Adding of a blue-green micro-algae, spirulina, to maize at ensiling

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Abstract
Maize was ensiled with or without the addition of spirulina (a blue-green micro-algae) to increase the protein content of maize silage in laboratory silos (1.5 liter). The protein content of maize silage was increased from 8.9 to 15.4% by the addition of 3.4 g spirulina/100g of fresh chopped maize plant. Both the control and protein enriched silages were well preserved at a pH of 3.73 and 3.98 and ammonia nitrogen as percentage of total nitrogen was 6.7 and 7.1 for the respective silages. Spirulina can be used to increase the protein content of maize silage. Further studies are needed to determine the palatability and production potential of protein enriched maize silages.

Keywords: Maize silage, algae, spirulina, protein
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Introduction
Maize (Zea mays) is regarded as an ideal silage crop with a low buffering capacity and adequate water soluble carbohydrates which results in rapid preservation (Meeske & Basson, 1998). The biggest limitation of maize silage as animal feed is its relative low crude protein value of 7.7% of DM (Auffrère et al., 1992). The protein content of maize may be increased by adding urea or ammonia at ensiling (McDonald et al. 1991). The addition of urea or ammonia will increase the ammonia-N content and the pH of silage. This may cause a reduced rate of preservation resulting in silage of low palatability and a high butyric acid content (Cilliers & Van Schalkwyk, 1976). It is possible to produce algae on the final effluent after anaerobic digestion of dairy factory effluent. Spirulina is a blue-green micro-algae that derives its name from the spiral shape of its filament. The protein content of spirulina varies from 60 to 70% on a dry matter basis (Switzer, 1982). The protein consists mainly out of amino acids and is therefore of a very high quality (Switzer, 1982). If the algae could be added to maize at ensiling without it having a negative effect on the preservation, the nutritional value of maize silage could be greatly improved. The aim of the study was to determine if algae (spirulina) can be used to increase the protein content of maize silage.

Materials and methods
Maize (Senkuil) was planted on the 24th of November 1993 at the Irene (longitude 28° 13’S: latitude 25° 55’E, altitude 1524m). The plant density was 38000 plants per hectare. Maize was harvested at the hard dough stage with a Feraboli 945 precision silage chopper. The maize was ensiled with or without the addition of spirulina at 3.4 g/100g of fresh chopped material in five mini silos for each treatment. The aim was to increase the crude protein level of maize silage from 8 to 13% on a DM basis. Mini silos were 1.5 litre glass jars, equipped with a special lid with springs which enables gas release (J. WECK, GmbH u. Co., Wehr-Oflingen, W. Germany). The silos were opened after 95 days of ensiling. A representative sample from each silo was taken for DM determination and chemical analysis (stored at -20 °C). Dry matter of the fresh material and silage was estimated by drying samples in an oven at 60°C for 72 hours. Water-soluble carbohydrate (WSC), pH and lactic acid (LA) were determined on filtrates of 40 g of frozen sample added to 360 ml of distilled water, homogenized for 3 minutes with a stomacher. Water soluble carbohydrates were determined by the phenol-sulphuric acid method according to Dubois et al. (1956) and lactic acid was determined by the colorimetric method of Barker & Summerson (1941). Volatile fatty acid
(VFA) was determined with a Carlo Erba 4200 gas chromatograph with flame ionisation detector with a 2.35m x 3mm stainless steel column packed with 10% SP 1200 containing 1% orthophosphoric acid (H₃PO₄). The column was conditioned for 48 hours at 165 °C with a nitrogen carrier gas flow of 40 ml per minute. *In vitro* organic matter digestibility (IVOMD) was determined according to Tilley & Terry (1963) and neutral detergent fibre (NDF) according to Van Soest *et al.* (1991). Total nitrogen was determined by the Kjeldahl method. The ammonia nitrogen content of silage was determined by homogenizing 50 g of silage in 250 mL of a 0.1N H₂SO₄ solution for three minutes. The homogenate was filtered through Whatman no 4 filter paper and the ammonia content in the filtrate was determined by distillation using a Buchi 342 apparatus and a Metrohm 655 Dosimat with a E526 titrator according to AOAC (1984). This method is based on the method of Pearson & Muslemuddin (1968) to determine volatile nitrogen.

At day 95 of ensiling, the silage was exposed to air for 5 days and the aerobic stability determined according to the method of Ashbell *et al.* (1991). According to this method CO₂ production during the aerobic deterioration is measured as an indicator of aerobic spoilage of silage. The minimum and maximum temperature during the aerobic stability test was 11 °C and 23 °C respectively. The dry matter, organic matter, IVOMD, crude protein, lactic acid WSC and pH of maize silage after 5 days of aerobic exposure was determined as described above.

