Fermentation characteristics and changes in nutritive value of guinea grass-leucaena ensiled with or without additive

J.E. FLEISCHER, Y. KAWAMOTO, M. SHIMOTO, Y. MASUDA & I. GOTO
(J.E.F., M.S., Y.M. & I.G.: Laboratory of Feed Science and Animal Behaviour, Faculty of Agriculture, Kyushu University, 46-06, Hakozaki, Fukuoka Shi 812 Japan; Y.K.: Department of Animal Science, College of Agriculture, University of Ryukyu, Senbara, Nishihara, Okinawa, 903-01 Japan; J.E.F.’s present address: Department of Animal Science, Faculty of Agriculture, University of Ghana, Legon, Ghana)

SUMMARY
Fifty-two-day-old guinea grass (Panicum maximum var. maximum cv. Gatton) which received 150 kg N per hectare and 10-month-old leucaena (Leucaena leucocephala (Lam.) De Witt.) were used. There were five treatments viz., guinea grass (T1), leucaena (T2), guinea grass-leucaena (T3), guinea grass-leucaena with starch (T4) and guinea grass-leucaena with formic acid (T5). Both guinea grass and leucaena were mixed in a grass:legume ratio of 7:3 in T1, T3 and T5. Samples were stuffed into laboratory silo (1.4-1.5 kg sample) and stored for 42 days. Samples were taken at and after ensiling and analysed for total nitrogen, volatile basic nitrogen as per cent of total N, pH, lactic acid, acetic acid, butyric acid, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, acid detergent lignin (ADL) and in vitro dry matter digestibility (IVDMD). Guinea grass had significantly lower (P<0.01) nitrogen content but higher content of fibre compared to leucaena. The IVDMD values (50%) were similar (P>0.05) in both guinea grass and leucaena. Combining guinea grass with leucaena significantly (P<0.05) raised the nitrogen content of the mixture but slightly reduced the cell wall components. It also raised the IVDMD value (54 per cent) though not significantly. Addition of starch reduced the nitrogen content and the cell wall consistency but increased the IVDMD value (56 per cent). These changes were significant (P<0.05). Guinea grass silage had a significantly (P<0.05) lower pH and volatile basic acid (VAN) than leucaena. Guinea grass with leucaena starch had the lowest pH but was not significantly different from T2, T6 and T7. The lactic acid content of T7 was significantly higher (P<0.05) than the others. T7 has the lowest VBN.

RÉSUMÉ
FLEISCHER, J.E., KAWAMOTO, Y., SHIMOTO, M., MASUDA, Y. & GOTO, I. : Les caractéristiques de fermentation et les changements en valeur nutritive de l’herbe guinéenne-leucaenaea ensilée avec ou sans additif. Une herbe guinéenne (Panicum maximum var. maximum cv. Gatton)à l’âge de 52 jours qui recevait 150 kg N par hectare et leucaena (Leucaena leucocephala (Lam.) De Witt.) à l’âge de 10 mois étaient utilisées. Il y avaient cinq traitements o-a, herbe guinéenne (T1), leucaena (T2), herbe guinéenne-leucaena (T3), herbe guinéenne-leucaena avec féculle (T4) et herbe guinéenne avec acide formique (T5). A la fois l’herbe guinéenne et leucaena étaient mélangées dans une proportion herbe:légumineuse de 7:3 en T1, T3 et T5. Les échantillons étaient remis dans un silo laboratoire (1.4-1.5 kg d’échantillon) et mis en réserve pour 42 jours. Les échantillons étaient prélevés pendant et après ensilage et analysés pour azote total, azote basique volatile, comme pourcentage de N total, pH, acide lactique, acide acétique, acide butyrique, fibre détergente neutre (NDF), fibre détergente acide (ADF), cellulose, acide détergent lignine (ADL) et in-vitro matière sèche (IVDMD). L’herbe guinéenne avait un contenu d’azote considérablement plus bas (P<0.01) mais un contenu plus haut de fibres comparé à leucaena. Les valeurs d’IVDMD(50%) étaient semblables (P<0.05) à la fois en herbe guinéenne et leucaena. Combiant l’herbe guinéenne avec leucaena élevait considérablement (P<0.05) le contenu d’azote de la mixture mais réduisait légèrement les composants de mur cellullosique. Il a également élevé la valeur de IVDMD (54 pour cent) bien que ce n’est pas considérable. L’addition de féculle réduisait le contenu d’azote et le mur cellullosique consistant mais augmentait la valeur (56 pour cent) de IVDMD. Ces changements étaient significatifs (P<0.05). L’ensilage de l’herbe guinéenne avait pH (P<0.05) considérablement plus bas et acide basique volatile (VAN) que leucaena. Herbe guinéenne avec féculle de leucaena avait pH le plus bas mais n’était pas considérablement différente de T1, T3 et T7. Le contenu d’acide lactique de T7 était
Introduction

