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FINAL TECHNICAL REPORT

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PROJECT TITLE: **STRATEGIC USE OF *LEUCAENA LEUCOCEPHALA* AND MOLASSES/UREA BLOCKS FOR FEEDING DUAL-PURPOSE GOATS**

USAID PROJECT NO: PCE 5053-G-00-3066-00

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EXECUTIVE SUMMARY

Leucaena is a leguminous forage tree with a similar nutritive value as alfalfa. However, continuous exposure of animals to mimosine, a toxic chemical in the plant causes reduced feed intake and fertility, alopecia, renal and hepatic dysfunction or even death. Therefore, a series of experiments were carried out to develop feeding strategies which will ensure that dual purpose goats receive adequate nutrition using leucaena as a dietary protein source.

In the initial trial several attempts were made to make leucaena-molasses/urea blocks acceptable for goats. In the next trial increasing levels of leucaena (o, 15, 30, 45 and 60% leucaena) was incorporated into a total mixed diet for both Angora and cashmere producing goats. Goats on the 45% leucaena diet consumed the highest intake while the goats on the control and 15% leucaena diets were lowest but ADG was not significantly affected by treatment. However, feed efficiency was lowest for the high leucaena levels (45 and 61%). In the final trial, twenty multiparous does were used to evaluate the effect of leucaena on the milk production and composition for Alpine goats. In this experiment 50% of the dietary protein was provided by either leucaena or alfalfa. No differences ($P > .1$) were found between alfalfa and leuceana treatments in milk production (3.21 vs 3.07 kg), milk fat (2.44 vs 2.65%), lactose (4.27 vs 4.29%) and SNF (7.75 vs 7.72%).

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EXPERIMENT ONE

Productivity of Angora Goats in Response to Addition of Protein to a Basal Diet of Cottonseed Hulls and Molasses/Urea Blocks

INTRODUCTION

In most less developed countries (LDC) protein meals are too expensive for small farmers. Therefore, there is a need to develop a protein supplementation strategy that will maximize the use of the local resources. This system would make strategic use of locally produced oil seed cakes (as sources of bypass protein) and provide a source of soluble nitrogen. Molasses, urea and oil seed cakes are available, however, their use as animal feed in LDC's is limited.

The leguminous tree, *Leucaena leucocephala,* is ubiquitous to many LDC's where it is used for firewood and as an animal feed. It is fast-growing once established, and because of its deep roots, tolerates adverse moisture conditions, growing actively during the dry season. Leucaena has been shown to have great potential as a source of high quality feed for ruminants. Its leaves are highly palatable with a high digestibility (up to 70% reported) and a high crude protein (CP) content (26%). In comparison, digestibility and CP content of feedstuffs consumed by most ruminants in LDC's would be in the range 40 - 50% and 6 - 10%, respectively. Leucaena can be used for cut and carry feeding systems, dried for leaf meal, used as a silage or browsed as standing bushes.

Leucaena has a low protein solubility in the rumen and has been shown to be an effective bypass protein supplement with urea-molasses in intensive beef production (Alvarez et al., 1977). However, low liveweight gains, depressed appetite, alopecia, enlarged thyroid glands, depressed serum thyroxine levels, ulceration of the oesophagus and death of newborn offspring were encountered when leucaena was the sole feed source (Jones and Hegarty, 1984). These effects are caused by the ruminal metabolite of mimosine, 3-hydroxy-4(1H)-pyridone (DHP),

a potent goitrogen (Hegarty et al., 1979). These problems are avoided when leucaena is fed at 30 to 60% of the diet (Jones and Hegarty, 1984; Adejumo and Ademonsun, 1991).

Though supplementation with protein rich meals is expensive if it is applied indiscriminately, proper resource management will ensure a good return on the investment. The technology to assist small farmers in LDC's is available but technology transfer has not occurred due to the lack of expertise and training at the local level.

MATERIALS AND METHODS

Forty-eight yearling Angora goats were blocked by body weight and sex and randomly assigned to one of eight treatments. To a basal diet of ad libitum cottonseed hulls and molasses/urea blocks (CON) the following treatments were added: 100 g/d leucaena (LEU); 70 g/d fishmeal (FM); 100 g/d cottonseed meal (CSM); 50 g/d blood meal (BM); FM plus 100 g leuc (FM+); CSM plus 100 g Leuc (CSM+); BM plus 100 g Leuc (BM+). Equal amounts of nitrogen was supplied by the FM, CSM and BM supplements. Animals were fed at 1000 h each day and had continuous access to fresh, clean water. The molasses/urea blocks were made according to the specifications given by Sansoucy (1986) and were available on an ad libitum basis. An adaptation period of 2 weeks was allowed to accustom the animals to consuming the block. For the first week of the adaptation period the goats were allowed 30 g/d of the M/U block and for the second week, 60 g/d and finally the block was available on an ad libitum basis for the duration of the trial. The composition of the blocks was molasses 50%, urea 10%, salt 5%, cement (dry) 10%, and wheat bran 25%. The mixture was mixed in 20 kg batches, put into small buckets and placed in a forced air oven at 60°C for two days to harden.

The experiment consisted of a 2 wk habituation period, and a 8 wk feeding period. The goats were housed at ambient temperature in raised, individual stalls indoors with natural lighting. During the 2 wk habituation period, goats were adapted gradually to ad libitum access to the molasses/urea blocks and the cottonseed hulls. Water was available at all times through nipple drinkers. Feed intake was recorded daily, and BW was measured once each week before feeding. The diets were sampled throughout the trial and analyzed for DM, CP (Technicon Instruments Co., Tarrytown, NY), gross energy using an adiabatic oxygen bomb calorimeter (Parr Instrument, Moline, IL), NDF, and ADF (Goering and Van Soest, 1970).

Mohair Measurements. All goats were shorn at the beginning of the trial, and at the end of the trial. A midside sample was collected from each fleece; clean mohair yield and staple length were determined according to the ASTM (1988) Standards; fiber diameter was determined using Peyer FDA 200 System (Walleran, Switzerland) as described in Sahlu and Fernandez 1992, and Sahlu et al., 1992.

Blood and Ruminal Traits. On days 30 and 60 ruminal fluid samples were obtained at 0, and 2 h postprandial by applying a vacuum to an esophageal tube fitted with a suction strainer as described by Sahlu et al. (1992). Ruminal fluid samples were analyzed for VFA and ammonia N as described by Lu et al. (1990). Jugular blood samples were collected on these days by venipuncture into tubes containing either sodium heparin or potassium oxalate and sodium fluoride (Becton Dickinson Vacutainer Systems, Rutherford, NJ). Tubes were chilled immediately in an ice bath and transported to the laboratory, and centrifuged at 1500 x *g* and 4°C for 20 min. Aliquots of plasma were stored at -20°C until analyzed.

Concentration of plasma nonesterified fatty acids (NEFA) was determined with a commercial kit using an enzymatic colorimetric procedure (Wako Pure Chemical Industries, Richmond, VA) as described in Sahlu et al. (1995). Plasma urea N, total protein, and glucose were quantified colorimetrically using a Technicon Autoanalyzer II System (Technicon Instruments, Tarrytown, NY).

Statistical Analysis

Feed conversion ratio and mohair fiber measurements were analyzed by analysis of variance by PROC GLM of the Statistical Analysis Systems Institute Inc. (SAS, 1982). Average daily gain for each goat was estimated by PROC REG of SAS (1982) using the regression of liveweight on the number of days of treatment. Dry matter intake was analyzed using repeated measures and further analyzed using covariate analysis to test the slopes of the regression lines. Blood parameters were analyzed as a split-plot in time, and when there were no treatment by time interactions, the overall treatment means are given in the results.

RESULTS

Production Variables. Two goats were withdrawn from the experiment as they would not consume any molasses/urea blocks. All other goats accepted the blocks and their intake of the block is given in Table 1. Average daily gain (ADG) is given in Figure 1. The goats on the fishmeal treatment had a higher ADG ($P < .06$) than the control goats. Goats on the other protein supplements and those supplemented with protein and leucaena had ADG's similar to that of the control ($P > .10$). Intake of cottonseed hulls (CSH) and CSH plus protein supplement are given in Figures 2 and 3. Goats on the FM, CSM, BM and BM+ treatments had higher intakes

($P<.001$) of cottonseed hulls than the control goats. Total dry matter intake was highest on the CSM treatment and lowest on the control ($P<.01$). Feed conversion ratios were similar between treatments with only BM+ being different from the control ($P<.07$). The FCR for the CON, LEU, FM, CSM, BM, FM+, CSM+ and BM+ goats were 11.8, 19.4, 9.1, 14.0, 9.5, 12.9, 15.9 and 24.5, respectively.

Mohair production data are given in Table 1. There were significant treatment differences in both grease and clean fleece production ($P<.02$). Goats on the LEU ($P<.02$), CSM+ ($P<.01$) and the BM+ ($P<.08$) produced significantly less clean mohair than did the CON goats. Fiber diameter was the finest for goats on the CSM+ treatment ($P<.01$) with no other significant differences found. Length growth, med and kemp percentages were similar among treatments ($P>.10$).

Results of blood metabolites are given in Table 2. No differences were found in plasma concentrations of glucose, NEFA, total protein or urea-N between goats on the different treatments.

Ruminal measurements are given in Table 3. No differences were found in rumen ammonia levels or total VFA levels ($P>.10$). Levels of acetate were higher in the CSM ($P<.08$) and the BM+ ($P<.01$) goats than in control goats. Levels of propionate were lower in the FM, CSM, BM and BM+ goats ($P<.05$) than in the control. Goats in the FM+ had higher ($P<.01$) isobutyric levels than goats in the LEU, CSM, BM and BM+ groups. Isovaleric levels were higher in FM goats than CSM ($P<.01$), BM ($P<.07$) and BM+ ($P<.03$) goats.

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TABLE 1. Mohair characteristics of Angora goats fed a basal diet of cottonseed hulls, molasses/urea blocks and supplemented with different protein sources

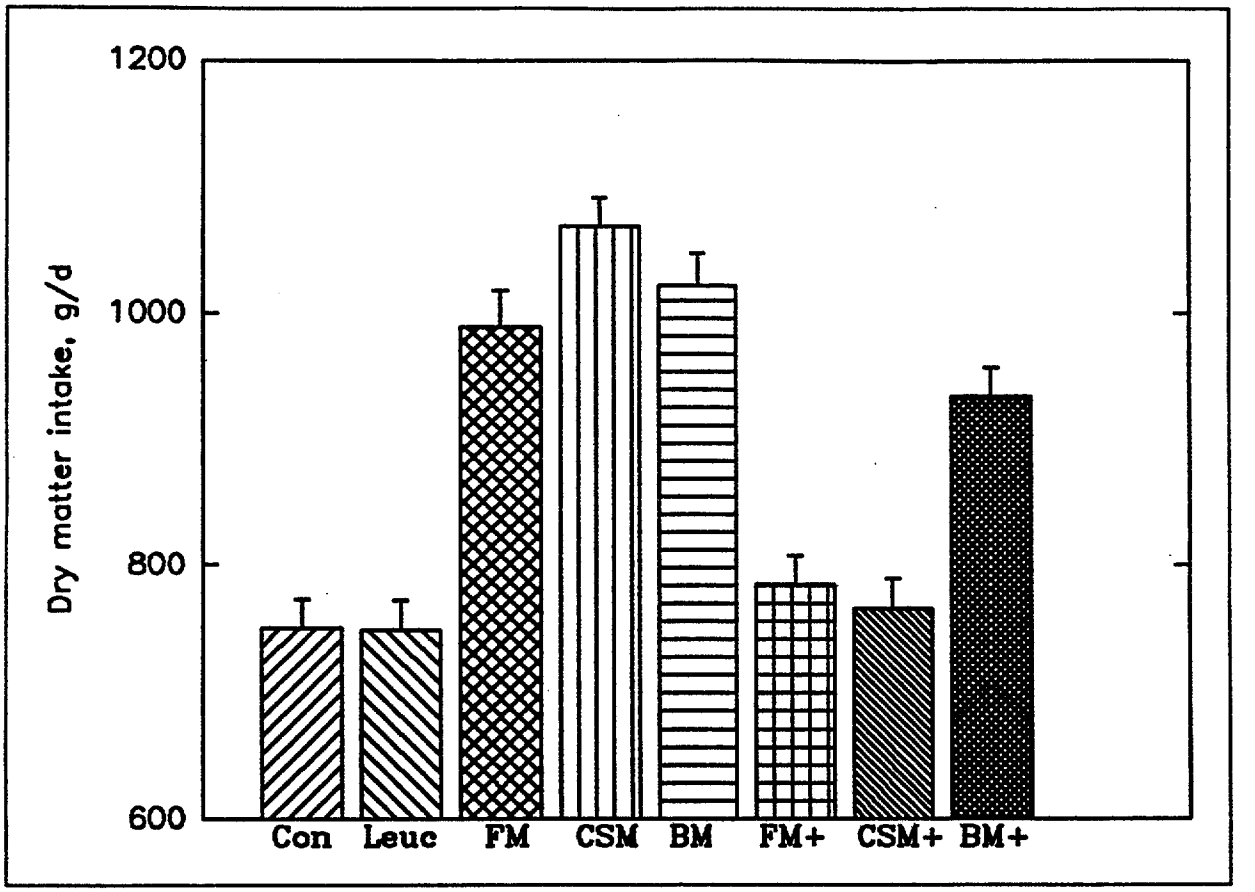
Item	Con	Leu	FM	CSM	BM	FM+	CSM+	BM+	SEM	P<
Fleece,g/d										
Grease	10.6	8.5	11.8	11.1	10.2	10.2	8.4	8.8	.9	.02
Clean	8.9	6.9	9.3	8.8	8.3	8.4	6.7	7.4	.7	.02
Length, μm/d	958	965	925	1005	1011	997	966	987	34	.53
Diameter, μm	28.2	27.7	26.6	27.5	28.5	26.7	25.3	28.8	1.0	.06
Med, %	.12	.07	.03	.17	.06	.07	0	.15	.05	.19
Kemp, %	.25	.22	.33	.20	.18	.19	.17	.48	.17	.76

