ENSILING OF PEARL MILLET *PENNISETUM TYPHOIDES* WITH CLUSTERBEAN *CYAMOPSIS TETRAGONOLOBA* FOR ARID REGIONS

ENSILAJE DE *PENNISETUM TYPHOIDES* CON *CYAMOPSIS TETRAGONOLOBA* EN REGIONES ARIDAS

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SUMMARY

Clusterbean *(Cyamopsis tetragonoloba)* was ensiled with pearl millet *Pennisetum typhoides* harvested at late flowering stage in four different proportions of 25-100 p.100 (D.M. basis). Good quality silage was produced in 25 p.100 and 50 p.100 and 75 p.100 clusterbean proportions (pH 4.03 - 4.2; lactate 3.63 to 7.25 p.100 DM), while in 100 p.100 clusterbean silage, rapid decline in pH (4.2) and lactic acid production to the minimum required (3 p.100, D.M. basis) could not be achieved.

Increased proportions of clusterbean upto 75 p.100 improved the crude protein (C.P.) levels of the silage and with 75 p.100 proportions, C.P. level of 14.6 could be achieved. Coliform, clostridial and yeast and mould counts also confirmed the biochemical findings. A combination of clusterbean-pearl millet silage can help solving problems of conservation of legume crops besides providing a suitable feed for arid livestock.

RESUMEN

Cyamopsis tetragonoloba fue ensilado con *Pennisetum typhoides* cosechados al final de la etapa de floración en cuatro proporciones, del 25 al 100 p.100 (en materia seca). Se produjo ensilaje de buena calidad con *C. tetragonoloba* a las proporciones de 25,50 y 75 p.100 (pH 4,03-4,2; lactato 3,63 a 7,25 p.100 de la M.S.), mientras que en el ensilaje puro de *C. tetragonoloba* no se consiguió un rápido descenso del pH (4,2) y producción de ácido láctico (3 p.100 de la M.S.) a los mínimos requeridos.

El aumento de las proporciones de *C. tetragonoloba* por encima del 75 p.100 mejoró los niveles de proteína bruta en el ensilaje, consiguiéndose para el 75 p.100 un nivel de 14,6 p.100 de proteína bruta. Los recuentos de coliformes, clostridios y levaduras y hongos confirmaron así mismo los hallazgos bioquímicos. La combinación de ambas plantas para el ensilaje puede ayudar a resolver los problemas de conservación de las leguminosas forrajeras suministrando un alimento adecuado para el ganado de zonas áridas.

INTRODUCTION

The continuous increase in livestock numbers and decline in area

under common grazing lands has resulted in shortage of forage in the arid regions. The decline in the quantity of available forage has the immediate implication of survival of animals and the wider implication for the preservation of the desert ecosystem. Irrigational infrastructure may not necessarily lead to increased dry matter supply owing to shift to commercial crops which yield less or no animal fodder. Similarly prospects of increasing forage supply through improved range practices or increasing grazing lands have their own limitations (Anonimous, 1992).

A better understanding of the potentialities of the existing poor quality feed and improved conservation practices is therefore required for most optimum utilisation of existing fodder. The process of ensiling, suitably modified for the arid regions appears to be of greatest advantage for conserving and stabilising the nutritive value of the forage (Pancholy and Mali, 1992).

Clusterbean (*Cyamopsis tetragonoloba*) is a drought resistant, salinity tolerant legume commonly grown in Indian arid regions and utilised both for human and animal consumption Pearl millet (*Pennisetum typhoides*) is another quick growing crop of arid and semi-arid regions and is a non maintenance type of fodder with 4.5 p.100 protein at flowering stage. It is difficult to conserve leguminous crops as a silage since they contain lower amount of soluble carbohydrates to ensure good type of fermentation, high moisture and high buffering capacity (Miller and Clifton, 1965). Good quality silage has been produced by mixing legumes with oats and wheat straw (Kamra *et al.*, 1983), dry roughages and molasses (Singh *et al.*, 1985). In the present study attempt has been made to produce silage by mixing clusterbean with millet forage harvested at late flowering stage.

