Studies on the variations in the chemical composition of leucaena (*Leucaena leucocephala* (Lam.) De Wit.) during ensiling

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SUMMARY

The fermentation quality and nutritive value of leucaena ensiled either as whole forage or separate stem and leaf fractions were investigated. About 10-month-old leucaena (*Leucaena leucocephala* (Lam.) De Wit.) grown in Okinawa Island, Japan, was used. Samples were chopped to about 4 cm lengths and stuffed into laboratory silos of 1.4-1.5 kg capacity. Samples were taken at 0, 1, 3, 5, 7 and 42 days of ensiling for the laboratory determination of total nitrogen (T-N), volatile basic nitrogen fibre (VBN) expressed as g kg^{-1} of T-N, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, acid detergent lignin (ADL), *in vitro* digestibility (IVD), lactic acid, acetic and butyric acid. The leaves had the highest T-N (52 g kg Dm^{-1})] IVDM (695.6 g kg^{-1}) and lowest cell wall components, while the stem had the lowest T-N (15.8 g kg Dm^{-1}) and IVDM (334.9 k kg Dm^{-1}) but highest cell wall components. However, the whole forage had intermediate values. These differences were significant (P<0.01). The *p*H of the silages declined with advancing ensiling time but was generally high ($P \geq 5.00$) in all three. Although lactic acid was the dominant acid in all fractions, the difference between leaf and stem was significant (P<0.01) but that between either the leaf or stem and, whole forage were not significant (P>0.05). Acetic acid was very low in all and showed no significant difference (P> 0.05) among silages. Ensiling only slightly decreased the IVD and T-N in whole forage and leaves but not in stem.

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RÉSUMÉ

FLEISCHER, J. E., KAWAMOTO, Y., SHIMOJO, M., GOTO, I. & MASUDA, Y.: *Comparaison de la qualité de la fermentation et de la valeur nutritive entre le fourrage ensilé fait de leucaena (*Leucaena leucocephala *(Lam.) de Wit) et de deux autres herbes tropicales.* Leucaena (*Leucaena leucocephala* (Lam.) de Wit.) à l' âge d' environ 10 mois cultivé au Japan et l'herbe guinéenne répoussée (*Panicum maximum* var. *maximum* cv. Gatton) à l'âge de 52 jours et sorgho (Sorghum bicolor Moench cv. FS40IR) étaient tranchés à 3-5 cm de longeurs et ensilés dans les silos laboratoires d'une capacité de 1.4 - 1.5 kg. Les échantillos étaient prélevés à 0, 1, 3, 5, 7 et 30 jours d'ensilage pour la détermination laboratoire d'azote total (T-N), l' azote basique volatil exprimé en pourcentage de T-N, *p*H, acide lactique, acide acétique, acide butyrique, fibre détersive neutre (NDF), fibre détersive acide (ADF) cellulose, lignine d' acide détersive (ADL) et *in-vitro* capacité digestive (IVD). Il y avaient des différences significatives (P<0.05) dans le T-N et les constituants de mur cellulosique mais pas dans IVD (P>0.05) permi les espéces. Les *p*H finals des fourrages ensilés étaient 4.00, 3.94 et 5.27 respectivement pour herbe guinéenne, sorgho et leucaena. Acide lactique était l' acide dominant dans tous les fourrages ensilés. Les différences significatives (P<0.05) en contenu d' acide lactique étaient observées parmi les fourrages ensilés. Aucune difference significative (P>0.05) n' était observé dans les contenus d'acide acétique des herbes ensilées mais celles-si étaient considérablement plus fortes que celui de leucaena ensilé. Acide butyrique était observé uniquement dans l' herbe ensilée. L'IVD des fourrages ensilés aprés 30 jours étaient 488.5, 519.6 et 515.5 g en kg DM respectivement de l'herbe guinéenne, sorgho et leucaena.