Tropical grasses have high dry matter yield potential (Caldwell, 1975). However, the production is very seasonal and is characterized by rapid deterioration in protein content as well as dry matter digestibility. Thus, animal production from the grasses is rather low. One way of overcoming these problems would be to combine the grass with leguminous shrubs or tree leaves and ensile. Such grass-legume combinations usually have improved protein content but varied effects on digestibility and fermentation quality (Fleischer & Tackie, 1993 Uchida & Kitamura, 1987; Moss et al., 1984). The low fermentation quality is expected since most tropical forages are low in water soluble carbohydrate content (Smith, 1973) and the legumes are additionally high buffering capacity (McDonald, 1981; Kaiser, 1984). To increase the fermentation quality of tropical forage silage, studies on the effect of additives have been made.

The objective of this study was, therefore, to investigate the fermentation quality and changes in nutritive value of guinea grass and/or leucaena with or without additive.

Materials and methods

The plant materials used were guinea grass (Panicum maximum var. maximum cv. Gatton) and leucaena (Leucaena leucocephala (Lam.) De Wit.). The guinea grass was seeded on 17 Apr 92 and first cut on 12 Jun 92. It received a basal fertilizer of N-P$_2$O$_5$-K$_2$O at the rate of 150-100-100 kg ha$^{-1}$ at seeding. Additional nitrogen fertilizer was applied immediately after the first cut at the rate of 150 kg ha$^{-1}$. The grass for this experiment was harvested on 3 Aug 92, i.e. after 52-day regrowth. Leucaena was collected from naturalized field and was about 10 months old and about 3 m tall.

Both the guinea grass and leucaena were chopped to about 3-5 cm length. The guinea grass was divided into four batches, three of which were combined with leucaena in a grass: legume ratio of 7:3. Thus, there were five treatments viz., guinea grass (T$_1$), leucaena (T$_2$), guinea grass-leucaena (T$_3$), guinea grass-leucaena with starch (T$_4$) and guinea grass-leucaena with formic acid (T$_5$). The starch formed 5 per cent of the fresh forage while the formic acid was added at the rate of 2.3 l t$^{-1}$ of fresh forage. Each of the treatment groups was then stuffed into a two litre laboratory silo and sealed. Each silo contained about 1.4-1.5 kg of sample. The silos were opened after 42 days of ensiling. Some samples were taken at the time of silo stuffing for analysis.

On opening the silos the top and bottom 3-5 cm silage respectively were discarded and samples taken from the middle portion. These were further chopped with scissors up to 2-5 mm length and used for analysis. One hundred grammes (100 g) of the samples was placed in one-litre conical flask and distilled water added to the one-litre mark. This was kept in a refrigerator for at least 12 h with occasional shaking. The extract was filtered using a No. 41 Whatman filter paper. The pH of the filtrate was immediately determined using a pH meter. The filtrate was used for the determination of organic acids and volatile basic nitrogen (VAN) (Mortimoto, 1971).

Fresh samples were used for total nitrogen (T-N) determination (AOAC, 1990). Cell wall components were determined according to the methods of Goering & Van Soest (1970) and in vitro dry matter digestibility (IVDMD) by the method outlined by Goto & Minson (1977) using oven dried sample.

Results

Chemical composition and IVDMD of forage before ensiling are shown in Table 1. The dry matter content of $T_1$ was significantly lower ($P<0.01$) than that of $T_2$. Combining guinea grass with leucaena ($T_3$) increased the DM compared to

considérablement plus élevé ($P<0.05$) que les autres. $T_1$ avait le plus bas VBN.
Fermentation characteristics of guinea grass-leucaena silage