MATERIAL AND METHODS

The experiments consisted of 4 feed sets in which *Pennisetum typhoides* (D.M. 32.5 p.100, C.P. 4.8 p.100, W.S.C.1.8) and clusterbean *C. tetragonoloba* (D.M.22.5 p.100, C.P.13.5 p.100, W.S.C.2.25) was precision chopped and mixed with minimum possible wilting, in different proportions of 75:25 (Treatment I), 50:50 (Treatment II) and 25.75 p.100 (Treatment III) on D.M. basis. In all the sets, urea and molasses were added at the rate of 1 p.100 and 8 p.100 respectively (on D.M. basis) and a non-specific lactic acid bacterial culture (LAB; cell concentration, adjusted to 1.8×10^6 cells ml^{-1}) was added at the rate of 6 p.100 on D.M. value of the premix in the form of fermented buttermilk. The components of each sets were mixed manually and the D.M. of the mixture was adjusted to 32-34 p.100.

The mixture was filled into miniature laboratory silos in triplicate (cap. 1.5 l, plastic jars double lid with air tight screw type closing arrangement) compactly excluding the entrapped air to maximum possible, and incubated at room temperature (26 ±2°C) for 75 days. Three replication

per set for each periodical opening were taken and thus silos were opened in triplicate per set on 15, 30, 45, 60 and 75th day and discarded after taking sample. Samples were drawn periodically for biochemical and microbiological analysis.

From each silo, 20 g of fermented product was taken and mixed with 180 ml of distilled water in a high speed blender for 3 minutes. The extract was double filtered and centrifuged at 8000 rpm for 15 min. The supernatant was used for measurement of biochemical parameters. Water soluble carbohydrates (WSC) was estimated by the anthrone method (Yemn and Willis, 1954). Lactic acid was estimated by the method described by Barker and Summerson (1941) and pH were also taken. Total nitrogen and crude protein were estimated on kjeltec-1003 by the microjeldahl method and D.M. was observed by the method described by AOAC (1975).

For the microbiological analyses, silage was extracted in the same way as for the chemical analysis except that autoclaved physiological saline was used instead of distilled water, and the samples were collected under aseptic conditions.

The extract was serially diluted and plated on various selective media. Total bacterial population was obtained on glucose and yeast extract medium (Kroulik *et al.*, 1955). The plates were incubated at 37°C for 24 hrs. coliforms were estimated by the most probable number (MPN) technique using McConkey broth medium (Indian Standards Institute, 1977) and confirmation done on eosine methylene blue agar; yeast and moulds were estimated of Rose-bengal agar (Jarvis, 1973), clostridial testing on sodium sulphate iron citrate medium (Mosell *et al.*, 1956) and lactic acid bacterial number were obtained by plating appropriate dilutions on Rogosa agar (Rogosa *et al.*, 1951).

Statistical analysis was done using two way analysis of variance (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The silages produced from treatment I, II and III (25, 50 and 75 p.100 clusterbean proportions) were dark brown in colour, had pleasant aroma and sufficient moisture and were almost similar in appearance to the original millet forage with a green colouration of clusterbean. Silage produced in the fourth set (Treatment IV: clusterbean 100 p.100 proportion) was slimy to touch, dark green to black in colour and had a strong offensive smell, indicative of clostridial-butyric fermentation.

Effect of fermentation on pH, lactate, and water soluble carbohydrates (WSC) have been presented in **table I** (a and b) and the biochemical and microbiological characteristics of the silage from all the four treatments have been presented in **table II**.

All the silages from treatment I to II were well preserved as indicated by low pH values and high lactic acid concentrations.

Individual pH averages of all the silages from T-I to T-III at 60 and 75

days i.e. completion of optimum ensiling period was less than 4.1 conforming good quality.

The sudden decrease in lactic acid levels after 30 days of ensiling in treatment IV (3.24 to 1.27) is significantly ($p < 0.05$) higher than the decrease in other three treatments.