Introduction

Leucaena (*Leucaena leucocephala* (Lam.) De Wit.) is a high nitrogen-fixing browse legume adapted to a well drained soil in the tropics and sub-tropics (Skerman, 1977). Its dry matter yield and crude protein content can be quite high, ranging between 7.5 and 25 t/ha (Skerman, 1977; Horgberg & Kuarnshtrom, 1982; Singh, Tripathi & Gill, 1991; Venkateswarlu, Korwar & Singh, 1991) and 250 and 360 g kg^{-1} DM (Skerman, 1977; Fleischer & Tackie, 1993) respectively. However, the potential benefits of this feed resource has been limited by its content of the free amino acid, mimosine, which is toxic to animals (Jones, 1979). Recent discoveries that the metabolism of mimosine in animals appear to be geographically dependent (Jones, 1979) and also that ensiling reduces the minosine content to non-toxic levels for animals (Hongo & Tawata, 1986; Hongo, Tawata & Kawashima, 1989) have renewed interest in its wide adoption to overcome the dry season feeding problems of ruminants. Indeed, the use of the leucaena is reported to increase not only organic matter intake and digestibility but also nitrogen intake (Bamualim *et al.*, 1984; Bamualim, 1985).

The objective of this work was to study the fermentation characteristics of leucaena either as whole forage or separate leaf and stem fractions, so as to gain an understanding of its possible nutritive value.

Materials and methods

Location

The plant material used was obtained from the University of Ryukyus in Okinawa, Japan, while the laboratory analyses were carried out at the Laboratory of Feed Science and Animal Behaviour, Kyushu University, Fukuoka, Japan.

Plant material

Leucaena (*Leucaena leucocephala* (Lam.) De Wit.) was collected from a naturalized field. The field was frequently harvested. The leucaena was about 10 months old and about 3 m tall. The plant was separated into leaf and stem fractions by hand and hence there were three treatments, viz., whole forage, leaf and stem.

Each sample was chopped to about 4 cm lengths and stuffed into 2-litre capacity laboratory silos and sealed. Each silo contained 1.4-1.5 kg of sample. There were three silos per treatment. The silos were opened after 1, 3, 5, 7 and 42 days of ensiling. Duplicate samples were taken at the time of ensiling and opening of silos for analyses.

Chemical analyses

At the time of opening the silos, the top and bottom 2-3 cm silage were discarded and samples were taken from the middle portion of the rest of the silage. These samples were further chopped up into 2-5 mm lengths and used for analyses. 100 g of each sample was placed in a one-litre conical flask and distilled water was added to it to the one-litre mark. This was kept in a refrigerator at 4 °C for at least 12 h with occasional shaking. The extract was filtered using a No. 41 Whatman filter paper. The *p*H of the filtrate was determined using a *p*H meter. The filtrate was used for the determination of organic acids and volatile basic nitrogen (Morimoto, 1971).

Fresh samples were used for total nitrogen determination (AOAC, 1990). Cell-wall components and *in vitro* digestibility were determined with the oven-dried samples according to the methods of Goering & Van Soest (1970) and Goto & Minson (1977) respectively.

Results

Chemical composition and *in vitro* digestibility of whole forage leucaena and its parts prior to ensiling are shown in Table 1. Nitrogen content and IVD were higher in leaf than in stem. On the contrary, dry matter and cell-wall components were lower in the leaf. Whole forage was intermediate between the stem and leaf in all the parameters.

Changes in organic acids and *p*H during ensiling are shown in Fig. 1. The *p*H of the three silages generally declines with time. The *p*H of the silage decreased from about 5.45 to 4.99;

TABLE 1

Chemical Composition and in vitro *Digestibility of Whole Forage Leucaena and its Parts before Ensiling (g kg^{-1})*

	DM	*T-N*	*NDF*	*ADF*	*Cellulose*	*ADL*	*IVD*
Whole forage	351.8	34.9	589.0	432.0	274.8	154.1	567.5
Leaf	289.0	52.6	433.2	180.2	122.4	57.8	695.6
Stem	508.5	15.8	833.2	627.3	427.2	200.6	334.9

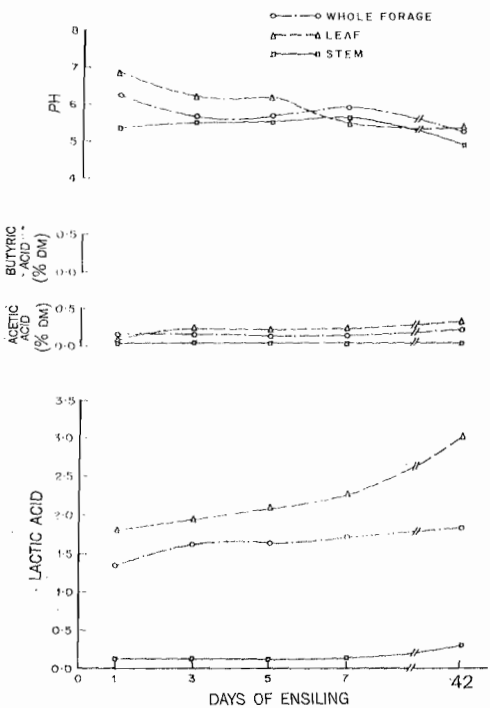

Fig. 1. Changes in organic acids and *p*H of leucaena silages with time

leaf from 6.85 to 5.35 and that of whole forage from 6.22 to 5.27.