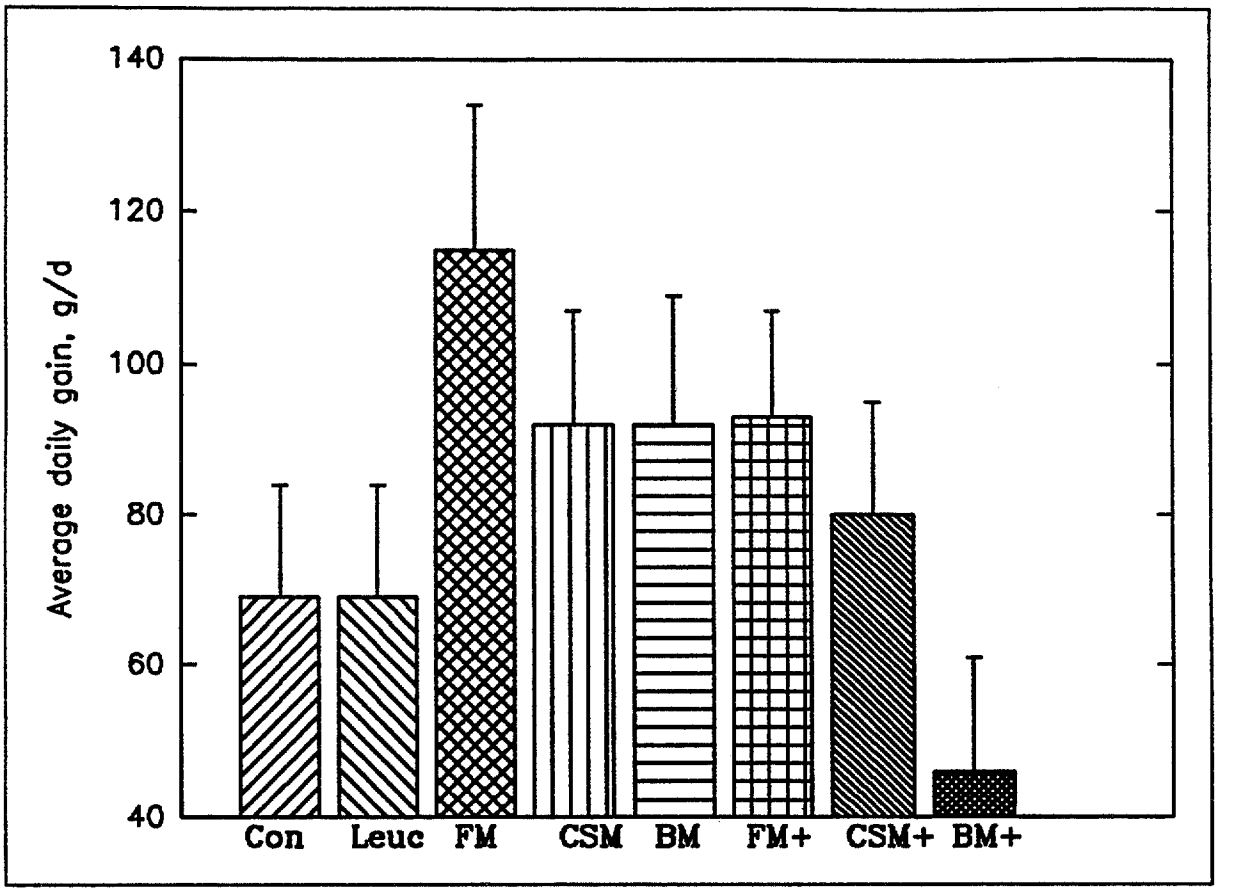
TABLE 2. Blood metabolites of Angora goats fed a basal diet of cottonseed hulls, molasses/urea blocks and supplemented with different protein sources

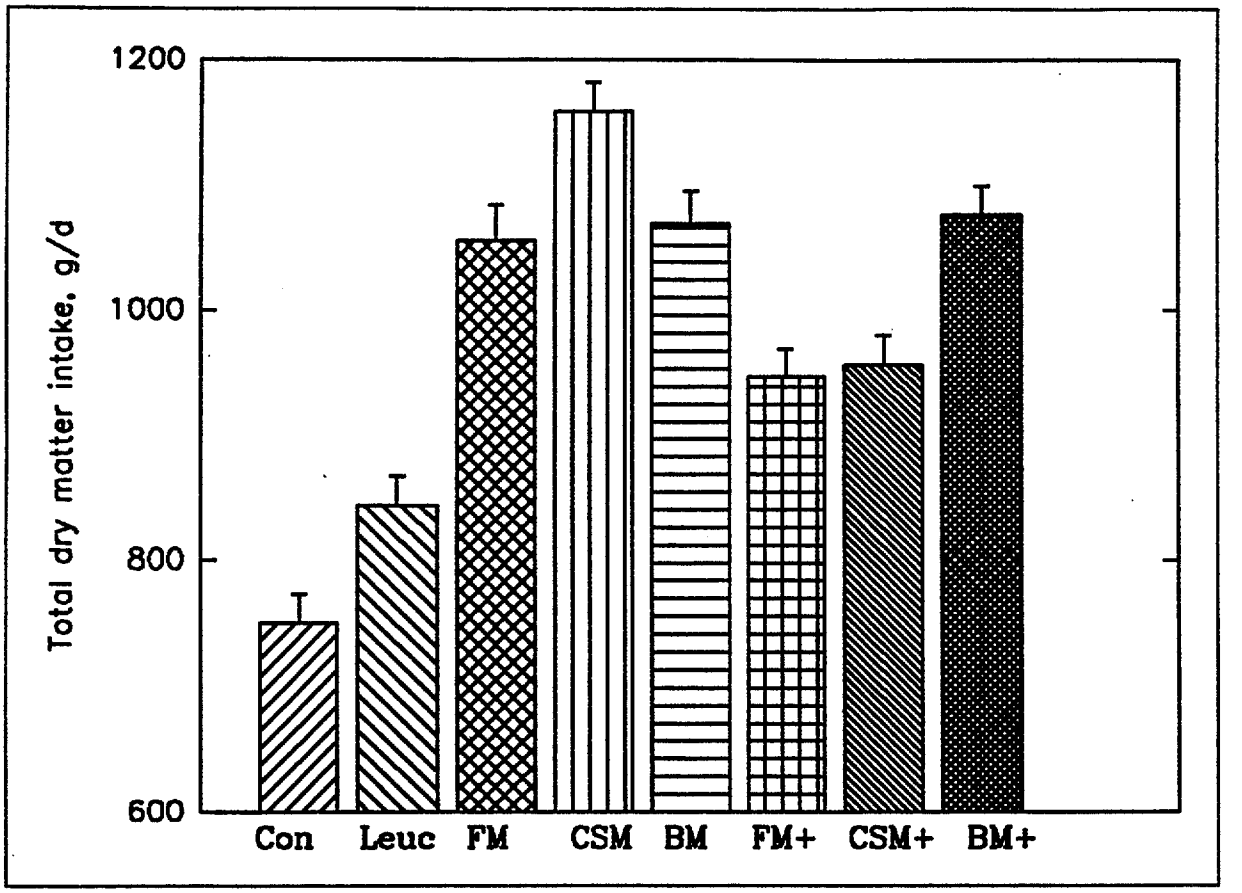
Item	Hr	Con	Leu	FM	CSM	BM	FM+	CSM+	BM+	SEM	P<
Glucose	0	57.7	56.4	56.7	58.3	57.1	56.1	54.7	51.6	2.8	.58
mg/dl	2	58.7	57.0	61.3	60.9	56.1	65.1	60.0	51.2	4.1	.18
NEFA	0	216	183	180	212	243	203	209	204	22	.78
μeq/L	2	192	171	149	172	221	181	182	182		
TP	0	54.7	53.2	57.2	58.2	54.1	62.9	57.7	55.0	6.4	.69
g/L	2	67.5	77.8	70.9	59.8	67.3	65.7	59.7	66.8		
Urea-N	0	17.9	18.7	16.1	15.5	15.5	17.5	16.4	13.9	1.3	.78
mg/dl	2	18.6	17.7	18.0	15.4	16.7	18.8	15.7	13.5		

TABLE 3. Rumen volatile fatty acids (VFA), Ph and ammonia (NH_3) in goats fed a basal diet of cottonseed hulls, molasses/urea blocks and supplemented with different protein sources

Item	H	Con	Leu	FM	CSM	BM	FM+	CSM+	BM+	SEM	P<
Rumen NH_3	0	10.3	10.0	11.5	9.7	8.6	8.0	10.2	7.5	1.5	.77
(mg/dl)	2	6.8	6.1	8.4	7.6	7.0	7.8	9.6	6.7		
VFA, mol %											
Acetate	0	75.1	75.9	77.4	77.2	76.6	76.5	75.5	78.7	.7	.09
	2	73.7	73.9	76.2	76.2	76.4	74.3	74.2	77.5		
Propionate	0	17.7	16.6	15.5	14.7	15.5	15.8	16.4	14.5	.7	.03
	2	19.1	18.9	16.4	15.6	15.9	17.5	18.4	15.3		
Isobutyric	0	.23	.15	.24	.17	.14	.25	.25	.14	.04	.02
	2	.22	.16	.22	.13	.16	.32	.17	.19		
Butyric	0	5.5	6.3	5.5	6.8	6.6	5.9	6.3	5.5	.4	.17
	2	5.8	6.2	5.9	7.1	6.4	6.6	6.2	5.9		
Isovaleric	0	.45	.23	.84	.39	.51	.70	.68	.44	.11	.01
	2	.34	.13	.69	.25	.45	.52	.33	.43		
Valeric	0	.95	.84	.75	.76	.67	.80	.84	.78	.06	.14
	2	.77	.72	.66	.69	.66	.73	.74	.69		
Total VFA	0	91.5	89.1	99.8	87.7	86.2	87.1	81.6	76.9	4.2	.72
(mM)	2	77.7	77.0	83.7	82.9	77.5	77.4	80.9	79.4		


1200
1000
800
600
Dry matter intake, g/d
Con
Leuc
FM
CSM
BM
FM+
CSM+
BM+


Average daily gain, g/d
140
120
100
80
60
40
Con
Leuc
FM
CSM
BM
FM+
CSM+
BM+


1200
1000
800
600
Total dry matter intake, g/d
Con
Leuc
FM
CSM
BM
FM+
CSM+
BM+

EXPERIMENT TWO

Leucaena Leucocephala as a Dry Season Supplement for Angora Goats

ABSTRACT

Thirty Angora goats (19 wethers, 11 doelings) 8 mo of age and 17 kg BW were used in an 85 d study to determine the potential of *Leucaena leucocephala* (10.21% CP), as a supplement for low quality roughages such as cottonseed hulls (CSH; 6% CP) used as rations for goats in tropical countries. The five treatments (Trt 1 - 5) were: Alfalfa (CSH + 10% alfalfa); and CSH plus 10; 20; 30 or 40% of leucaena. Vitamins and minerals were included in all rations. All ingredients of each ration were mixed together and fed as a TMR. The animals were offered feed ad libitum once daily at 0830 to ensure 10% feed residues. Water was available at all times. Goats were weighed once weekly before feeding and ruminal fluid samples were taken before and 2 h after feeding at the middle and end of the trial. Apparent DM digestibility of the rations was measured at the end of the trial. Dry matter intake (DMI) was highest in Trt 4 and 5 and lowest in Trt 3 (.563, .593, .528, .742 and .709 kg/d for Trt 1 - 5 respectively; $P<.08$). Apparent DM digestibility of the ration did not differ between treatments (50.0, 46.5, 47.8, 41.3 and 46.0%; $P>.38$). Goats fed the 40% leucaena ration had a lower liveweight loss than goats on the other treatments (-19, -31, -36, -8 and -2 g/d for Trt 1 - 5 respectively; $P<.002$). No differences were found in fiber quality (diameter, med and kemp) or in clean fleece production (7.1, 7.2, 7.6, 8.3, AND 8.7 g/d; $P>.29$). Total ruminal VFA concentration was highest for Trt 4 and 5 and lowest for Trt 2 (45.3, 40.6 38.9, 51.0 and 50.0 mmol/L, Trt 1 - 5, respectively; $P < .02$). Molar proportions of individual acids did not differ between treatments and the fermentation pattern was 78:16:6 for acetate:propionate:butyrate. Rumen pH was similar for all groups. It was concluded that Leucaena could be used at 30 - 40% of the DM in a low quality roughage diet to maintain goats in tropical countries.