Moreover, initial lactate levels after 15 days of ensiling are also significantly ($p < 0.05$) lower than other three treatments where millet was added to clusterbean in the premix. It is possible that in treatment IV, the rapid decline in pH of silage is difficult to achieve because legumes have high buffering capacity and a low content of WSC (McDonald, 1981). Failure to achieve a rapid decline in silage pH leads to extensive protein degradation during ensiling, proliferation of undesired microorganisms and a reduction in nutrient value of silage (Flores *et al.*, 1986). Due to high buffering capacity, the substrate requirement for lowering the pH by fermentation increases. Clusterbean having a relatively low fermentable

Table I. *Effect of fermentation on pH and lactic acid (p.100 D.M.).* (Efecto de la fermentación sobre el pH y concentración de ácido láctico (p. 100 M.S.)).

Treatment	Period in days					Mean
	15	30	45	60	75	
pH						
TI	5.23b	4.10f	4.03f	4.06f	4.03f	4.29
TII	4.70c	4.03f	4.03f	4.03f	4.03f	4.16
TIII	5.13b	4.30e	4.10f	4.03f	4.03f	4.31
TIV	5.60a	4.70c	4.56d	4.50d	4.50d	4.77
Mean	5.16	4.28	4.18	4.15	4.14	
Critical difference 1 p.100 for			treatment (A) (0.083)	period (B) (0.092)		interaction (AxB) (0.185)
Lactic acid						
T1	5.81de (13.94)	6.34cd (14.61)	7.77ab (16.18)	6.79cd (15.03)	7.25bc (15.64)	6.79 (15.08)
T2	6.23cd (14.46)	7.15bc (15.52)	8.30a (16.74)	8.12ab (16.57)	8.07ab (16.50)	7.57 (15.95)
T3	5.73de (13.89)	6.15cd (14.38)	4.19ef (11.88)	4.11ef (11.73)	3.63f (10.98)	4.76 (12.57)
T4	13.19f (10.30)	3.24f (10.35)	1.51g (7.03)	1.27g (6.46)	1.62g (7.34)	2.16 (8.29)
Mean	5.24 (13.14)	5.72 (13.71)	5.44 (12.95)	5.07 (12.44)	5.14 (12.61)	
Critical difference 1 p.100 for			treatment (A) (0.443)	period (B) (0.495)		interaction (AxB) (0.990)

Figures in parentheses shouw angular (arcsin) transformation value. Means with different superscripts differ significantly ($p<0.01$)

Table II. *Biochemical and microbiological characteristics of silage.* (Características bioquímicas y microbiológicas del ensilado).

Treatment	T I	T II	T III	T IV
pH	4.03	4.03	4.03	4.50
Lactlc acld (p. 100 D.M.)	7.25	8.07	3.63	1.62
Dry matter (p. 100)	40.l0	39.70	39.20	37.80
WSC (p. 100 D.M.)	1.70	1.39	1.08	0.71
Total nitrogen (p.100)	1.436	2.02	2.33	1.77
Crude protein (p. 100)	8.97	12.67	14.57	11.10
Coliforms*	Absent	Absent	Abs ent	1.42
Yeast/moulds*	5.25	5.75	5.33	2.92
Total bacteria*	6.12	6.25	6.42	7.12
Clostridia*	Absent	Absent	Absent	1.72
Lactic acid bacteria*	7.85	7.74	7.68	3.22

*(log number of cells/gram)

substrate content may not be able to meet this extra demand for substrate and thus the pH may not be lowered sufficiently when clusterbean is ensiled in 100 p.100 proportions, as previously reported with lucerne (Melvin, 1965). Even the addition of urea at the rate of 1 p.100 (D.M. basis) and molasses 8 p.100 (D.M. basis) has not shown any effect on unstable nature of clusterbean silage probably due to consumption of sugars by proliferation of undesirable micro-organisms as seen in microbiological examination. The addition of millet in varying proportions of 25-75 p.100 has reduced the buffering effect and increased the fermentable substrate leading to desired silage fermentation and subsequent quality.