Only in the stem silage, at the end of the storage period, was found some butyric acid (1.9 kg^{-1}). Acetic acid content was relatively low in all three silages. The differences among the silages were similar to the pattern observed with lactic acid but were not statistically significant (P>0.05). The dominant acid in all three silages at all times was lactic acid. Lactic acid was highest in leaf, rising from about 18.0 to 29.8 g kg^{-1}. Stems had the lowest content of lactic acid and was significantly different (P<0.01) from those of leaf and whole forage at all the periods. The lactic acid of whole forage was intermediate between the stem and the leaf; this was about 13.5 g kg^{-1} initially and rising only slightly to 18.0 g kg^{-1} by 42nd day. It was not significantly different from either stem or leaf at the respective times.

The changes in volatile basic nitrogen (VBN) content of the silages are shown in Fig. 2. Development of VBN, expressed as per cent of total

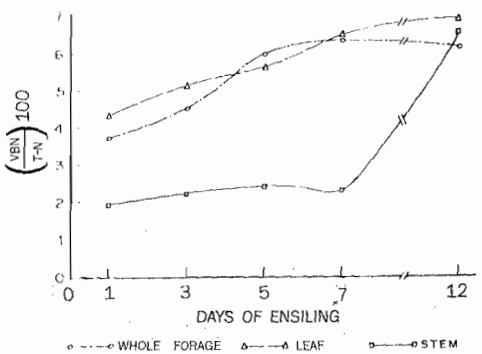

Fig. 2. Changes in volatile basic nitrogen content of leucaena silages with time

nitrogen, was very slow in stem silage in the first seven days, being up to about 2.3 per cent but rose thereafter. Consequently, by the 42nd day it had reached 6.7 per cent. The difference between the whole forage and leaf was not significant (P>0.05), but these were significantly higher (P<0.05) than that of stem in the first 7 days but not at the 42nd day. In all these silages the VBN content was 60-70 g kg^{-1} of T-N by the 42nd day.

Chemical composition and *in vitro* digestibility (IVD) of the silages stored for 42 days are shown in Table 2. Total nitrogen decreased dramatically in stem silage to 10.6 g kg^{-1} but only

TABLE 2

Chemical Composition and in vitro *Digestibility of Silage of Whole Forage Leucaena and its Parts (g kg^{-1})*

Chemical characteristic	*Whole forage*	*Leaf*	*Stem*
DM	365.3±15.1	286.6±16.2	501.0±20.1
Total Nitrogen	33.6±1.3	52.5±1.2	10.6±0.4
IVD	541.9±19.8	668.2±36.6	342.0±21.1
NDF	584.0±25.4	409.1±13.8	843.5±22.9
ADF	430.7±11.7	185.5±10.0	668.4±14.2
Cellulose	283.9±09.7	131.3±09.5	476.8±14.5
ADL	143.3±9.6	55.2±7.5	213.5±14.5

n = 5

slightly in whole forage to 33.6 g kg^{-1} and not in leaf. The variations in cell-wall components were only slight and non-significant (P>0.05) in IVD and decreased only slightly in leaf by 3.9 per cent units and whole forage by 4.5 per cent units but showed a marginal increase in stem by 2.1 per cent. These changes were not statistically significant (P>0.05).

Discussion

The variation in chemical composition and IVD of the stem and leaf as observed in the work reported here is consistent with reports in the literature (Norton, 1982; Hacker & Minson, 1982). Consequent upon such variation, that of whole forage is influenced by the proportion of these fractions as well as the stage of maturity at the time of harvest.