### TABLE 1

**Chemical Composition and IV DMD of Forages before Ensiling.**

<table>
<thead>
<tr>
<th>Forage type and treatment</th>
<th>Dry matter</th>
<th>T-N&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Neutral detergent fibre</th>
<th>Acid detergent fibre</th>
<th>Cellulose</th>
<th>Acid detergent lignin</th>
<th>In vitro dry matter digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea grass (T&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>23.39a</td>
<td>1.47a</td>
<td>74.75a</td>
<td>46.60a</td>
<td>35.69a</td>
<td>8.69a</td>
<td>51.60a</td>
</tr>
<tr>
<td>Leucaena (T&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>35.18b</td>
<td>3.86b</td>
<td>52.87b</td>
<td>42.70b</td>
<td>27.48b</td>
<td>15.41b</td>
<td>53.70a</td>
</tr>
<tr>
<td>Guinea grass + leucaena (T&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>26.52a</td>
<td>2.62c</td>
<td>68.37c</td>
<td>43.14b</td>
<td>33.03c</td>
<td>8.87a</td>
<td>53.04a</td>
</tr>
<tr>
<td>Guinea grass + leucaena + Starch (T&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>31.40ab</td>
<td>2.45d</td>
<td>54.89b</td>
<td>37.66c</td>
<td>29.12d</td>
<td>7.41a</td>
<td>60.41b</td>
</tr>
<tr>
<td>Guinea grass + leucaena + formic acid (T&lt;sub&gt;5&lt;/sub&gt;)</td>
<td>25.42a</td>
<td>2.44d</td>
<td>66.73c</td>
<td>42.37b</td>
<td>33.52c</td>
<td>7.86a</td>
<td>54.22a</td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td><strong>4.812</strong></td>
<td><strong>0.009</strong></td>
<td><strong>1.043</strong></td>
<td><strong>0.499</strong></td>
<td><strong>0.309</strong></td>
<td><strong>0.309</strong></td>
<td><strong>6.575</strong></td>
</tr>
</tbody>
</table>

<sup>1</sup>) T-N = Total nitrogen  
<sup>2)</sup> SE = Standard error  
<sup>3)</sup> Figures in the same column with different letters are significantly different at 5 per cent probability level.

T<sub>1</sub>, though not significantly (P<0.05) yet still lower (P<0.05) than that of T<sub>2</sub>. Addition of formic acid (T<sub>5</sub>) slightly depressed the DM compared to T<sub>1</sub>, but addition of starch (T<sub>4</sub>) raised it.

T<sub>2</sub> had significantly higher (P<0.05) T-N and acid detergent lignin (ADL) but lower neutral detergent fibre (NDF), acid detergent fibre (ADF) and cellulose compared to T<sub>1</sub>. The IV DMD values of both T<sub>2</sub> and T<sub>1</sub> were, however, not significantly different (P<0.05) from each other.

The grass-legume combination (T<sub>3</sub>) had significantly higher (P<0.05) T-N but lower fibres compared to T<sub>1</sub>. These, however, did not affect the IV DMD. On the contrary, T<sub>3</sub> had significantly (P<0.05) lower T-N but higher fibres compared to those of T<sub>5</sub>. The inclusion of additives, T<sub>4</sub> and T<sub>5</sub>, significantly (P<0.05) reduced the T-N compared to T<sub>3</sub>. However, the addition of starch, T<sub>4</sub>,

### TABLE 2

**Fermentation Characteristics of Guinea Grass-Legume Silages with or without Additives**

<table>
<thead>
<tr>
<th>Forage type and treatment</th>
<th>Dry matter</th>
<th>pH</th>
<th>Lactic acid (per cent)</th>
<th>Acetic acid (per cent)</th>
<th>T-N&lt;sup&gt;1&lt;/sup&gt;</th>
<th>VAN×100&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea grass (T&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>24.94a</td>
<td>3.95a</td>
<td>1.71a</td>
<td>0.31ab</td>
<td>1.53a</td>
<td>3.87ab</td>
</tr>
<tr>
<td>Leucaena (T&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>37.83b</td>
<td>5.27b</td>
<td>1.95a</td>
<td>0.41a</td>
<td>3.36b</td>
<td>6.27a</td>
</tr>
<tr>
<td>Guinea grass + leucaena (T&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>29.09a</td>
<td>4.17a</td>
<td>1.91a</td>
<td>0.26b</td>
<td>2.32ab</td>
<td>4.95a</td>
</tr>
<tr>
<td>Guinea grass + leucaena + Starch (T&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>27.05a</td>
<td>3.87a</td>
<td>2.64b</td>
<td>0.27b</td>
<td>2.42ab</td>
<td>4.02ab</td>
</tr>
<tr>
<td>Guinea grass + leucaena + formic acid (T&lt;sub&gt;5&lt;/sub&gt;)28.40a</td>
<td>3.94a</td>
<td>1.67a</td>
<td>0.38ab</td>
<td>2.44ab</td>
<td>2.47b</td>
<td></td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td><strong>4.939</strong></td>
<td><strong>0.587</strong></td>
<td><strong>0.391</strong></td>
<td><strong>0.067</strong></td>
<td><strong>0.649</strong></td>
<td><strong>1.409</strong></td>
</tr>
</tbody>
</table>

<sup>1)</sup> T-N = Total nitrogen  
<sup>2)</sup> VAN × 100 = Volatile basic nitrogen expressed as percentage of total nitrogen.