Key Words: Leucaena; Mohair growth; Angora goats

INTRODUCTION

The leguminous tree, *Leucaena leucocephala,* is ubiquitous to many tropical countries where it is used for firewood and as an animal feed (NRC, 1984). It is fast-growing once established, and because of its long taproot, tolerates adverse moisture conditions, growing actively during the dry season (Adejumo and Ademosun, 1991). Leucaena has been shown to have great potential as a source of high quality feed for ruminants (Jones, 1979). Its leaves are highly palatable with a high digestibility (up to 70% reported) and a high crude protein (CP) content (26%). In comparison, digestibility and CP content of feedstuffs consumed by most ruminants in tropical countries would be in the range of 40 - 50% and 6 - 10%, respectively. Leucaena can be used for cut and carry feeding systems, dried for leaf meal, used as a silage or browsed as standing bushes.

Leucaena has a low protein solubility in the rumen and has been shown to be an effective bypass protein supplement with urea-molasses in intensive beef production (Alvarez et al., 1977). However, low liveweight gains, depressed appetite, alopecia, enlarged thyroid glands, depressed serum thyroxine levels, ulceration of the oesophagus and death of newborn offspring were encountered when leucaena was the sole feed source (Jones and Hegarty, 1984). These effects are caused by mimosine and its ruminal metabolite, 3-hydroxy-4(1H)-pyridone (DHP), a potent goitrogen (Hegarty et al., 1979). These problems are minimized when leucaena is fed at 40% or less of the diet (Jones and Hegarty, 1984; Adejumo and Ademonsun, 1991).

In many tropical countries the dry season continues for several months and the only feed resources available to farmers for feeding their livestock are poor quality roughages (dry standing grass or crop residues). Under dry season conditions the worst scenario, but one which

happens frequently, is death of the animals through starvation. The challenge under these circumstances is not to increase or maintain BW and body condition but to find a feed source that results in the least amount of BW loss. In the following experiment dry season conditions were simulated when Angora goats were fed a basal diet of cottonseed hulls supplemented with different amounts of leucaena. The leucaena used was low in protein (10.2% CP) as it was mechanically harvested after a period of continued rain and contained a large proportion of stem and branches. In the harvesting process the stem and branches were milled with the leaf resulting in a lower crude protein percentage.

MATERIALS AND METHODS

Thirty Angora goats (19 wethers, 11 doelings; age = 8 mo and BW = 17 kg) were used in an 85 d study to determine the value of *Leucaena leucocephala* (10.2% CP) as a supplement for a basal diet of cottonseed hulls. The dietary treatments were cottonseed hulls with either 10, 20, 30 or 40% of leucaena. A reference diet (cottonseed hulls plus 10% alfalfa) was included to serve as a comparison. The compositions of the diets are given in Table 1. The experiment consisted of a 2 wk habituation period, an 8 wk feeding period and 5 day digestibility study. The goats were housed at ambient temperature in raised, individual stalls indoors with natural lighting. During the 2 wk habituation period, goats were adapted gradually to ad libitum access to their experimental diets. Water was available at all times through nipple drinkers. Feed intake was recorded daily, and BW was measured weekly before feeding. The totally mixed diets were sampled throughout the trial and analyzed for DM, CP (Technicon Instruments Co., Tarrytown,

NY), gross energy using an adiabatic oxygen bomb calorimeter (Parr Instrument, Moline, IL), NDF, and ADF (Goering and Van Soest, 1970).

Mohair Measurements. All goats were shorn at the beginning and end of the trial. A midside sample was collected from each fleece; clean mohair yield and staple length were determined according to the ASTM (1988) standards; fiber diameter was determined using Peyer FDA 200 System (Walleran, Switzerland) as described in Sahlu and Fernandez (1992), and Sahlu et al. (1992).

Blood and Ruminal Traits. On days 30 and 60 ruminal fluid samples were obtained at 0, and 2 h postprandial by applying a vacuum to an esophageal tube fitted with a suction strainer as described by Sahlu et al. (1992). Ruminal fluid samples were analyzed for VFA and ammonia N as described by Lu et al. (1990). Jugular blood samples were collected on these days by venipuncture into tubes containing either sodium heparin or potassium oxalate and sodium fluoride (Becton Dickinson Vacutainer Systems, Rutherford, NJ). Tubes were chilled immediately in an ice bath, transported to the laboratory, and centrifuged (1500 x *g*; 4°C; 20 min). Aliquots of plasma were stored at -20°C until analyzed.

Concentration of plasma nonesterified fatty acids (NEFA) was determined with a commercial kit using an enzymatic colorimetric procedure (Wako Pure Chemical Industries, Richmond, VA) as described in Sahlu et al. (1992). Plasma urea nitrogen (PUN), total protein, and glucose were quantified colorimetrically using a Technicon Autoanalyzer II System (Technicon Instruments, Tarrytown, NY).

Digestion Trial

In the last week of the experiment feces were collected for 4 days from each wether using fecal bags. The total amounts of feces were recorded daily. Each sample was mixed and a 10% subsample was bulked over the 4 day period. The total amounts of feed offered and refused were recorded and subsamples bulked for subsequent analyses. All fecal samples were stored at -20°C until analyzed. Samples of feed ingredients, feed refusal and feces were dried in a forced-air oven (60°C), ground to pass a 3 mm screen and analyzed for DM, total N (Technicon Instruments Co., Tarrytown, NY), gross energy using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL), and ADF (Goering and Van Soest, 1970). Apparent digestibility of dry matter was calculated as the difference between the amount of dry matter consumed by the goats and that excreted in the feces. The digestible energy content of the diet was measured using an adiabatic bomb calorimeter and was calculated as the difference between energy intake and energy excreted in the feces.

Statistical Analysis

Feed conversion ratio and mohair fiber measurements were analyzed by analysis of variance using PROC GLM (SAS, 1982). Average daily gain for each goat was estimated by PROC REG (SAS, 1982) using the regression of liveweight on the number of days of treatment. DMI was analyzed using repeated measures and further analyzed using covariate analysis to test the slopes of the regression lines. Blood metabolites were analyzed as a split-plot in time, and where no treatment by time interactions were identified, the overall treatment means are given in the results. Effects were judged to be statistically significant when $P < .10$.

RESULTS

Animal Performance. The effects of increasing levels of leucaena on animal performance are given in Figure 1 and Table 2. Most animals lost weight during the experiment. Goats in the 10 and 20% leucaena treatments lost the most weight and their daily liveweight changes were different from the alfalfa ($P<.07$) and the 30 and 40% leucaena groups ($P<.001$). The goats in the alfalfa treatment tended ($P<.10$) to loose more weight than goats in the 30 or 40% leucaena groups. Using linear regression (SAS, 1982) it was found that the mean ADG of the 10% alfalfa diet would be equivalent to using 26% leucaena in the diet. The regression equation ($r^2 = .38$) was ADG = -47.4 + 1.1x; where x is the percentage of leucaena in the diet.

Dry matter intake was affected by treatment ($P<.08$; Table 2). The 30% leucaena group had the highest intake but this was not different ($P>.10$) from the 40% group. The 20% leucaena group had the lowest DMI followed by the alfalfa and 10% leucaena groups (528 vs 563 and 593 g/d; $P<.08$). When covariance analysis was performed it was shown that the slopes differed ($P<.06$). Though the 40% leucaena group had a similar average DMI to the 30% group their intake was decreasing throughout the trial (Figure 3). Intake of goats in the other groups stabilized after dropping from their initial amounts. Dry matter digestibility (Table 2) did not differ ($P>.13$) between groups and was 50.0, 46.5, 47.8, 41.3 and 46.0% for Trt 1-5, respectively.

Fleece production and characteristics are given in Table 3. There were no significant differences between treatments in any of the fleece measurements made. The 40% leucaena treatment produced a 23% heavier fleece than the alfalfa group but this difference did not reach significance ($P>.10$).

Blood Metabolites. The effects of treatment on concentrations of blood metabolites are summarized in Table 4. Treatments had no significant effects on plasma concentrations of glucose, PUN, NEFA or total protein.

Rumen Variables. VFA proportions and total VFA concentrations are given in Table 5. Increasing the level of leucaena in the diet had no significant effect on rumen VFA proportions but total VFA were different between treatments ($P < .02$). Overall, total VFA for groups fed 10 and 20% leucaena were lower ($P < .05$) than the values for the groups fed 30 and 40% leucaena. The total VFA for the alfalfa group did not differ ($P > .10$) from any other treatment.

DISCUSSION

The liveweight losses that occurred in this experiment are indicative of what occurs in the dry season in many tropical countries. Indeed in many situations animals die as a result of undernutrition during the dry season (REF**). Inclusion of 30 to 40% leucaena in the diet was sufficient to stabilize liveweight (Figure 1); the goats were able to maintain their liveweight even though they were consuming rations with a crude protein content of only 6.6 - 6.8%. The metabolizable energy of these rations was correspondingly low at only 1.4 to 1.7 Mcal/kg. Angora goats, because of their high protein requirement for fiber production (Hart et al., 1993), probably lost more weight under this feeding regime than would be typical of a dual purpose native goat.

The leucaena used in this trial had been grown and harvested in Southern Texas. Due to a very wet period, harvesting was delayed and subsequently there was a lot of stem in the harvested leucaena. This reduced the CP from the expected 17 - 20% down to 10.2%. Because

the leucaena had been passed through a mill it was ground to very fine particles and the goats were unable to sort it. Under normal management conditions in a tropical country the leucaena would be offered either by allowing the goats to browse the standing tree or it would be cut and branches would be offered to the goats. Under these situations the digestibility and protein content of consumed material would be much higher than that found in the present experiment.

The rumen ammonia-N levels at 6 - 8 mg/dL are above the 5 mg/dL level that Satter and Slyter (1974) reported was necessary for maximizing microbial growth. However, recent data from cattle fed low quality roughages, have shown that digestibility of cellulose and fiber from nylon bags in the rumen was optimized at a rumen ammonia-N level below 10 mg/dL while intake was optimized at 20 mg/dL (Perdok et al., 1988). Thus, in the present experiment the rumen ammonia levels may have been too low to support high dry matter intake.

Bamualim et al. (1984) estimated that 60% of the protein in leucaena escaped ruminal degradation. They found a significant increase in DMI of sheep fed a tropical pasture hay supplemented with urea and minerals when leucaena was given. In the present experiment the diet was marginal in protein content and the goats responded to the increase in protein by increasing intake. It was not possible to determine the extent of the ruminal escape of the leucaena protein. Goats in the 40% leucaena treatment decreased their intake over the trial and may have been subjected to toxicity from the toxic compounds that arise from the break down of mimosine in the rumen. Jones (1979) reported that goats in Hawaii had rumen microorganisms capable of degrading DHP to non-toxic compounds, however, it has been shown that ruminants in the USA do not have these organisms. Megarrity and Jones (1983) showed that

after 7 weeks of being on a leucaena diet goats without DHP degraders reduced their intake drastically due to DHP toxicity.

