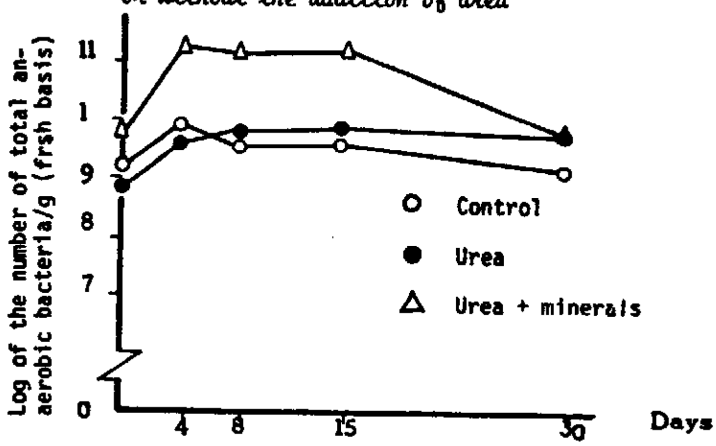



*Figure 3:*

*Changes in population of lactobacilli in silages made from mixtures of manure and molasses with or without the addition of urea*

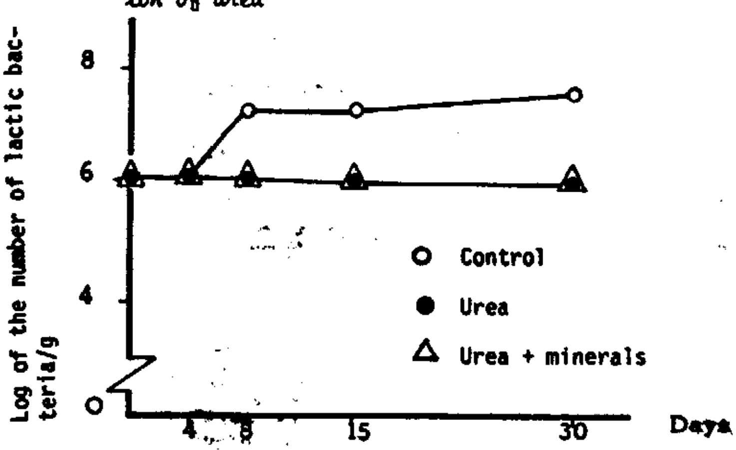


*Figure 4:*

*Changes in population of streptococci in silages made from mixtures of manure and molasses with or without the addition of urea*

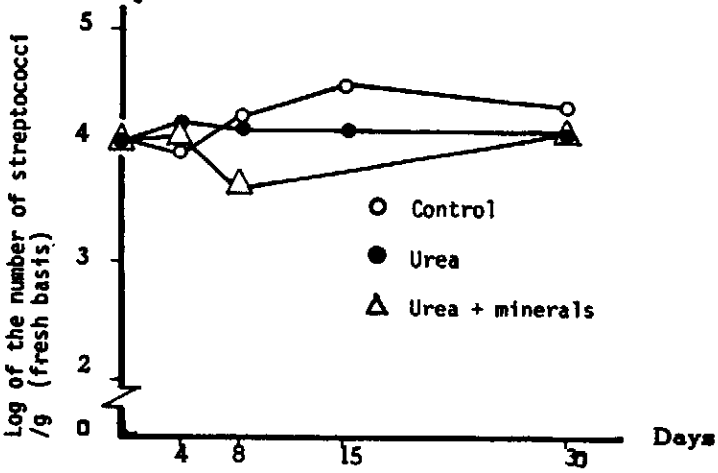



*Figure 5:*

*Changes in population of cellulolytic bacteria in silages made from mixtures of manure and molasses with or without the addition of urea*

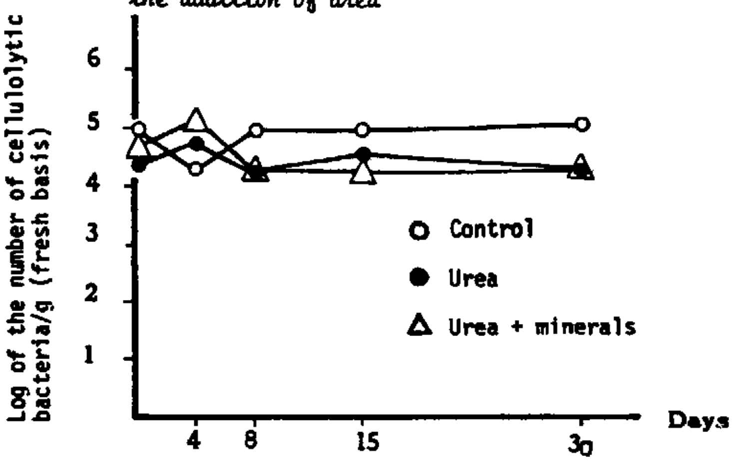
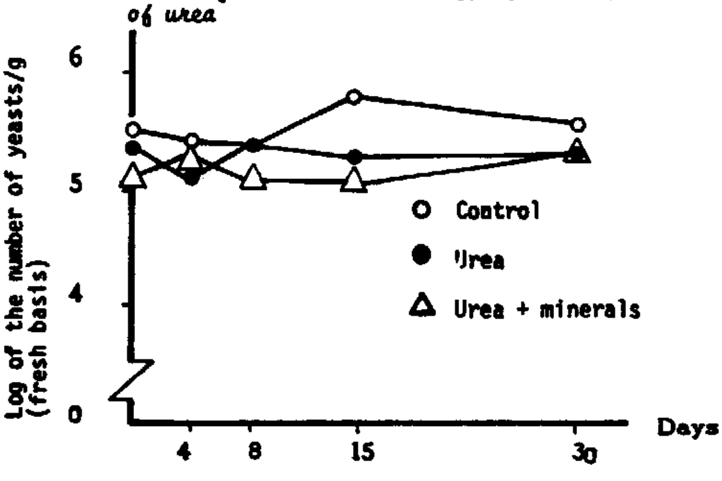