Data was processed by one way analysis of variance determining the least significant differences using Statgraphics (1988).

Results and Discussion

The silos were stored at a minimum temperature of 18.1 ± 4.1 °C and a maximum temperature of 21.9 ± 2.9 °C. The average chop length of the maize was 5.04 ± 0.69 mm (n = 12). The chemical composition of maize silage after 95 days of ensiling is given in Table 1.

Table 1 Composition (% of DM) of maize silage made with or without the addition algae.

<table>
<thead>
<tr>
<th></th>
<th>Maize silage 95 days of ensiling</th>
<th>Maize silage 5 days aerobic exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Algae</td>
</tr>
<tr>
<td>Dry matter</td>
<td>27.7ᵇ</td>
<td>29.0ᵃ</td>
</tr>
<tr>
<td>Organic matter</td>
<td>89.5ᵃ</td>
<td>93.4ᵇ</td>
</tr>
<tr>
<td>IVOMD</td>
<td>74.5ᵇ</td>
<td>75.5ᵇ</td>
</tr>
<tr>
<td>Crude protein</td>
<td>9.3ᵃ</td>
<td>15.4ᵇ</td>
</tr>
<tr>
<td>NDF</td>
<td>45.8ᵃ</td>
<td>43.1ᵇ</td>
</tr>
<tr>
<td>NH₃-N (% of TNᵇ)</td>
<td>6.7ᵃ</td>
<td>7.1ᵇ</td>
</tr>
<tr>
<td>pH</td>
<td>3.73ᵃ</td>
<td>3.98ᵇ</td>
</tr>
<tr>
<td>WSC</td>
<td>7.1ᵃ</td>
<td>4.9ᵇ</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>6.7ᵃ</td>
<td>7.1ᵃ</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.95ᵃ</td>
<td>1.3ᵇ</td>
</tr>
<tr>
<td>N-Butyric acid</td>
<td>0.01ᵃ</td>
<td>0.01ᵇ</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>NFᵇ</td>
<td>NF</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>n-Valeric acid</td>
<td>NF</td>
<td>NF</td>
</tr>
</tbody>
</table>

ᵃᵇᶜ Means with different superscripts in the same row differ significantly (P < 0.05)
⁴Sem = Standard error of means, ⁵ND = Not determined, ⁶WSC = Water soluble carbohydrates, ⁷NF = Not found, TNᵇ = Total nitrogen.

The control and protein enriched silages were well preserved as indicated by the low pH, high lactic acid content and the amount of protein that was broken down to ammonia. The protein enriched maize silage had a significant (P< 0.05) higher protein content than the control silage while the ammonia-nitrogen as a percentage of the total nitrogen did not differ significantly between the two silages. This indicates that the added protein was well preserved and protein
breakdown was limited to only 7.1%. The IVOMD and crude protein (CP) of the control maize silage found in the present study (Table 1) is comparable to the 74 ± 3% IVOMD and 7.7 ± 0.8% CP found by Aufrère et al. (1992) on 118 whole maize plant samples. The adding of algae did result in maize silage with a higher (P < 0.05) pH and acetic acid content while the WSC content was lower than that of the control maize silage. The lower WSC and higher lactic acid content of the protein enriched silage indicate a longer fermentation time compared to the control silage. The drop in pH was however rapid enough to prevent extensive growth of clostridia as indicated by the low butyric acid content and limited protein breakdown in the enriched silage. The composition of the maize silages after five days of aerobic exposure is given in Table 1. The control maize silage was more unstable when exposed to air for five days than the protein enriched silage. The pH of the control maize silage increased significantly (P < 0.05) when exposed to air while the LA and WSC content decreased significantly (P < 0.05). A significant drop in WSC did occur in the protein enriched maize silage when exposed to air while the lactic acid and pH remained constant (Table 1). During aerobic exposure of control and protein enriched silage, 16.7 and 7.5 g CO2 kg-1 DM was produced respectively (Standard error of means = 2.7, P-value = 0.8).

Conclusion
It can be concluded that spirulina can be used as a silage additive to increase the protein content of maize silage. Further studies are needed to determine the palatability and production potential of protein enriched maize silage. The viability of using spirulina or other algae as silage additives depends on the cost compared to other available protein sources, the milk production response, the availability of algae and possible practical constraints.

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References