Both clean mohair production and fiber diameter were lower than the values reported for Angora goats (mean = 15.1 g/d and 35.4 μm) by Davis et al. (1995). Fiber yield and diameter are known to be affected by the amount of dietary protein and energy intake (Reis and Sahlu, 1994). In a previous study, Sahlu et al. (1993) used a regression equation to describe the positive relationship between CP intake and both grease mohair yield and diameter. Hart et al. (1993) reported a 59% decrease in greasy fiber production and a 16% decrease in fiber diameter when Angora goats were grazed on dormant bermuda pasture (similar in quality to the basal diet of CSH used here) compared to a winter wheat pasture.

Mean plasma glucose, PUN, NEFA and total protein measurements are shown in Table 4. There were no differences ($P > .05$) between the dietary treatments in overall means; plasma glucose (average 45.5 mg/dL), PUN (average 5.8 mg/dL), NEFA (average 552 meq/L) and total protein (average 60.5 g/L). The contribution of absorbed glucose to glucose turnover in ruminants is limited; the main source of circulating glucose is absorbed propionate which is converted to glucose in the liver (Weekes, 1991). Sahlu et al. (1995) reported a linear increase in blood glucose concentration with increases in dietary energy concentration. The mean glucose concentrations reported here are lower than the values for Alpine dairy goats reported by Sahlu et al. (1993; 1995) and Angora goats (Hart et al., 1993; Sahlu and Fernandez, 1992). In all those experiments goats were fed a high quality diet with high energy content in the diet equal to or greater than 2.3 Mcal ME/day. Weekes (1991) indicated that 95% of absorbed propionate

may be converted to glucose accounting for 87% of the glucose production. In low quality diets with high levels of fiber, such as the one used in this trial, acetate is the main source of energy.

The principal source of PUN is from NH_3 produced by liver conversion of amino acids and ruminal degradation of dietary CP (Carter et al., 1989). PUN tended to be higher in goats fed higher concentrations of dietary protein (Sahlu et al., 1993; 1995). Preston et al. (1965) have observed a close relationship (r = .986) between PUN concentration and protein intake in lambs. The low PUN reported here is typical of low dietary protein intake. Sahlu et al. (1995) reported values of 8.4 mg/dL for a diet containing 8.5% dietary protein and 16.2 mg/dL for a 14.5% CP diet.

Even though there was no treatment effect on NEFA concentration the mean values reported here are higher than the mean concentration reported by Sahlu et al. (1995) of 285 meq/L in pregnant alpine does fed a diet containing 8.5 to 14.5% CP and 1.8 to 2.5 Mcal/kg ME. Plasma glucose, free fatty acids and ketone bodies such as B-HOB have been used as indicators of energy status (Bowden, 1971; Sahlu et al., 1992, 1995). Higher levels are indicative of mobilization of fat depots. It is not surprising, therefore, to observe higher values of these metabolites for goats in this study that are losing BW compared to previous studies with goats maintaining or gaining weight (Sahlu et al., 1992, 1995).

The total protein values are similar to values reported previously for both Angora (Sahlu et al., 1992) and Alpine goats (Sahlu et al., 1995). Total protein is know to vary with dietary protein intake or due to dehydration (Sahlu et al., 1993).

IMPLICATIONS

Results from this trial indicate that Leucaena Leucocephala can be used as a dry season supplement for goats. Leucaena is known for its drought tolerance and palatability. This trial has shown that even low quality leucaena (10.2% CP) could maintain liveweight and produce a fleece in goats fed diets containing the minimum recommended crude protein content (6%).

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TABLE 1. Diet composition, % of DM

Item	Alfalfa Ration	% Leucaena			
		10	20	30	40
Ingredient					
Cottonseed Hulls	88	88	78	68	58
Alfalfa	10	-	-	-	-
Leucaena	0	10	20	30	40
Trace mineral salts[1]	0.8	0.8	0.8	0.8	0.8
Dicalcium phosphate	0.95	0.95	0.95	0.95	0.95
Vitamins ADE[2]	0.25	0.25	0.25	0.25	0.25
Chemical composition, % of DM					
CP	5.3	5.4	5.8	6.6	6.8
ME[3], Mcal/kg	1.7	1.6	1.6	1.4	1.6
NDF	76.8	76.6	73.1	70.5	68.9
ADF	55.1	56.2	54.1	51.8	52.1

[1]Containing (in percentages) NaCl, 94 to 95; Mn, >.2; ferrous Fe, >.16; ferric Fe, >.14; Cu; >.033; Zn, >.10; I, >.007; Co, >.005

[2]Each gram contained 2,200 IU of Vitamin A, 2,200 IU of Vitamin D, and .2IU of Vitamin E

[3]Calculated energy in diet (NRC, 1981)

TABLE 2. Liveweight change, dry matter intake and dry matter digestibility of Angora goats fed a basal diet of cottonseed hulls supplemented with 10% alfalfa or different levels of Leucaena

Item	Alfalfa Ration	% Leucaena				SE[1]	P<
		10	20	30	40		
ADG, g/d	-18.6	-30.7	-35.6	-8.1	-2.3	6	.004
DMI, g/d	563	593	528	742	709	14	.08
DMD[2] , %	50.0	46.5	47.8	41.3	46.0	2.5	.13

[1]SE=largest standard error of least square means

[2]DMD - dry matter digestibility

TABLE 3. Mohair characteristics of Angora goats fed a basal diet of cottonseed hulls supplemented with 10% alfalfa or different levels of Leucaena

Item	Alfalfa Ration	% Leucaena				SE[1]	P <
		10	20	30	40		
Grease fleece, g/d	9.81	9.60	9.79	11.44	11.47	.95	.43
Clean fleece, g/d	7.11	7.22	7.58	8.29	8.71	.61	.29
Diameter, μm	23.53	23.29	24.05	24.60	25.21	.65	.24
Fiber Length, μm/d	803	829	849	750	814	33	.30
Kemp, %	.17	.13	.18	.18	.22	.05	.80
Med, %	.37	.33	.40	.40	.38	.09	.98

[1]SE=largest standard error of least square means

TABLE 4. Blood metabolites of Angora goats fed a basal diet of cottonseed hulls supplemented with 10% alfalfa or different levels of Leucaena

Item	Alfalfa Ration	% Leucaena 10	20	30	40	SE[1]	P <
Glucose, mg/dL	47.5	44.3	44.7	44.4	46.4	1.9	.64
Urea N, mg/dL	4.53	6.87	6.74	6.29	4.75	.82	.13
NEFA, meq/L	622	518	640	482	500	121	.88
Total Protein, g/L	59.8	60.2	61.1	61.4	60.2	1.1	.83

[1] SE=largest standard error of least square means

TABLE 5. Rumen variables of Angora goats fed a basal diet of cottonseed hulls supplemented with 10% alfalfa or different levels of Leucaena

	Hour	Alfalfa Ration	% Leucaena 10	20	30	40	SE[1]	P<
pH	0	6.8	6.8	6.8	6.7	6.7	.05	.50
	2	6.5	6.5	6.6	6.5	6.6		
Ammonia N,	0	7.8	8.0	7.3	6.9	6.9	.3	.13
mg/dL	2	8.5	7.4	6.0	7.0	7.7	.7	.24
VFA, mol %								
Acetate	0	78.3	78.4	79.1	79.4	78.4	0.6	.84
	2	75.5	76.7	76.9	76.9	76.6		
Propionate	0	15.2	14.0	13.6	12.9	14.0	0.6	.47
	2	18.8	16.9	17.3	16.8	17.3		
Isobutyrate	0	0.31	0.25	0.67	0.35	0.41	0.09	.30
	2	0.08	0.09	0.22	0.14	0.17		
Butyrate	0	5.72	6.76	5.93	6.82	6.14	0.33	.34
	2	4.95	5.76	4.87	5.62	5.31		
Isovalerate	0	0.29	0.25	0.51	0.24	0.46	0.09	.63
	2	0.09	0.05	0.14	0.07	0.10		
Valerate	0	0.19	0.36	0.21	0.35	0.53	0.06	.39
	2	0.62	0.44	0.51	0.50	0.58		
Total	0	31.8	35.1	32.3	43.1	43.3	2.5	.02
VFA, mM	2	58.7	46.0	46.0	58.8	57.1		

[1]SE=largest standard error of least square means

FIGURE 1. Liveweight change of Angora goats fed a basal diet of cottonseed hulls supplemented with 10% alfalfa or different levels of Leucaena.

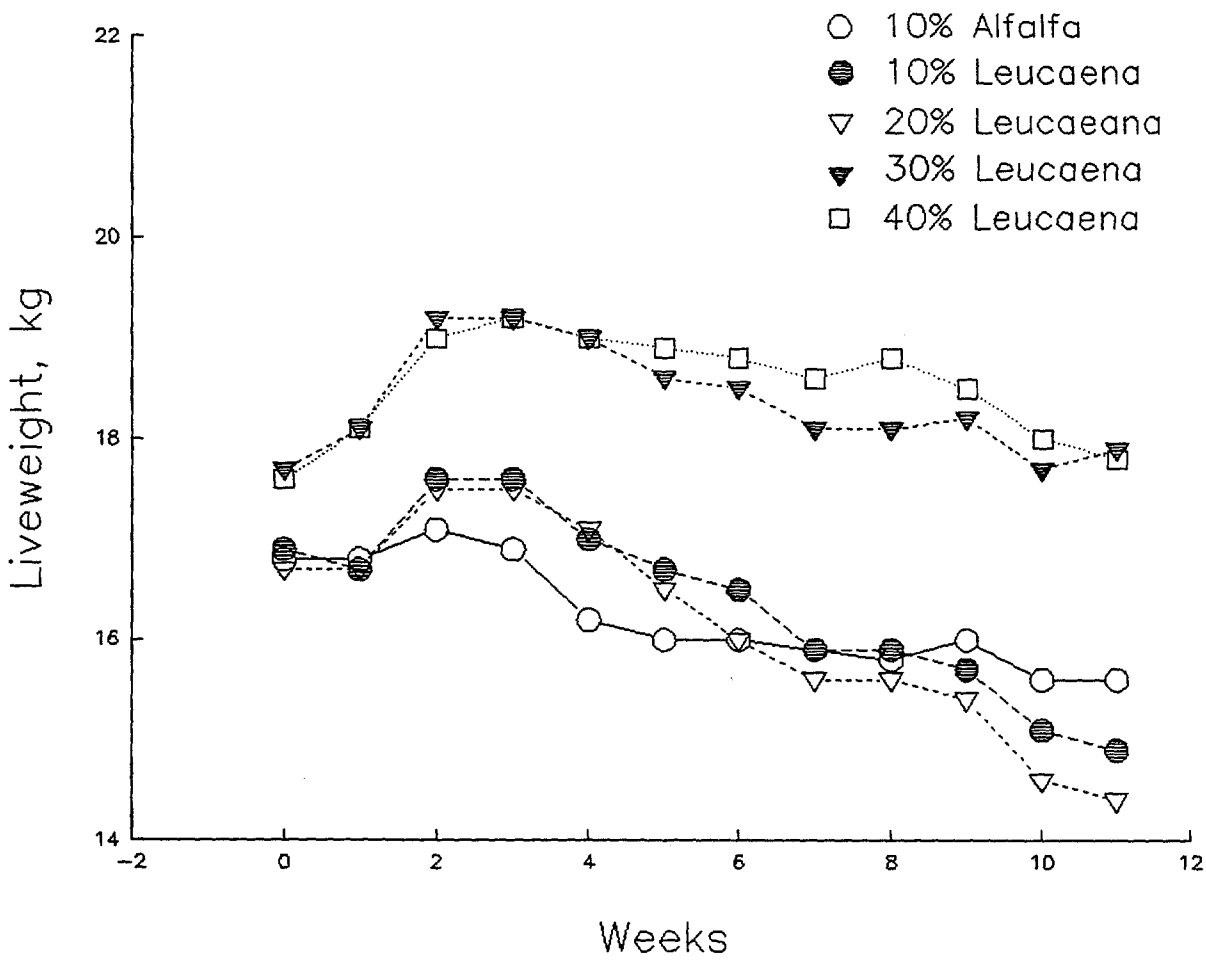


FIGURE 2. Dry matter intake of Angora goats fed a basal diet of cottonseed hulls supplemented with 10% Alfalfa or different levels of Leucaena