The minimum herbage WSC necessary to produce well fermented silage without additive has been reported as 25-30 g kg^{-1} (on dry matter basis), although a higher value of 30-35 g kg^{-1} has also been suggested (Haigh, 1990). In the present experiment WSC content of millet was less than minimum required (18.7 kg). While the supplementation of green millet to clusterbean in the present experiment, may provide minimum required level of SWC, the additional molasses has been added for the urea addition at 1 p.100 level (D.M. basis). Since the sugars in the silage should be sufficient to produce enough acids to bind ammonia-N evolved during fermentation and addition of urea without molasses has a prominent effect on ammonia production in earlier reports (Kamra *et al.*, 1983)

An intense metabolic activity within 15 days of fermentation indicative of proper silage fermentation is shown in treatment 1, II and III by increasing lactic acid levels and rapidly

falling pH associated with decreasing residual WSC levels, which is in accordance with previous reports (Patterson *et al.*, 1990). However, only rapid degradation of sugars without correlation with pH and lactate levels could not lead to good quality silage production as in case of treatment IV.

The microbiological analysis of the silage (**table III**) shows significantly lower value of LAB in treatment IV conforming to biochemical values, while there is no major difference between lactobacillus counts in treatment I, II and III. The total bacterial count showed a correlation with the treatment and treatment IV showed higher count probably indicating that the conditions favoured growth of organisms other than lactobacillus which might have finally consumed the fermentable substrate. Coliform bacteria were present only in treatment IV. It is reported earlier that pH of the silage must be as low as 4.2, so that the coliform growth is inhibited and the silage is free from coliform bacteria (Kamra *et al.*, 1983).

In treatment IV the final pH was 4.77 which was not sufficient in abolish the coliforms completely from silage. Silage from treatment IV also showed presence of clostridia which renders the silage unfit for consumption, apart from diverting the direction of VFA production.

Table III. *Effect of fermentation on water soluble carbohydrates (p.100 D.M.).*
(Efectos de la fermentación sobre los carabohidratos solubles en agua (p.100 M.S.).

Treatment	Water soluble carbohydrates (p.100 D.M.) Period in days					Mean
	15	30	45	60	75	
T I	3.82^{a}	2.26^{cd}	2.16^{de}	1.79^{ef}	1.70^{f}	2.34
	(11.29)	(8.73)	(8.46)	(7.63)	(7.49)	(8.72)
T II	3.21^{ab}	2.67^{bc}	2.07^{de}	1.65^{f}	1.39^{fg}	2.19
	(10.35)	(9.40)	(8.26)	(7.41)	(6.80)	(8.44)
T III	2.10^{de}	1.86^{ef}	1.36^{fg}	1.18^{g}	1.08^{g}	1.51
	(8.33)	(7.85)	(6.71)	(6.29)	(6.02)	(7.04)
T IV	1.53^{f}	1.21^{g}	1.09^{g}	1.00^{g}	0.71^{h}	1.10
	(7.11)	(6.29)	(6.02)	(5.83)	(4.80)	(6.01)
Mean	2.66	2.00	1.67	1.40	1.22	
	(9.27)	(8.06)	(7.36)	(6.79)	(6.27)	

CD 1 p.100 for treatment (A)	(0.171)
CD 1 p.100 for period (B)	(0.191)
CD 1 p.100 for interaction (AxB)	(0.382)

Figures in parentheses show angular (arcsin) transformation values. CD= Critical difference. Means with different superscripts differ significantly ($p<0.01$).

Therefore from the microbiological point of view also, the silage in treatment IV was not of desired quality and the bacterial counts confirmed the biochemical values.

Good silage can traditionally be made from a mixture of legume forage with a suitable proportion of green fodder or any material which contains more sugar (Kamra *et al.*, 1983) or straw supplemented with molasses (Singh *et al.*, 1985). The results from the present study indicates possibilities of ensiling millet harvested at a late stage with 25-75 p.100 proportions of clusterbean to provide protein rich feed to animals normally fed on crop residues of low crude protein content especially during dry season. Urea-molasses supplementation at the rate of 1 p.100 and 8 p.100 (D.M. basis) respectively can provide NPN and fermentable nitrogen and energy sources, while effective LAB population ensure effective fermenta-tion of the crop being ensiled. In arid regions LAB inoculant addition has been shown to be beneficial owing to lesser natural epiphytic level of lactobacillus (Pancholy and Mali, 1994).

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