*Figure 6:*

*Changes in population of yeast in silages made from mixtures of manure and molasses with or without the addition of urea*



days (15 x $10^6$ organisms/g). Urea addition provoked a gradual decrease until 7 x $10^6$ organisms/g. However, the addition of urea plus minerals encouraged an increase in the microbial population at 4 days after storing (112 x $10^6$ organisms/g). These values remained constant thereafter. The development of micro-organisms compared to the control increased to 294% through adding urea plus minerals but was only 53% with urea alone.

The total count of anaerobic bacteria was higher than that found for total facultative aerobic bacteria. The figures were in the order of $10^8$ organisms/g in all treatments (Figure 2). All mixtures showed an increase of these micro-organisms in the first 4 days. A larger population (341%) than that found in the control was observed when urea plus minerals were added, although 15 days later the population declined to 25 x $10^8$ organisms/g. In the control treatment there was an initial increase of micro-organisms (the fourth day), and a decrease was observed in the remaining period down to 10 x $10^8$ organisms/g.

When urea alone was added there was no increase in these micro-organisms until the 8th day, after which the value remained the same (38 x $10^6$ to 41 x $10^8$ organisms/g).

Lactobacilli presented a different situation from the previously mentioned groups (Figure 3). There were no differences between treatments during the first 4 days. In the control treatment a great increase occurred after the 4th day and the same level was observed at the end of the study period (53 x $10^6$ organisms/g).

Streptococci were also influenced by the presence of urea in the mixtures (Figure 4). No changes were apparent in the treatments during the first 4 days. From the 4th day to the 8th day an increase in the control and the urea plus minerals treatments were observed. The control continued increasing until 15 days, but thereafter the values dropped to 36 x $10^6$ organisms/g at 30 days. There was little variation in the mixture containing urea beyond the 8th day. In mixtures containing urea plus minerals the increase was slow until the 30th day.

Few changes were present in the cellulolytic bacterial population (Figure 5). Values ranged between 3.5 x $10^4$ and 12 x $10^{14}$ organisms/ g in all treatments.

Yeast counts were affected by the presence of urea in the mixtures (Figure 6), but there were no differences after 30 days of storage. The treatment without urea presented values as high as 72 x $10^4$ organisms / g.

## Discussion

The addition of urea to silages of bovine manure and final molasses showed a significant effect on the fermentative indices which was more noticeable in the pH but less evident in organic acid production (Table 1). The former was to be expected, but during fermentation urea hydrolysis originates ammonia, which in turn increases pH and it may thus influence the growth of organic acid producing micro - organisms (mainly lactobacilli and streptococci). This suggestion is supported by the results found in these groups of micro-organisms (Figures 3 and 4), since growth inhibition in comparison with the control with no urea was observed. Similar pH values have been reported by Saylor and Long (1974) and Caswell et al (1975) working with poultry litter, high in uric acid.

On the other hand, total VFA values reported in this paper are higher than those found by Caswell et al (1977) with poultry litter and maize (grains with high humidity), but are in agreement with those published by Knight et al (1977) with bovine manure and concentrate (60:40). This high total VFA production (in comparison with that encountered when ensiling poultry manure), could be influenced by the type of micro-organisms present in bovine manure, which are fed on fibrous materials, not rapidly digested, and producing in their great majority VFA's as end-products(Hungate 1966b). Furthermore, they could be fermented by being in contact for a longer period.

The aerobic facultative microflora tended to decrease with time,which is in agreement with the findings of Caswell et al (1977) and Knight et al (1977). However, the high counts of viable strictly anaerobic micro-organisms obtained ($10^9$-$10^{10}$ organisms/g of ensiled matter) has not been reported by other authors. Knight et al (1977) only reported aerobic or anaerobic spore-forming bacteria. It is possible that this group of micro-oragnisms plays a most important role in the fermentation occurring during this process.

The high counts of cellulolytic bacteria found during the whole period in all mixtures are possibly an important part of the anaerobic microflora. This leads to suggest the possibility of using these silages as a means of inoculating these micro-organisms in low or medium quality fibrous diets to improve their digestibilities. On the other hand, the high yeast population found in the silages possibly produces some B - complex vitamins, which are known to be required by many ruminal bacterial as growth factors (Hungate 1966b).

The fact that no coliforms or salmonellae were found could be due to a rapid decrease of these micro-organisms during the first days of ensiling or to the use of an inaccurate technique. Knight et al (1977) and McCaskey and Anthony (1975) have reported the decrease of these micro-organisms in the first 10 days after ensiling, although the pH's found by these authors were lower than 4.7. On the other hand, Harmon et al (1975) reported coliforms even 71 days after the ensiling process started. It is possible that the non-detection of these micro-organisms could be due to the fact that the population was not enriched in an adequate medium, as reported by Knight et al (1977). New studies on these micro - organisms must be performed to arrive at a definitive conclusion.

Results encountered showed that urea addition affects the fermentation process when bovine manure and final molasses are ensiled, by acting on the development of certain groups of micro-organisms, mainly, organic acid formers and possibly on coliforms and salmonellae, the effects being more marked when minerals are added. However, the products resulting from fermentation preserve the odour, colour and palatability characteristics of the silage even without urea.

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