Figures in the same column with different letters are significantly different at 5 per cent probability level.
significantly decreased the cell wall components and increased IVDM.

Though differences were observed among the in vitro dry matter digestibility (IVDMD) values, only that of T₄ was significantly different (P<0.05) from others.

Fermentation characteristics of the silages are shown in Table 2. Except for leucaena silage (T₂) which had a significantly higher pH (P<0.05) compared to all the others no significant differences (P<0.05) were observed among T₁, T₃, T₄ and T₅. Followed by T₃, T₁ had the lowest pH. Lactic acid which formed the dominant acid ranged between 1.67 and 2.64 per cent. T₄ had the highest lactic acid content and this was significantly different (P<0.05) from the others. The differences among the others were not significant (P<0.05). Acetic acid production was rather low ranging between 0.26 and 0.41. The differences in acetic acid production among T₁, T₂ and T₃ were not significant (P<0.05). On the contrary, T₅ was significantly higher (P<0.05) than T₂ and T₄. No butyric acid was observed in any of the silages.

The T-N content showed a slight depression in T₃ and T₄ compared to those in Table 1.

The volatile basic nitrogen (VAN) content of the silages ranged between approximately 2.5 and 6.3 per cent of T-N. T₂ had the highest VAN content and this was significantly different from that of T₅, significantly different (P>0.05) from that of T₂. Though T₅ was lower than T₁ the difference was not significant (P>0.05). Cellulose content of T₂ was lower than that of T₁ but higher than that of T₅. However, none of the differences was significant (P>0.05). Those of T₃, T₄ and T₅ were higher than that of T₂ but the difference was not significant (P>0.05). The ADL content of T₅ was significantly higher (P>0.05) than all the others. The differences among the others were not significant (P>0.05). There was an increase in all the cell wall components of T₅ on ensiling (cf Tables 1 and 2).

IVDMD of T₂ was similar to that of T₁. Both T₃ and T₄ had slightly higher but not significant iVDMD compared to those of T₁ and T₂. On the

<table>
<thead>
<tr>
<th>Forage type and treatment</th>
<th>Neutral detergent fibre</th>
<th>Acid detergent fibre</th>
<th>Cellulose</th>
<th>Acid detergent lignin</th>
<th>In vitro dry matter digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea grass (T₁)</td>
<td>73.19a</td>
<td>51.95a</td>
<td>41.16a</td>
<td>7.43a</td>
<td>49.69a</td>
</tr>
<tr>
<td>Leucaena (T₂)</td>
<td>48.94b</td>
<td>42.24b</td>
<td>25.43b</td>
<td>14.26b</td>
<td>50.43a</td>
</tr>
<tr>
<td>Guinea grass + leucaena (T₃)</td>
<td>65.38</td>
<td>41.98b</td>
<td>32.67ab</td>
<td>7.94</td>
<td>53.95ab</td>
</tr>
<tr>
<td>Guinea grass + leucaena + Starch (T₄)</td>
<td>58.86ab</td>
<td>40.19b</td>
<td>30.81b</td>
<td>7.92a</td>
<td>55.84b</td>
</tr>
<tr>
<td>Guinea grass + leucaena + formic acid (T₅)</td>
<td>66.15a</td>
<td>45.10ab</td>
<td>34.35ab</td>
<td>9.10a</td>
<td>52.43a</td>
</tr>
</tbody>
</table>

SE 9.124 4.628 5.713 2.823 2.522

n = 5

Figures in the same column with different letters are significantly different at 5 per cent probability level.
Fermentation characteristics of guinea grass-leucaena silage

contrary, IVDMD of T₄ was significantly higher ($P<0.05$) than all the rest except that of T₃.

**Discussion**

The differences in chemical composition of guinea grass and leucaena is consistent with the differences reported in grasses and legumes in the literature (Norton, 1982). Consequently, combining them gives values intermediate to both. Of primary significance, however, is the dramatic increase in the T-N. Indeed, the T-N of the guinea grass was just above the threshold level at which point intake decreases in beef but inadequate for dairy animals (Minson, 1981). The additives depressed the T-N content but was still reasonably high enough to meet the needs of both the beef and dairy animals. Starch is a non-structural carbohydrate (Smith, 1973) hence addition to the combined product though raised the dry matter yet depressed all the cell wall components. This, therefore, led to an increase in the IVDMD.