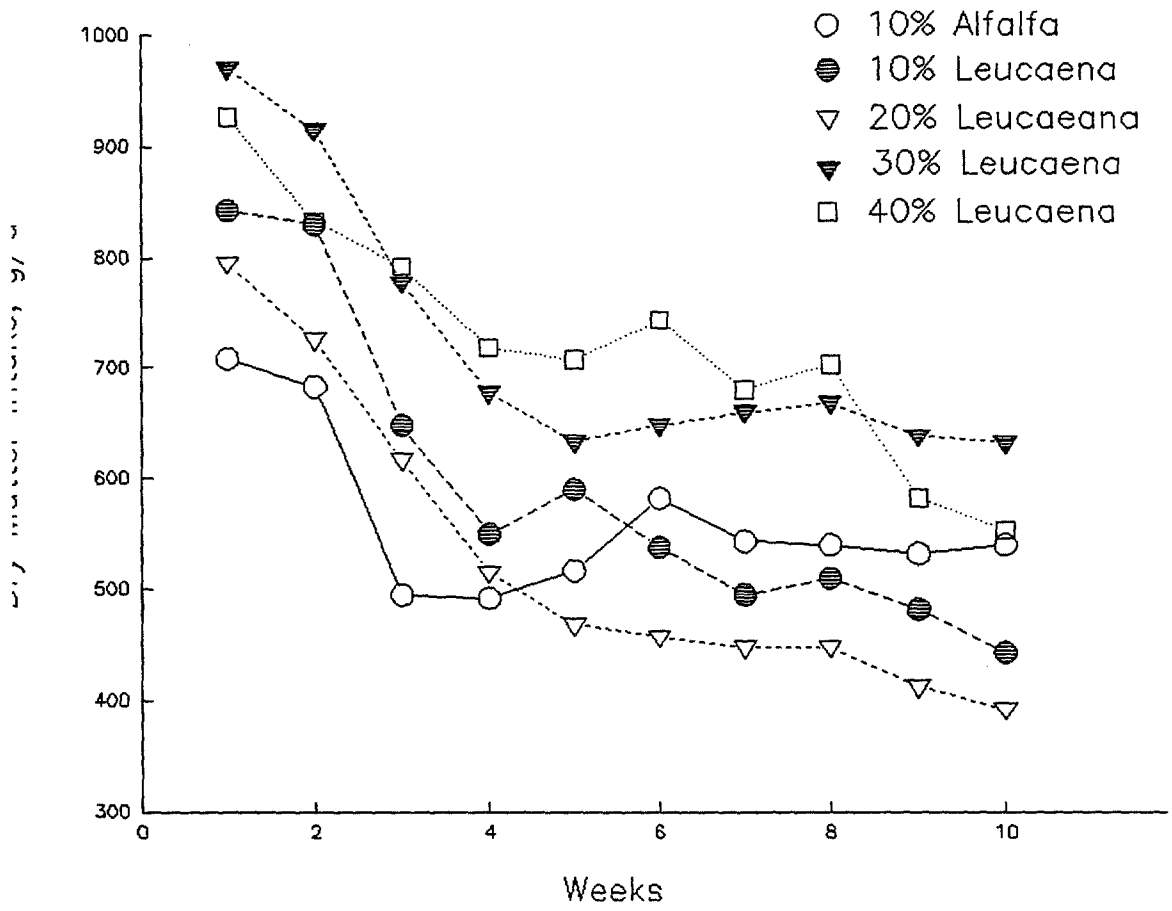
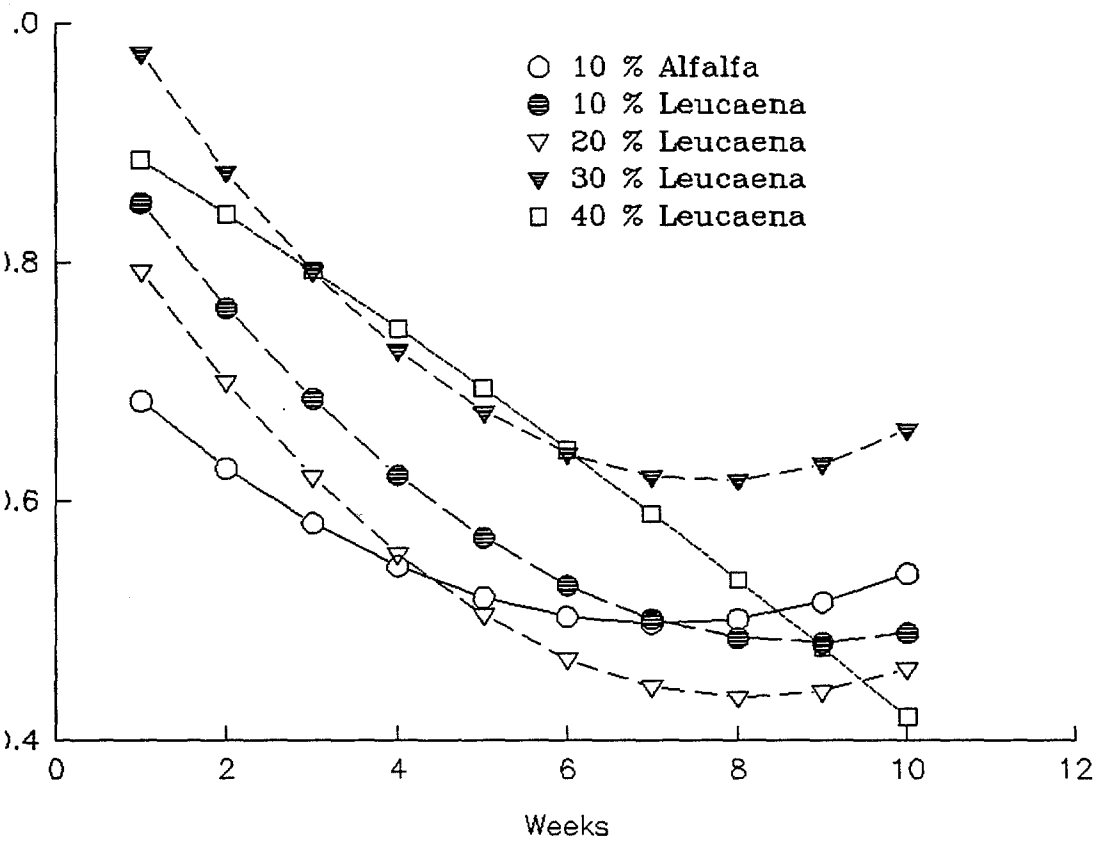


FIGURE 3. Regressions of dry matter intake of goats fed a basal diet of cottonseed hulls and supplemented with 10% alfalfa or different levels of leucaena.



EXPERIMENT THREE

Effects of Increasing Levels of *Leucaena Leucocephala* in Angora and Cashmere-Producing Spanish Goats

INTRODUCTION

Leucaena leucocephala, is a tropical, drought resistant, leguminous tree, which is found throughout the tropical world. In less developed countries (LCD) leucaena was, until recently, used for control of soil erosion, firewood and construction purposed. However, recently it has been evaluated as a forage source for ruminants in LDC during droughts (NRC, 1984).

Leucaena can be toxic to ruminants, particularly when stock have not been previously adapted (Jones and Hegarrity, 1984). The toxic effects of Leucaena are caused by mimosine and its ruminal metabolite, 3-hydroxy-4(1H)-pyridone. Reported toxic effects of mimosine include reductions in feed intake and fertility, alopecia, esophageal lesions, renal and hepatic dysfunction, cataracts, uroschesis and general unthriftiness (Smuts et al., 1995). However, Davis et al. (1995) reported that 30-40% of the dry matter in diets of Angora goats could be composed of leucaena without effecting animal performance. There is a dearth of information on the nutritive value of leucaena for goats, particularly at high percentages of the diet. It is possible that leucaena could be a suitable feed source for use during drought in LDC's where 96% of the world goat's population are found.

Fiber growth, in comparison to liveweight gain, in Angora goats is particularly responsive to protein supply (Sahlu et al., 1992) and rumen-protected protein (Ash and Norton, 1987). Leucaena, contains moderate levels of tannins which when binding to proteins protects them from rumen degradation. Therefore, leucaena may be a particularly suitable feed source for goats with high fiber production. The fiber growth of low-producing cashmere goats, and presumably low fiber tropical goats, is largely unresponsive to increasing

protein content and increasing proportion of rumen protected protein (Ash and Norton, 1987). In these goats more protein is diverted for production of live weight (Cronje, 1995; Jia et al., 1995).

The objective of the present study was to evaluate the effects on liveweight and fiber production of increasing levels of *Leucaena* in the diet of Angora and cashmere goats. The leucaena diets were compared to a diet containing a source of rumen-protected protein.

MATERIALS AND METHODS

Thirty Angora (16 $\pm$ 2 kg) and twenty Cashmere (19 $\pm$ 2 kg) doelings (eight months of age) were blocked by breed and randomly allocated on liveweight and stretched fiber length to one of five treatments. The basal diet consisted of cotton seed hulls and corn and either 15 (L15), 30 (L30), 45 (L45) or 60% (L60) leucaena on a dry matter (DM) basis (Table 1). A reference diet of cotton seed hulls and corn and 100 g DM of 1% formaldehyde treated casein (Sigma) was also included (Ca). The diets (Table 1) were formulated to be isocaloric (2.13 Mcal ME/Kg DM) and ranged in crude protein content from 10 to 13%. The goats received *ad libitum* access to their diets (10% orts) and water. The experiment consisted of a two week habituation period, 11 week experiment period and 5 day digestibility.

Feed intake was recorded daily and liveweight measured weekly before feeding. On day 45 of the experiment, blood and rumen samples were collected at 0 and 3 hours post prandially. Rumen samples were measured for volatile fatty acids and ammonia and plasma concentrations of glucose, urea N, total protein, essential amino acids, triiodothyronine,

thyroxine and cortisol were determined. In the digestibility study, feces and urine were collected twice daily, recorded, subsampled and preserved at -20°C. Feeding was at 90% *ad lib* and water was freely available. Pooled fecal samples were dried for DM determination (100°C) and nutrient analysis (55°C). Urine samples were freeze dried. Feeds, orts, fecal and urine samples were analyzed for DM percentage, crude protein (Nx6.25) (Technicon Instruments Co., Tarrytown, NY), gross energy (GE; adiabetic bomb calorimeter (Parr Instrument, Moline, IL) and ash (AOAC, 19??). Feed, orts and fecal samples were analyzed for acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Goering and Van Soest (1970).

Patch fiber regrowth rate was determined on a measured (approximately 15 by 15 cm^2), right midside patch 45 days after treatment and at the end of the experiment (wk 11) by clipping to skin level (Oster blade 40). Fiber diameter and estimated cashmere yield by weight were determined on 2000 snippets using an optical fiber distribution analyzer.

Data were analyzed statistically using the general linear models procedure of (GLM) (SAS, 1994) with treatment, breed and time fitted as classes and interactions determined. Mean weekly DM intake and average daily gain (ADG) were analyzed using repeated measures in time (SAS, 1994).

RESULTS AND DISCUSSION

As leucaena level increased goats responded by increasing *ad lib* DM intake. *Ad lib* intake (Table 2) was highest in 30L, 45L and 60L (811-899 g/goat/day) goats and lowest in 15L (648 $\pm$ 57 g/goat/day) while Ca goats had intermediate intakes of 710 $\pm$ 57 g/goat/day).

A similar trend was observed when intake was corrected for metabolic liveweight (Table 2). There was a quadratic effect on DM intake ($P<0.01$) mirroring increases leucaena percentage in the diet from 15 to 45L, but intake declined at 60L. Angora goats (765 $\pm$ 32 g/goat/day) had similar intakes to cashmere goats (789 $\pm$ 39 g/goat/day).

The feed efficiency for gain was between 50 and 200% greater ($P<0.05$) in Ca goats than leucaena fed goats (Table 2) and was unaffected by breed (Angora 0.048 $\pm$ 0.005; Cashmere 0.038 $\pm$ 0.005). The likely explanations for the reduced feed efficiency for gain with increasing levels of leucaena include the decline in DM digestibility, toxic metabolic effects, or the overprotection of the protein in leucaena by tannins.