The development of organic acids was very slow especially in the stem and whole forage leucaena. Alli, Baker & Gracia (1983), working with whole forage leucaena, also observed that organic acid development and fall in *p*H of silage was very slow. These authors found that leucaena had very low levels of water-soluble carbohydrate. They did not, however, indicate the stage of maturity or leaf: stem ratio. In the experiment reported here, the leaf: stem ratio was about 1:1 and the material was about 10 months old. Like other legumes, leucaena is low in water-soluble carbohydrates (Smith, 1973; Kaiser, 1984) and has high buffering capacity (McDonald, 1981; Kaiser, 1984). Furthermore, the *p*H of the initial fresh forage material is relatively high (Alli, Baker & Gracia, 1983). Because the water-soluble carbohydrate is relatively low, the initial rapid fermentation is not sustained hence the fall in *p*H is very slow. In spite of these limitations, leucaena can still be successfully ensiled if it is combined with grass in reasonable ratio (Fleischer & Tackie, 1993) or some easily fermentable carbohydrate such as molasses or starch (Kaiser, 1984).

Extensive protein hydrolysis occurs during ensiling, giving rise to a number of products, one of which is volatile basic nitrogen (Ooshima & McDonald, 1978). This protein hydrolysis is rapid when the *p*H is high and slows down as the *p*H decreases (McKersie, 1985). Though the *p*H was not measured at the time of preparation the values obtained after one day were very high. The pattern of development of VBN in stem and leaf was different; that of the whole forage being closely related to that of the leaf. This is probably due to the differences in their content of water soluble carbohydrates. Lechtenberg *et al.* (1971) as quoted by Smith (1973) reported differences in the water soluble carbohydrate content of leaf and stem of alfalfa. Even though this was not determined in the experiment reported here, perhaps a similar pattern was the case here. McKersie (1985) also reported that the proteolytic potential activity in leaf varies among forages. Whether such difference exists between leaves and stems of the same species is not clear. In spite of that, the VBN at the end of the 42 days was about 70 g kg^{-1} of T-N. Ooshima and McDonald (1978) reported that the VBN in lactic acid silages is usually less than 100 g kg^{-1} of T-N. However, Kaiser (1984) has indicated that for silage intake to approximate that of the parent forage the VBN should be less that 50 g kg^{-1} of T-N. Thus, the silage, though could be considered good, the intake of this silage can be expected to be slightly reduced.

Decrease in total nitrogen content of silage compared to the fresh herbage has been observed in other trials (Fleischer *et al.*, unpublished). Ooshima & McDonald (1978) have reported that

not all the nitrogen in the herbage is accounted for on ensiling. Protein breakdown into various products in most forages is very high when cut but declines with advancing ensiling times as a result of the fall in *p*H (McKersie, 1985; Buchanan-Smith, 1982). Probably as a result of the breakdown in protein, some of the products might have been lost at opening and during the sample preparation. Oozing out of plant exudates (wounding) is known to be high. This might account for the large decrease in nitrogenous substances in the stem sample.

The non-significant changes in the cell-wall components is contrary to some observations made in earlier trials. Fleischer & Tackie (1993) observed a significant decrease in NDF but not in the other cell wall components. Wilkins (1981) quoting Kuntzel & Zimmer (1972) has indicated that structural carbohydrates may make a contribution to the fermentation of silage. Usually the cell-wall components reflect the degree of maturity and may influence the voluntary intake. Differences in the IVD is consistent with observations in the literature (Hacker & Minson, 1982).

Because of the large differences between stem and leaf and in view of the fact that the legume would form only 10-30 per cent of the total diet of ruminants, it is better to use a more immature forage of which the twigs could also be useful. This would not only improve the nitrogen intake of the animal but also the digestibility of the entire feed. Indeed, in using such materials, Tackie & Fleischer (1993) raised the nitrogen and IVD of *Sorghum arundinaceum* mixture from 60 and 170 to 230 and 600 in g kg^{-1} DM respectively.

Conclusion

The work reported here has shown that even though the development of organic acid in whole forage and leaf silages of leucaena was fast, the fall in *p*H was slow because of the high buffering capacities of the acids. The dominant acid in all three was lactic acid. Although the pattern of development of VBN was different between the leaf and the stem, that of the whole forage being similar to that of the leaf, in neither of them was this more than 100 kg^{-1} of the total nitrogen. There was a slight reduction in the total nitrogen in all the silages. The cell-wall components and the IVD of the silages did not significantly differ from those of the fresh material.

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