Apart from the the leucaena silage which had relatively high pH, all the other silages had reasonable pH levels. The relatively low pH values of the other silages were due to the fact that in this experiment the dominant acid was lactic acid (McDonald, 1981; Kaiser, 1984) and also to the addition of acid in T₄. This may appear contrary to the observations with most tropical forage ensiled without additive (Miller, 1969; Catchpoole & Williams, 1969; Catchpoole & Henzel, 1971; Miaki, Tanaka & Kawamura, 1986; Uchida & Kitamura, 1987). Legumes tend to have a high buffering capacity as well as low water soluble carbohydrate content (McDonald, 1981). Besides, leucaena in particular is found to be very slow fermenting material (Alli, Baker & Garcia, 1983; Fleischer *et al.*, unpublished). Consequently, it tended to have relatively high pH.

The higher pH of grass-legume combination silage compared to grass silage have been reported by Uchida & Kitamura (1987) using rhodes grass (*Chloris gayana* cv. Masaba) and stylo (*Stylosanthes guianensis* cv. Schofield) or siratro (*Macroptilium atropurpureum* cv. Siratro) and by Enishi, Shijiyama & Shioya (1991) using corn (*Zea mays* L.) and Sesbania (*Sesbania cannabina* Press.). Making a good quality silage of grass-legume mixture, grass needs to have potential characteristics to be of good fermentation quality as in this experiment. Another way to improve the fermentation quality is to use some additives. The use of additive, especially starch, improved the fermentation of the silage dramatically. This is soluble in view of the fact that starch, unlike formic acid, could easily be obtained as a by-product of the cassava-processing industry and technically poses no health risk in its application.

The decline in T-N of the forages during ensiling has also been observed by Ooshima & McDonald (1978). These notwithstanding, the values obtained in this experiment were still high indicating that the process may overcome one of the major problems of dry season feeding of ruminants. Furthermore, the development of volatile basic nitrogen (VAN) is a normal process encountered during ensiling (Ooshima & McDonald, 1978). Of significant interest here, therefore, is the fact that the level of VAN produced was relatively low compared to what has been observed for most tropical forage silages (Catchpoole & Henzel, 1971; Moss *et al.*, 1984). Dermaquilly & Dulphy (1977), as quoted by Kaiser (1984), have suggested that a good silage should have VAN to be 50 g/kg total N and acetic acid to be 25 g/kg DM. Thus, the silage produced in this experiment can be considered good. The fermentation quality of grass-legume combination silage depends on the potential characteristics of fermentation of each component. In this experiment, combination decreased pH value owing to the good fermentation characteristics of guinea grass and depression in production of VAN. This means that it is possible to ensile these materials.

The slight decline in NDF and the non-significant changes in the other cell wall components have been observed by other workers (Fleischer & Tackie, 1993 in press). The increase in fibres of silage with starch as an additive is because the starch which is a non-structural carbohydrate.
was effectively used up as the fermentation substrate.

The decline in IVMD on ensiling though slight was expected because no correction was made of the volatile fatty acids and soluble components that were used in the fermentation process. Though in this experiment slight but non-significant increases were observed by making grass-legume silage, reports on similar work have been varied. Carneiro et al. (1984) did not observe any change when they mixed elephant grass (*Pennisetum purpureum* cv. Cameroonian) and Lablab (*Dolichos lablab*). Uchida & Kitamura (1987) did not observe any change in the IVMD of rhodes grass-stylo combination but observed a decline in rhodes grass-siratro combination. Enishi et al. (1991) also observed a decline in digestible organic matter with increase in sesbania proportion in corn-sesbania silage. Despite these discrepancies, the values obtained in the present experiment were very encouraging especially with regard to the grass-legume combination with starch. It remains to be seen, however, as to how this nutritive quality improvement would affect intake and hence animal performance.

**Conclusion**

The present work has shown that when guinea grass is combined with leucaena in the grass-legume ratio of 7:3, the nitrogen content is raised but the neutral detergent fibre, acid detergent fibre and cellulose contents are decreased except the increase of acid detergent lignin content. The *in vitro* dry matter digestibility of the combined product may not be affected. This combined product can be ensiled and the fermentation quality improved using formic acid or starch as an additive. The use of starch is more favoured since it can be easily obtained locally and may also improve the digestibility.

**Acknowledgement**

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Fermentation characteristics of guinea grass-leucaena silage