Increasing concentrations of leucaena in the diet had no effect on mean liveweight gain (Table 2). The leucaena diets were able to support a mean liveweight gain of 36 g/d in young goats and gain was unaffected by breed (Angora 37 $\pm$ 4 g/goat/day; Cashmere 32 $\pm$ 5 g/goat/day).

Therefore, as in other studies with goats (Davis et al., 1996) leucaena could be used to stabilize liveweight in drought periods.

Clean fiber growth rate was unaffected by increasing leucaena or protected casein treatment (Table 2) though there was a trend ($P = 0.14$) for fiber growth rate to decline with increasing leucaena concentrations in the diet. This trend was most apparent in cashmere goats. Clean fiber growth rate of Angoras (1.10 mg/cm^2/day) was higher ($P<0.0001$) than cashmere goats (.3 mg/cm^2/day). Mohair fiber diameter was between 1.8 and 3.9 microns coarser ($P<0.001$) in Ca goats than leucaena fed goats (Table 2). There was no effect of leucaena level or protected casein on cashmere fiber parameters or kemp percentage in Angora goats.

Blood urea ($P<0.001$) and protein ($P<0.05$) were higher in 60L goats (20.5 mg/dl; protein 46 g/l). In addition, 60L goats had lower concentrations of propionate (15.5 mol%; $P<0.05$) and higher concentrations of butyrate (11.5 mol% $P < 0.01$) than the other goats (propionate 17.1-18.6 mol%; butyrate (9.4-10.4 mol%). Angora goats had higher blood urea (17.8 mg/dl; $P<0.05$) and rumen ammonia (13.5 mg/dl; $P<0.001$) than cashmere goats (15.6 mg/dl and 8.6 mg/dl, respectively).

CONCLUSION

In conclusion, goats can consume up to 60% of their diet as leucaena for 11 weeks without developing acute mimosine symptoms. However, changes in digestibility, intake and blood and rumen measurements with increasing levels of leucaena indicate the possibility of subclinical disturbances in rumen and metabolic function.

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TABLE 1. Composition of experimental diets

	Experimental diets				
	Ca	15%L	30%L	45%L	60%L
Ingredients[1] (DM%)					
Leucaena	0	15	30	45	60
Cotton seed hulls	61	50	42	34	27
Corn	19.5	24	17	10	20
Formaldehyde casein	9	0	0	0	0
Urea	0.5	1	1	1	1
Chemical composition (DM)					
Dry matter (%)	93	93	93	93	93
Crude protein (%)	14	10	12	13	13
NDF, %	58	49	59	55	52
ADF, %	46	39	39	41	39
GE, kcal/kg	4470	4400	4380	4400	4450

[1]Additional components. Molasses 7% DM. Di Calcium Phosphate 1% DM. Pellet binder 1% DM; Trace elements (0.8% DM; 95% NaCl; .2% Mn; .16% Fe, .033% Cu; .1% Zn; .007% I; .005% C0), 0.2% DM Vitamins ADE (2200 iu Vitamin A; 2200 iu Vitamin D; .2 iu Vitamin E)

TABLE 2. Effect of increasing leucaena levels and protected casein on intake, liveweight and blood rumen parameters

	Experimental diets						
	Ca	15%L	30%L	45%L	60%L	SEM	TRT
DMI[1] (g DM/goat/day)	710[cb]	648[c]	815[ab]	899[a]	811[ab]	57	.03
Metabolic DMI ($g/kg^{0.75}$)	.072	.071[b]	.087[a]	.100[a]	.087[ab]		.002
DMD[2] (%)	59[a]	59[a]	56[ab]	53[bc]	51[c]	1	.002
FE[3] for gain (g/g)	.066[a]	.041[ab]	.45[b]	.035[b]	.29[c]	.008	.02
ADG[4] (g/goat/day)	48	28	38	34	26	8	.3
CFGR[5] ($mg/cm^2/day$)	.72	.67	.56	.59	.42	.08	.14

[1]DMI - Dry matter intake; [2]DMD - Dry matter digestibility; [3]FE - Feed efficiency; [2]ADG - Average daily gain

[5]CFGR - Clean fiber growth rate

EXPERIMENT FOUR

Effect of *Leucaena Leucocephala* on the Performance of Alpine Dairy Goats

ABSTRACT

The effect of *Leucaena leucocephala* (leucaena) on the production and composition of milk from lactating Alpine does was investigated. Twenty multiparous does (mean body weight of 44.5 kg) in early lactation were stratified by expected kidding date and assigned randomly to one of two diets, in which approximately 50% of the dietary protein was provided by either leucaena or alfalfa (Table 1). Rations were fed as complete mixed diets and provided adequate protein and energy for lactating does. Does were milked twice daily (0600 and 1800 h), milk weights were recorded at each milking and samples for milk composition analysis were collected once every week. The experimental period lasted 16 weeks. No differences ($P > .10$) were found between alfalfa and leucaena treatments in milk production (3.21 vs 3.07 kg) milk fat (2.44 vs 2.65%), lactose (4.27 vs 4.29%), and SNF (7.75 vs 7.72%). However, protein (2.92 vs 2.86%) and total solids (10.2 vs 10.37%) of milk were affected by diet x time interactions. Ruminal and blood metabolites were not affected by treatments. Mimosine, a free amino acid found in leucaena, was not detected in milk samples.

Key Words: Alfalfa; Leucaena; Alpine goat; Milk

INTRODUCTION

Interest is currently centered on the introduction of various forage trees and browses species as supplementary forages for ruminants. Such plants are deep rooted and therefore drought tolerant; in addition they provide forage of high nutritive value. Forage trees can also be used for a variety of purposes such as provision of firewood and for construction purposes. *Leucaena leucephala* (leucaena) is a tree legume and has a similar nutritive value to alfalfa (12). The tannin content of leucaena is higher than alfalfa (10.15 vs 0.13 mg/g) and this may reduce the digestibility of dry matter and enhance the amount of protein escaping degradation in the rumen. By-pass protein is commonly incorporated into the diet of high-producing dairy cows in order to increase milk production (4). Supplementation with leucaena increases milk production in lactating dairy cows (13, 10, 14) and body weight gain in sheep (7, 19). However, most leucaena foliage contains 3 to 5% mimosine on a DM basis. Mimosine is a non-protein, free amino acid that is toxic to animals when absorbed into the blood stream. In ruminants, mimosine us partially degraded in the rumen to 3,4-DHP and 2,3-DHP (8), which also have toxic effects. Ruminants in some parts of the world have rumen microorganisms capable of degrading DHP (8). It is generally believed that leucaena can constitute up to 30% of the diet without toxic effects in ruminants that do not possess these organisms. The nutritive value of leucaena for dairy goats has not been evaluated. This study was designed to investigate the nutritive value of leucaena, relative to that of alfalfa, in diets for lactating Alpine goats.

MATERIALS AND METHODS

Twenty multiparous Alpine does (mean body weight (BW) of 44.5 kg) from the milking herd of the E (Kika) de la Garza Institute for Goat Research were used in this study. As soon as individual does kidded they were introduced to the experiment. Kidding took place over a 6-week period. Does were blocked by kidding date and assigned randomly to one of two dietary treatments in which approximately 50% of the dietary protein was provided by either leucaena or alfalfa (Table 1).

Does were offered their rations as a complete mixed diet (16.4 CP vs 14.6% CP .3 Mcal of metabolizable energy/kg of DM) to minimize particle size differences and to prevent selection by animals. Does were offered their ration at 0800 h daily to ensure at least 10% orts. A Calan goat feeding system (American Calan Inc., Northwood, NH) allowed freedom of movement while monitoring individual feed intake.

Does were milked twice daily (0600 and 1800 h) and milk weights were recorded at each milking using a computerized flow metering device (Westfalia Systemat, Elk Grove, IL). Milk samples were collected at both milkings once a week throughout the experimental period. Samples were analyzed for fat, protein (N x 6.38), lactose, total solids (TS), and solid not fat (SNF) by infrared spectrophotometry (Multispec 2, Mulyispec, Wheeldrake, York, UK). Samples of feed were collected after each mixing, oven dried at 60°C for 48 h and ground to pass through a 2 mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). The samples were analyzed for dry matter (DM), crude protein (CP) (12), gross energy using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL), acid detergent fiber (ADF) and neutral detergent fiber (NDF) by the procedure of Goering and Van Soest (6).

Mimosine and 2,3-DHP in leucaena were measured using the method of Tangendjaja and Wills (18). Approximately 0.5 g of dried leucaena was mixed with 30 ml of citric acid, homogenized, and allowed to stand for 30 min. After filtration, samples were dispensed into auto-sampler vials, and measured using reverse-phase HPLC with an AminoQuant apparatus (Hewlett Packard, San Fernando, CA).

Blood and ruminal fluid were sampled at 0 and 4 h after feeding at weeks 6, 9 and 12 of the experimental period. Rumen fluid samples were taken and quantified for pH immediately after sampling and volatile fatty acids (VFA) using Gas Chromatography (Hewlett Packard, Model 5880) and ammonia using a Technicon Auto Analyzer II System (Technicon Instrument, Bran and Luebbe Inc., Buffalo Grove, IL). Blood was collected via jugular venipuncture into 7 ml vacuum tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ) containing either K_3-EDTA or potassium oxalate with sodium fluoride (a glycolytic inhibitor). Blood sample tubes were immediately chilled in an ice bath, transported to the laboratory, and centrifuged at 1500 x g and 4°C for 20 min. Plasma was separated and stored at -20°C until analysis. Non-esterified fatty acids (NEFA) and total protein were measured using spectrophotometric techniques and glucose were measured by the techniques of Gochman and Schmitz (5) and Chaney and Marbach (3), respectively using a Technicon Autoanalyzer II System (Technicon Instrument, Bran and Luebbe Inc., Buffalo Grove, IL).

Plasma concentration of insulin (ICN kit #07-160102), thyroxine (T_4) (ICN kit #07-290102), and triiodothyronine (T_3) (ICN kit #07-292102) were assayed using commercially available radioimmunoassay (RIA) kits (ICN Biomedics, Costa Mesa, CA). Plasma hormone samples were assayed in duplicate within a single RIA run. The assay used hormone calibrators

in human serum; the known sensitivities of the insulin, thyroxine and triiodothyronine RIA were 1.4 μU/mL, 0.5μg/dL, and 18 ng/d, respectively. Intraassay CV for the same were 7.3, 6.4, 7.9%, respectively.

For analysis of DHP 1 ml of milk was mixed in 1 ml of TCA, vortexed and centrifuged at 2500 x g for 15 min. The supernatant was transferred into a glass tube, 3 ml of ethyl ether was added and the mixture was again vortexed for 3 min. Using a suction pump, ethyl ether and TCA were discarded, and filtered and dispensed into auto-sampler vials. The quantity of DHP was measured using the method of Tangendjaja and Wills (18).

Data for milk production, feed intake, body weight and blood and ruminal parameters were analyzed using the multivariate repeated measures procedure as a split-plot in time (11) with the GLM procedures of SAS (5). When treatment or treatment x time interactions were significant ($P<0.10$), means were separated using Duncan's Test.

RESULTS AND DISCUSSION

Daily dry matter intake (DMI) and DMI as a percentage of body weight were similar for the two diets (Table 2). Although body weight (BW) change did not differ between the two diets for the first seven weeks of lactation there was a diet x time interaction (Figure 1). After seven weeks the BW change for the alfalfa treatment was higher ($P<0.01$) than that of leucaena treatment. The mean BW gain for the entire experimental period was higher ($P<0.01$) for the alfalfa fed group (109 vs 60 g/day; Table 2). It was interesting to note that neither treatment group lost weight at peak lactation. However, the low milk production (Av 3.21 kg/day)

measured here may have enabled a greater amount of dietary nutrients to be partitioned to BW gain.

Milk production data (Table 3) indicated similar milk yield (3.21 vs 3.07 kg/d) and fat corrected milk (2.32 vs 2.70 kg/d) for the two treatments. Peak lactation occurred at seven weeks postpartum for the alfalfa group, whereas the leucaena group reached peak lactation at three weeks and maintained it up to 7 weeks postpartum (Figure 2). The milk production of the leucaena and alfalfa groups declined by 3.9 and 2.8% per week, respectively, after the peak, which may indicate better persistency for the alfalfa group. Milk fat, lactose, SNF, total solid percentages and somatic cell count were not affected by treatment (Table 3). Milk protein indicated a diet x time interaction. The difference between treatments in milk protein was greater ($P<0.10$) as lactation progressed (Figure 3).

