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

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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 Wit.) were used. 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). Both guinea grass and leucaena were mixed in a grass:legume ratio of 7:3 in T_3, T_4 and T_5. 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, *p*H, 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 *p*H and volatile basic acid (VAN) than leucaena. Guinea grass with leucaena starch had the lowest *p*H but was not significantly different from T_1, T_3 and T_5. The lactic acid content of T_4 was significantly higher ($P<0.05$) than the others. T_5 has the lowest VBN.

RÉSUMÉ

FLEISCHER, J.E., KAWAMOTO, Y., SHIMOJO, M., MASUDA, Y. & GOTO, I. : *Les caractéristiques de fermentation et les changements en valeur nutritive de l'herbe guinéenne-leucaena-ensilée avec ou sans additifs.* 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 Wit.) à l'âge de 10 mois étaient utilisées. Il y avaient cinq traitements c-à-d, herbe guinéenne (T_1), leucaena (T_2), herbe guinéenne-leucaena (T_3), herbe guinéenne-leucaena avec fécule (T_4) et herbe guinéenne avec acide formique (T_5). A la fois l'herbe guinéenne et leucaena étaient mélangées dans une proportion herbe:légumineuse de 7:3 en T_3, T_4 et T_5. Les échantillons étaient rembourrés dans un silo laboratoire (1.4 - 1.5 kg d'échantillon) et mis en réserve pour 42 jours. Les échantillons étaient prélevés pendant et aprés ensilage et analysés pour azote totale, azote basique volatile, comme pourcentage de N total, *p*H, acide lactique, acide acétique, acide butyrique, fibre détersive neutre (NDF), fibre détersive acide (ADF), cellulose, acide détersif lignin (ADL) et *in-vitro* digestibilité de matiére sèche (IVDMD). L'herbe guinéenne avait un contenu d'azote considérablement plus bas ($P< 0.01$) mais un contenu plus haut de fibre comparé à leucaena. Les valeurs de IVDMD (50%) étaint semblables ($P<0.05$) à la fois en herbe guinéenne et leucaena. Combinant l' herbe guinéenne avec leucaena élevait considérablement ($P<0.05$) le contenu d'azote de la mixture mais réduisait légèrement les composants de mur cellulosique. Il a également élevè la valeur de IVDMD (54 pour cent) bien que ce n'est pas considérable. L' addition de fécule reduisait le contenu d'azote et le mur cellulosique consistant mais augmentait la valeur (56 pour cent) de IVDMD. Ces changements étaient significatifs ($P <0.05$). L' ensilage de l'herbe guinéenne avait *p*H ($P<0.05$) considérablement plus bas et acide basique volatile (VAN) que leucaena. Herbe guinéenne avec fecule de leucaena avait *p*H le plus bas mais n' était pas considérablement différente de T_1, T_3 et T_5. Le contenu d' acide lactique de T_4 était

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considérablement plus élevé (P<0.05) que les autres. T_5 avait le plus bas VBN.

Introduction

Tropical grasses have high dry matteryield 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 probems 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 of 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_2O_5-K_2O 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 *p*H of the filtrate was immediately determined using a *p*H meter. The filtrate was used for the determination of organic acids and volatile basic nitrogen (VAN) (Morimoto, 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

TABLE 1

Chemical Composition and IVDMD of Forages before Ensiling

Forage type and treatment	*Dry matter*	*T-N*[1]	*Neutral detergent fibre*	*Acid detergent fibre*	*Cellulose*	*Acid detergent lignin*	*In vitro dry matter digestibility*
Guinea grass (T_1)	23.39a	1.47a	74.75a	46.60a	35.69a	8.69a	51.60a
Leucaena (T_2)	35.18b	3.86b	52.87b	42.70b	27.48b	15.41b	53.70a
Guinea grass + leucaena (T_3)	26.52a	2.62c	68.37c	43.14b	33.03c	8.87a	53.04a
Guinea grass + leucaena + Starch (T_4)	31.40ab	2.45d	54.89b	37.66c	29.12d	7.41a	60.41b
Guinea grass + leucaena + formic acid (T_5)	25.42a	2.44d	66.73c	42.37b	33.52c	7.86a	54.22a
SE[2]	4.812	0.009	1.043	0.499 n = 5	0.309	0.309	6.575

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

T_1 though not significantly (P<0.05) yet still lower (P<0.05) than that of T_2. Addition of formic acid (T_5) slightly depressed the DM compared to T_1 but addition of starch (T_4) raised it.

T_2 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_1. The IVDMD values of both T_2 and T_1 were, however, not significantly different (P<0.05) from each other.