Ruminal measurements are shown in Table 4. Ruminal pH was higher ($P<0.01$) for the leucaena diet. Ruminal ammonia-N showed a higher tendency ($P<0.10$) for leucaena than the alfalfa treatment (17.7 vs 13.7 mg/dL). The highest concentration of ruminal ammonia-N was recorded at 4 h after feeding (Table 5). A higher concentration of plasma urea was also observed at 4 h than zero h (Table 6). The amounts of ruminal ammonia-N measured in this study (13.7 and 17.7 mg/dL) are higher than the 5 mg/d suggested as a minimum for optimal bacterial activity in the rumen, and subsequent adequate microbial-N flow to the duodenum (16). The total concentrations of VFA and molar proportion of acetate, propionate, butyrate, isobutyrate, isovaleric acid and valeric acid were similar in both diets.

The blood metabolites NEFA, glucose, TP, and urea were not affected ($P>0.10$) by treatments. Mean plasma glucose concentration was higher ($P<0.01$) at week 12 than at weeks

6 and 9 (90.2, 60.4, and 60.6 mg/dL, respectively; Table 6). This could be due to higher uptake of glucose by the udder during early lactation. Blood urea concentration was higher ($P<0.01$) at 4 h than at 0 h after feeding and also higher ($P<0.01$) at week 12 than weeks 6 and 9 weeks (42, 30, and 31 mg/dL; Table 6). Plasma insulin concentration was similar for the two dietary treatments. The leucaena treatment reduced ($P>0.05$) T_3 production by the thyroid gland. The reduction in T_3 may be an early indication of hyperthyroidism. These results agree with previous reports of Al-Dehneh et al. (1) using Alpine goats and Jones and Hegarty (9) using steers. There was a treatment x time effect ($P>0.05$) on plasma T_4. The highest T_4 concentration was measured at week 12 of sampling (5. 5, and 6.5 μU/dL for week 6, 9, and 12, respectively).

No DHP was observed in the goats' milk, except on two milk samples from the leucaena group. The amount detected in their milk was 0.7 μM on average, which is well below likely harmful levels.

The concentrations of mimosine and DHP were 0.39 and 0.015%, respectively. From the mimosine value it can be calculated that an individual goat of 50 kg BW and consuming 1.26 kg of dried leucaena per day would ingest 4.9 g of mimosine per day (0.103 g/kg BW). Szyska and Meulen (17) observed no symptoms of poisoning when sheep were fed 0.14 g mimosine per kg BW. Similarly, no symptoms of mimosine poisoning were observed in this experiment.

CONCLUSION

The replacement of alfalfa by leucaena resulted in similar milk production and composition. Owing to the high cost of production of alfalfa, under tropical conditions, tree

legumes like leucaena could be used to supply a greater portion of the dietary protein to ruminant livestock.

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TABLE 1. Ingredients and chemical composition of Experimental diets

Ingredients and Composition	Diet	
	Alfalfa	Leucaena
	% of DM	
Leuceana	0	40.0
Alfalfa	40.0	0
Cottonseed hulls	15.0	10.0
Ground corn	16.0	20.0
Oats	17.5	17.5
Soybean meal	9.3	9.3
Trace mineral salt[a]	1.0	1.0
Calcium carbonate	0.5	0.5
Vitamin ADE[b]	0.2	0.2
Urea	0.5	1.4
Composition		
CP	16.38	14.56
NDF	39.78	42.23
ADF	24.48	30.33
Energy Mcal/kg	4.04	4.07
Calcium	0.88	0.79
Phosphorus	0.29	0.30
Ash	7.52	6.09

[a]Containing (in percentage) NaCl, 94 to 95; Mn, > .2; Ferrous, >16; Ferric Fe, > .14; Cu, > .33; Zn, 7.10; I, > .007; Co, > .005

[b]Each gram contained 2,200 IU of Vitamin A, 2,200 IU of Vitamin D, and .2 IU of Vitamin E

TABLE 2. Feed intake and BW for does fed leucaena and alfalfa diets

Item	Diets		SE	P>F	
	Alfalfa	Leucaena		Diet	Diet x Time
DMI[a] (kg/d)	3.2	6.1	0.2	0.23	-
DMI (% of BW[b])	6.3	6.5	0.3	0.68	0.70
Intake of CP[c] (g/d)	519	625	30	0.92	-
Initial BW (kg)	43.9	45.9	-	-	-
Final BW (kg)	53	51	3.	0.99	0.001
Average daily BW gain (g/d)	109.3	60.1	10	0.01	-

[a]DMI = Dry matter intake

[b]BW = Body weight

[c]CP = Crude protein

TABLE 3. Averge yield and composition of milk from does fed leucaena or alfalfa based diets during the experimental period

Item	Diet		SE	P>F	
	Alfalfa	Leucaena		Diet	Diet x Time
Milk, kg/d	3.2	3.1	0.3	0.69	0.77
Fat %	2.4	2.7	0.1	0.25	0.33
Protein %	2.92	2.86	0.04	0.32	0.08
Lactose %	4.37	4.29	0.02	0.67	0.83
SNF[a] %	7.76	7.72	0.09	0.79	0.21
Total solids, %	10.2	10.4	0.2	0.57	0.21
4% FCM[b], kg/d	2.3	2.7	0.2	0.18	0.16
Milk/DMI	1.07	0.92	0.07	0.3	0.13
SSC[c] (10^3/ml)	459	237	150	0.52	0.36

[a]SNF = Solid not fat

[b]FCM = Fat corrected milk

[c]SSC = Somatic cell count

TABLE 4. Ruminal characteristics of lactating does fed alfalfa or Leucaena diets

Item	Diets			
	Alfalfa	Leucaena	SE	P>F
pH	6.21	6.38		0.01
Ammonia-N, mg/dl	13.7	17.7	0.8	0.25
Total VFA[a], mM	98.2	87.2	4.8	0.25
Acetate, mol/100 mol	64.8	65.9	0.8	0.48
Propionate, mol/100 mol	19	18	1.3	0.60
Isobutyrate, mol/100 mol	0.67	0.74	0.02	0.09
Butyrate, mol/100 mol	13.4	12.9	0.3	0.34
Isovalerate, mol/100 mol	0.1	1.4	0.1	0.11
Valerate, mol/100 mol	1.17	1.24	0.07	0.58
Acetate:Propionate ratio	3.6	3.8	0.3	0.69

[a]Volatile fatty acids

TABLE 5. Blood components of lactating does fed leucaena or alfalfa diets

Item	Diets		SE	P>F
	Alfalfa	Leucaena		
NEFA, Meq/L	185	136	29	0.33
Glucose, mg/dL	72.2	68.6	2.6	0.41
Total protein, g/L	70.7	72.1	2.7	0.75
Urea N, mg/dL	34.6	35.7	0.4	0.17
Insulin, μIU/ml	38.7	31.2	3.3	0.24
T_3, ng/dL	200	179	6	0.02
T^4, μu/ml	5.7	5.4	0.4	0.73

T_3 = Triiodothyronine

T_4 = Thyroxine

TABLE 6. Effect of leucaena or alfalfa diets on ruminal ammonia, blood urea and glucose (mg/dL)

Weeks	Ruminal NH_3-N	Plasma urea	Plasma glucose
6	16	30	60
9	14	31	60
12	15	42	90
Hour			
0	11	32	64
4	19	36	75
Significance level			
Weeks	-	0.06	0.79
Hours	0.001	0.001	0.001
S.E. of weeks	0.76	0.55	2.65
S.E. of hours	1.38	1.08	2.52

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APPENDIX

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LEUCAENA IN A RATION FOR HIGH PRODUCING ALPINE GOATS

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SUMMARY

In this study the effect of substituting leucaena for alfalfa in a ration for Alpine does on milk production and composition was investigated. Twenty multiparous Alpine does (mean BW 44.5 kg) in early lactation were stratified by expected kidding date and assigned randomly to two treatment diets in which 50% of the dietary protein consisted of either leucaena or alfalfa. Rations were fed *ad libitum* as complete mixed diets. Does were milked twice daily (0600 and 1800 h) and weights were recorded at each milking. The experimental period lasted 15 weeks. No differences (P>.10) were found for milk production (3.21 vs. 3.07 kg/d); percentage of milk fat (2.44 vs. 2.65%) or milk protein (2.92 vs. 2.86%) for alfalfa and leucaena treatments, respectively. Mimosine, a free amino acid found in leucaena, was not detected in milk samples.
Keywords: Alfalfa, leucaena, goats, milk

INTRODUCTION

The main use of leucaena in Ethiopia was, until recently, for control of soil erosion. Most nutritional studies on leucaena in Ethiopia have used sheep or cattle. Neither the nutritive value of leucaena nor its feeding value for goats has been appropriately evaluated. Less developed countries (LDC's) possess 96% of the global population of goats. These goats, however, produce 88% less milk and 38% less carcass yield than goats in more developed countries (Winrock International, 1983). These differences are due to breed, nutrition, health status and management practices applied in these countries (Devendra 1988, Johnson et al., 1986). Milking goats cost less than cattle, kid between one and two years of age and require less feed per head for maintenance than a cow (Boor et al., 1987). Therefore, there is a tremendous opportunity for improving productivity of goats in LDC's. Forage trees or browses are deep rooted, offer some drought tolerance, and, being leguminous, provide forage of high nutritive value, and wood which can be used for a variety of purposes such as firewood and construction purposes. Legumes associated with moderate levels of tannins (e.g. *Leucaena leucocephala*) tend to have more by-pass protein than standard high leguminous forages like alfalfa (*Medicago Stavia*) (Woodward and Reed, 1989). This characteristic is important when the synthesis and flow of microbial protein to the intestine is insufficient to meet the needs of high producing dairy cows and goats, typically during early and peak lactation. Supplementation with leucaena has been shown to maintain production in milking cows (Lema, et al., 1988; Pollard and Richardson, 1995), and body weight gain in sheep (ILCA, 1986; Shenkoru and Mekonnen, 1994). However, most leucaena contains about 3 to 5% of mimosine on a DM basis. Mimosine is a non-protein, free amino acid that is toxic to non-ruminants and unadapted ruminants. It is generally believed that leucaena can constitute up to 30% of the diet of unadapted ruminants without signs of toxicity. Leucaena's feeding value for dairy goats has not been appropriately evaluated. Therefore, this study was designed to investigate the feeding value of leucaena and its substitutional value for alfalfa in a ration for lactating Alpine goats.

MATERIALS AND METHODS

Twenty multiparous Alpine does in early lactation (BW = 44.5 kg) were utilized for this study. Individual does were started on the experiment over a 6 week period. They were stratified by expected

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kidding date and assigned randomly to one of the two dietary treatments. The treatments were a) alfalfa, and b) leucaena. The composition of the two diets was similar (16% CP and 2.3 Mcal ME/kg; Table 1) with 50% of the dietary protein consisting of either leucaena or alfalfa. They were fed as a complete mixed diet to minimize particle size separation and to prevent sorting by animals. Does were fed daily (0800 h) sufficient to ensure at least 10% orts. A Calan goat feeding system (American Calan Inc., Northwood, NH) was used to allow maximum freedom of doe activity while monitoring individual feed intake. Milk production during the first 3 weeks was used as a covariate period for collection of pre-treatment data for milk production and composition. Does were milked twice daily (0600 and 1800 h) and milk weights were recorded at each milking using a computerized flow metering device (Westfalia Systemat, Elk Grove, IL). Milk was sampled both morning and evening once weekly throughout the experimental period. Samples were analyzed for fat, protein (N x 6.38), lactose and SNF by infrared spectrophotometer (Multispec 2, Multispec, Wheldrake, York, U.K.). One doe was removed from the experiment in week 4 as she was stealing food from other does. Data for milk production, feed intake, and body weight were analyzed by multivariate repeated measures (SAS, 1989).

Table 1. Experimental Diet Composition, % DM

Ingredients	Alfalfa	Leucaena
Leucaena	0	40.0
Alfalfa	40.0	0
Cottonseed hulls	15.0	10.0
Ground corn	16.0	20.0
Oats	17.5	17.5
Soybean meal	9.3	9.3
Trace mineral salt[a]	1.0	1.0
Calcium carbonate	0.5	0.5
Vitamin ADE[b]	0.5	0.5
Urea	0.5	1.4
Composition		
CP	16.4	14.6
NDF	39.8	42.2
ADF	24.5	30.3
Energy, Mcal/kg[c]	4.0	4.1
Calcium	0.88	0.79
Phosphorus	0.29	0.30
Ash	7.52	6.09

[a]Containing (in percentages) NaCl, 94 to 95; Mn, >.2; ferrous Fe, >.16; ferric Fe, >.14; Cu, >.033; Zn, >.10; I, >.007; Co, >.005.
[b]Each gram contained 2,200 IU of vitamin A, 2,200 IU of vitamin D, and .2 IU of vitamin E.
[c] Calculated from metabolizable energy (ME) values (NRC, 1981).