The grass-legume combination (T_3) had significantly higher (P<0.05) T-N but lower fibres compared to T_1. These, however, did not affect the IVDMD. On the contrary, T_3 had significantly (P<0.05) lower T-N but higher fibres compared to those of T_2. The inclusion of additives, T_4 and T_5, significantly (P<0.05) reduced the T-N compared to T_3. However, the addition of starch, T_4,

TABLE 2

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

Forage type and treatment	*Dry matter*	*pH*	*Lactic acid (per cent)*	*Acetic acid (per cent)*	*T-N(1)*	*VAN×100(2) / T-N*
Guinea grass (T_1)	24.94a	3.95a	1.71a	0.31ab	1.53a	3.87ab
Leucaena (T_2)	37.83b	5.27b	1.95a	0.41a	3.36b	6.27a
Guinea grass + leucaena (T_3)	29.08a	4.17a	1.91a	0.26b	2.32ab	4.95a
Guinea grass + leucaena + Starch (T_4)	27.05a	3.87a	2.64b	0.27b	2.42ab	4.02ab
Guinea grass + leucaena + formic acid (T_5)28.40a	3.94a	1.67a	0.38ab	2.44ab	2.47b	
SE	4.939	0.587	0.391	0.067 n = 5	0.649	1.409

Figures in the same column with different letters are significantly different at 5 per cent probability level.
1) T-N = Total nitrogen
2) UBN × 100 = Volatile basic nitrogen expressed as percentage of total nitrogen.
T-N

significantly decreased the cell wall components and increased IVDMD.

Though differences were observed among the *in vitro* dry matter digestibility (IVDMD) values, only that of T_4 was significantly different (P<0.05) from others.

Fermentation characteristics of the silages are shown in Table 2. Except for leucaena silage (T_2) which had a significantly higher *p*H (P<0.05) compared to all the others no significant differences (P<0.05) were observed among T_1, T_3 T_4 and T_5. Followed by T_5, T_4 had the lowest *p*H. Lactic acid which formed the dominant acid ranged between 1.67 and 2.64 per cent. T_4 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 T_1, T_2 and T_5 were not significant (P<0.05). On the contrary, T_2 was signifcantly higher (P<0.05) than T_3 and T_4. No butryric acid was observed in any of the silages.

The T-N content showed a slight depression in T_2 and T_5 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. T_2 had the highest VAN content and this was significantly different from that of T_5. The differences among T_1, T_2, T_3 and T_4 were not significant (P>0.05). The VAN contents of T_1, T_4 and T_5 were not significantly different (P>0.05) from each other.

Cell wall components and IVDMD of the silages are shown in Table 3. The NDF, ADF and cellulose content of T_1 were significantly higher (P<0.05) than that of T_2. The NDF contents of T_3, T_4 T_5 were not significantly different (P>0.05) from each other. Even though they were lower than that of T_1 the differences were not significant (P>0.05). The NDF content of T_4 was not significantly different from that of T_2.

The ADF content of T_3, T_4 and T_5 were not significantly different (P>0.05) from that of T_2. Though T_5 was lower than T_1 the difference was not significant (P>0.05). Cellulose content of T_3 was lower than that of T_1 but higher than that of T_2. However, none of the differences was significant (P>0.05). Those of T_3, T_4 and T_5 were higher than that of T_2 but the difference was not significant (P>0.05). The ADL content of T_2 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 T_4 on ensiling (*cf* Tables 1 and 2).

IVDMD of T_2 was similar to that of T_1. Both T_3 and T_5 had slightly higher but not significant IVDMD compared to those of T_1 and T_2. On the

TABLE 3

Cell Wall Components and IVDMD of Guinea Grass-Legume Silage

Forage type and treatment	*Neutral detergent fibre*	*Acid detergent fibre*	*Cellulose*	*Acid detergent lignin*	In vitro *dry matter digestibility*
Guinea grass (T_1)	73.19a	51.95a	41.16a	7.43a	49.69a
Leucaena (T_2)	48.94b	42.24b	25.43b	14.26b	50.43a
Guinea grass + leucaena (T_3)	65.38	41.98b	32.67ab	7.94	53.95ab
Guinea grass + leucaena + Starch (T_4)	58.86ab	40.19b	30.81b	7.92a	55.84b
Guinea grass + luecaena + formic acid (T_5)	66.15a	45.10ab	34.35ab	9.10a	52.43a
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.

contrary, IVDMD of T_4 was significantly higher ($P<0.05$) than all the rest except that of T_3.

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 *p*H, all the other silages had reasonable *p*H levels. The relatively low *p*H 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 T_5. 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 *p*H.

The higher *p*H 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). Dermarquilly & 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 *p*H 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 or no change 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 IVDMD 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. Cameroon) and Lablab (*Dolichos lablab*). Uchida & Kitamura (1987) did not also observe any change in the IVDMD 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.

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REFERENCES

Alli, I., Baker, B. E. & Garcia, G. (1983) The ensilage of chopped, whole-plant leucaena. In *Proc. Vth Wld Conf. Anim. Prod.* **2**, 621-622.

AOAC (1990) *Official methods of analysis*, 15th ed. Washington D.C.: A.O.A.C.

Caldwell, M. M. (1975) Primary production of grazing land. In *Photosynthesis and productivity of different environments* (ed. J.P. Cooper), pp. 41-73. Cambridge: Cambridge University Press.

Carneiro, A. M., Sanchez, R. L., Rodriguez, N. M., Vileila, H. & Barbosa, A. M. A. (1984) Intake and apparent digestibility mixed silages of elephant grass cv. Cameroon and of Lablab. *Arquivo Brasileiro de Medicina Veterinaria Zootecinia* **36**, 597-608.

Catchpoole, V. R. & Henzel, E. F. (1971) Silage and silage-making from tropical herbage species. *Herb. Abstr.* **41**, 213-221.

Catchpoole, V. R. & Williams, W. T. (1969) The general pattern in silage fermentation in two subtropical grasses. *J. Br. Grassl. Soc.* **24**, 317-324.

Enishi, O., Shijiyama, K. & Shioya, T. (1991) Quality of sesbania *(Sesbania canabina* Pers) - corn (*Zea mays* L.) mixed silage and its intake and digestibility by goat. *J. Japan Grassl. Sci.* **37**, 213-218.

Fleischer, J. E. & Tackie, A. M. (1993) The possibility of making silage from "wild sorghum" (*Sorghum arndinaceum*). *Bull. Anim. Hlth. Prod. Afri.* (In press).

Goering, H. K. & Van Soest, P. J. (1970) *Forage fibre analyses*. Agriculture Handbook (USADA) No. 397, pp. 1-20.

Goto, I. & Minson, D. J. (1977) Prediction of the dry matter digestibility of tropical grasses using a pepsin cellulase assay. *Anim. Feed Sci. Technol.* **2**, 247-253.

Kaiser, A. G. (1984) The influence of silage fermentation on animal production. In *Silage in the 80's*. (ed. T. J. Kempton, A. G. Kaiser and Trigg), pp. 106-135. *Proc. natn. Workshop, Armidale, New South Wales, Australia, 8-9 Aug. 1984.*

McDonald, P. (1981) *The biochemistry of silage*. Chichester, New York: John Wiley and Sons.

Miaki, T., Tanaka, S. & Kawamura, O. (1986) Production and utilization of tropical forage silage. *Anim. Prod. Res.* **40**, 1291-1296 (In Japan).

Miller, T. B. (1969) Forage conservation in the tropics. *J. Br. Grassl. Soc.* **24**, 158-162.

Minson, D. J. (1981) Forage Quality: Assessing the plant-animal complex. (ed. J.A. Smith and V.W. Hays), pp. 23-29. In *Proc XIV int. Grassl. Congr. Lexington Kentucky, U.S.A. June 15-24, 1981* .

Morimoto, H. (1971) *Experimental methods in animal nutrition.* Yokendo, Tokyo, Japan, pp. 319-323 (in Japanese).

Moss, R. J., Byford, I. J. R., Winks, L., Chambers, G. & O'Grady, P. (1984) *In vivo* digestibility of tropical grass-legume pastures conserved as silage or hay. In *Silage in the 80's* (ed. T. J. Kempton, A. G. Kaiser and T. E. Trigg), pp. 137-140. *Proc. natn. Workshop, Armidale, New South Wales, Australia, 8-9 August, 1984.*

Norton, B. W. (1982) Differences between species in forage quality. In *Nutritional limits to animal production from pastures* (ed. J. B. Hacker), pp. 89-110. Farnham Royal, U.K.: Commonwealth Agricultural Bureau.

Ooshima, M. & McDonald, P. (1978) A review of the changes in nitrogenous compounds of herbage during ensilage. *J. Sci. Fd Agric.* **29**, 497-505.

Smith, D. (1973) The nonstructural carbohydrates. In *Chemistry and biochemistry of herbage* (ed. G.W. Butler and R. W. Bailey), Vol. 1, pp. 105-155. New York: Academic Press.

Uchida, S. & Kitamura, Y. (1987) Silage making from tropical pasture plants growing in South Western Islands of Japan. 1. Effect of various treatments at the ensiling time on quality of Rhodes grass and Napier grass silages. *J. Japan. Grassl. Sci.* **32**, 369-374.