RESULTS AND DISCUSSION

The daily dry matter intake was similar for the two diets (Table 2). Milk production (Figure 1), milk fat, lactose, SNF, and total solid percentage were not affected by diet (Table 2). These results agree with those found by Richards et al. (1994) who reported that leucaena could replace 50% of the N

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supplied by a concentrate in a forage-based diet for lactating goats with no adverse effects on milk production. Using dairy cattle Pollard and Richardson (1995) showed no effect on milk production or composition when leucaena was substituted for alfalfa in the diet. No signs of mimosine or DHP toxicity were observed in this experiment. This agrees with the results of Adejumo and Ademosun (1991) who reported that West African Dwarf sheep and goats could be fed up to 40% (sheep) and 60% (goats) of leucaena in their rations without showing any symptoms of leucaena toxicity. The high milk production (Figure 1) of these animals supported by leucaena is comparable to that produced by local cattle on small farms in Kenya (Boor et al., 1987).

CONCLUSION

The replacement of alfalfa by leucaena resulted in similar milk production and composition. No signs of leucaena toxicity were observed and dry matter intake was similar between the two groups. Cwing to the high cost of production of alfalfa, tropical legumes like leucaena could be used to supply a greater portion of the dietary protein for dairy goats.

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Table 2. Dry matter intake and milk composition of does fed leucaena or alfalfa based diets

Item	Alfalfa	Leucaena	SE	P <
DMI, kg/d	3.07	3.14	0.19	.23
Fat, %	2.44	2.65	0.01	.32
Protein, %	2.92	2.86	0.07	.59
Lactose, %	4.27	4.29	0.02	.33
SNF, %	7.76	7.72	0.09	.31
Total Solids, %	10.20	10.38	0.18	.30

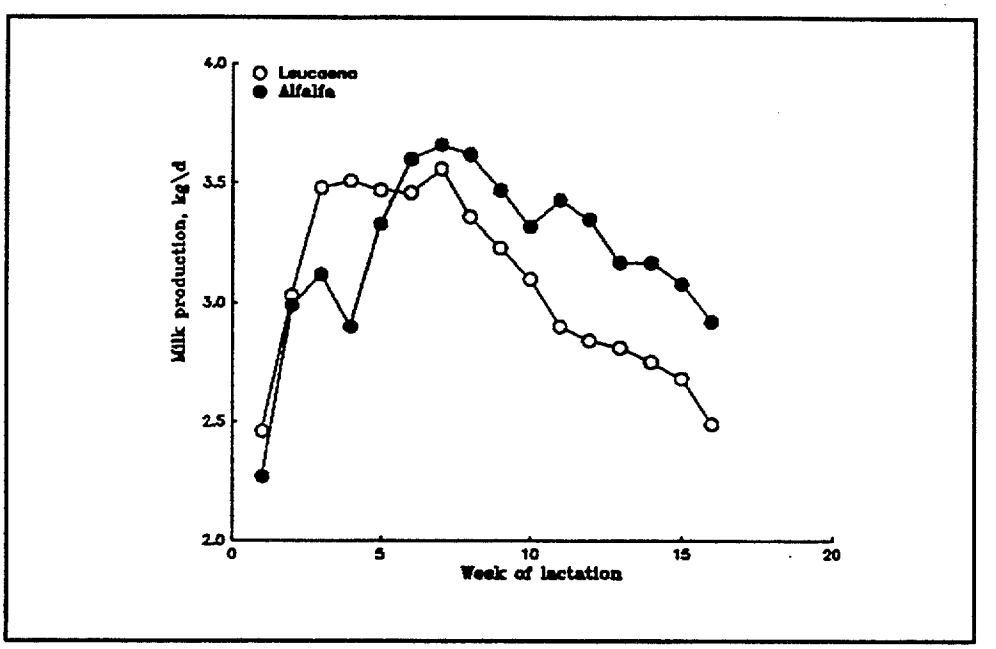

Figure 1. Milk production of Alpine goats fed diets with leucaena or alfalfa.

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AN ANOMALY IN THE REACTION OF TEST MEDIA TO *Synergistes jonesii*

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SUMMARY

Synergistes jonesii, a dihydroxypyridine-degrading bacteria, in ruminal fluid collected from steers grazing *L. leucocephala* was pulse-dosed via stomach tubes into the rumen of goats consuming *L. leucocephala*. Color reactions of test media Fe-1 and Fe-2 were used to determine the success of inoculation. Fe-1 and Fe-2 media proved a simple and reliable technique to test for the presence of *S. jonesii* in inoculum and ruminal contents. The Fe-1 test media probably does not supply sufficient substrate for sulphide producing bacteria in the mixed bacterial population. This substrate precipitates a sulfide deficiency resulting in a reduced formation of ferric sulphide and instead of the expected black color response readily obtained under similar conditions with Fe-2, free Fe(III) precipitates as a yellow color. However, the unexpected yellow color reaction of Fe-1 to a mixed ruminal population and *S. jonesii*, should be noted as a positive response to the presence of DHP-degrading bacteria in a mixed ruminal population.

Keywords: Synergistes jonesii, Leucaena leucocephala, dihydroxypyridine, mimosine

INTRODUCTION

L. leucocephala is a tropical leguminous shrub or tree which contains mimosine, a toxic amino acid. When ingested by ruminants, most mimosine is naturally degraded to a toxic (Christie et al. 1979) goitrogenic metabolite, dihydroxypyridine (3-hydroxy-4[1H]-pyridine; DHP) by the mixed microbial population in the rumen (Tangendjaja et al. 1983; Jones 1985).

A DHP-degrading bacteria, now classified as *Synergistes jonesii* (Allison et al. 1992) can be transferred to ruminants that experience symptoms of mimosine toxicity. This bacteria is capable of degrading both the 3,4 and 2,3-isomers of DHP (Allison et al. 1987) into non-toxic compounds. Allison et al. (1990), Allison (1991) and Hammond et al. (1992) described cultural tests and media that can be used in the detection of *S. jonesii* in ruminal fluid. Such techniques can be used to screen ruminants for the presence of these microorganisms before they are permitted to browse on *L. leucocephala*.

In this paper we report on an unexpected color reaction when ruminal fluid containing *S. jonesii* was inoculated into the test media.

METHODS

Experimental animals, housing and diets

Nine adult Angora wethers (average BW = 26.5 kg SE 1.3) were weighed, housed in individual metabolic cages with head gates and adapted to a 100% *L. leucocephala* diet (CP = 17.2%; DM basis) offered *ad libitum* with *ad libitum* access to water for 1 week. Feed was offered at 0800 daily after feed refusals of the previous day had been collected and weighed. Four goats were randomly allocated to receive no DHP-degrading bacteria (CON) and the remaining five goats were designated to receive DHP-degrading bacteria inoculation (INOC). The two treatment groups were physically removed to two distinctly separate areas of the experimental building. Special care was taken to keep inoculated goats isolated from the control group to prevent cross-contamination of DHP-degrading bacteria. Daily, handling (feeding, cleaning and sampling) of the control goats was completed first, after which the control experimental room was sealed and not re-entered on the same working day. Separate sets of laboratory clothing were used for each treatment group of animals.

Inoculation

Ruminal fluid (1 L) containing *S. jonesii* was obtained from steers grazing *L. leucocephala* from Dr. A. C. Hammond of the Department of Pharmacology and Toxicology of the USDA, ARS in Brooksville, FL. The ruminal fluid was shipped overnight in plastic containers with screw-top lids. The five goats selected for inoculation with DHP-degrading bacteria were dosed with ± 200 mL of the ruminal fluid via stomach tube according to the technique of Jones and Megarrity (1986) within 1 hour of receiving the ruminal fluid.

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Tests to determine viability of bacteria in steer ruminal fluid

To test the viability of the *S. jonesii* cells in the ruminal fluid collected from steers grazing *L. leucocephala* the test media Fe-1 (containing DHP) and Fe-2 (containing mimosine) were used as described by Allison (1991). Aliquots of the steer ruminal fluid (.1 mL) were anaerobically injected into each of two Fe-1 and Fe-2 culture media vials, which were then incubated at 38°C in a water bath.

Tests to determine success of inoculation

Immediately before, 1 day after and 1 month after inoculation with ruminal fluid from steers, ruminal fluid samples (± 30 mL) were collected from INOC and CON goats via stomach tube. Aliquots (.5 mL) were injected into Fe-1 and Fe-2 media containers and incubated at 38°C.

RESULTS

Viability of bacteria in steer ruminal fluid

Both sets of media turned black within 5 days.

Viability of bacteria in goats after inoculation

Samples collected from inoculated and non inoculated goats prior to inoculation remained orange. Samples collected 1 day and 1 month after inoculation from inoculated goats produced a yellow color reaction in Fe-1 media and a black color reaction in the Fe-2 media within 5 days of incubation. Fe-1 and Fe-2 media of non inoculated goats remained orange.

DISCUSSION

According to Allison (1991), the color reactions of culture media Fe-1 and Fe-2 to the addition of ruminal fluid of inoculated goats and incubation in a water bath at 38°C are indicative of the presence of DHP-degrading bacteria. The color changes of Fe-2 media from orange to black is the result of a series of reactions (Figure 1). Firstly, the alanine side-chain is removed from mimosine to form 3,4-DHP. Allison (1991) suggested that microbes were responsible for this step, but Jones (1984) suggested that mastication with alkaline saliva may also result in the autolytic degradation of mimosine to DHP. He noted that the cell-free supernatant of ruminal fluid was incapable of degrading mimosine and suggested that is was because the enzymes responsible for the degradation were not secreted into the ruminal fluid. Furthermore, endogenous plant enzymes (Smith and Fowden 1966) in fresh *L. leucocephala* but not in dried *L. leucocephala* (Jones 1984) meal also facilitated mimosine degradation to DHP. Tangendjaja et al. (1983) suggested that degradation occurs simply when plant tissue cells are disrupted, but later (Tangendjaja et al. 1986) was responsible for isolating 'mimosinase', an enzyme capable of degrading mimosine found in *L. leucocephala* leaf. It seems likely that mimosine degradation to DHP may occur in the presence of microbial enzymes, endogenous plant enzymes or by simple hydrogenation. Nevertheless, hydrolysis of mimosine produces 3,4-DHP and pyruvic acid, and it is the pyruvic acid which is further transaminated to produce alanine (Hart et al. 1977).

Following mimosine degradation, *S. jonesii* is responsible for the degradation of 3,4-DHP, first to 2,3-DHP (Allison et al. 1992) and then to acetate, propionate and ammonia. These end products have not yet been determined with certainty (Allison et al. 1992; Allison et al. 1994), but Allison et al. (1994) have suggested that the degradation of 3,4-DHP does not follow the classic pyridendiol degradation operative in aerobes such as *Agrobacterium* as shown by Watson et al. (1974). Instead, they propose that acetate and propionate are probably produced instead of pyruvate and formate (Figure 1). The degradation of 3,4-DHP leads to the subsequent release of Fe(III) from its chelate with DHP. It is not believed that the Fe(III) chelate is broken when mimosine is degraded to DHP because DHP is more effective at chelating iron than mimosine (Brock et al. 1990). Sulphide producing bacteria (normally present in a mixed ruminal population) must then be present to produce ferric sulphide with the released Fe(III). It is believed that the ferric sulphide is responsible for the black color formation in the Fe-1 and Fe-2 tests.

However, the formation of the yellow color in the Fe-1 media was unexpected. Allison (1991) stated that when Fe-1 media was inoculated with pure cultures known to degrade 3,4-DHP the media developed a yellowish color, but when inoculated with both a pure culture that could degrade 3,4-DHP and a mixed ruminal bacterial population, the media developed a dark black color for the same reasons as described for Fe-2 media. In our experiment Fe-1 media developed a yellow color despite the fact that a mixed ruminal population was present in the inoculum. It is speculated that this media may possibly not provide sufficient substrate for the mixed ruminal bacterial population. In Fe-2 media, where mimosine is the major substrate, the pyruvic acid or alanine produced from mimosine degradation may serve as an additional substrate for sulfide producing bacteria. The production of sulfide by this

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population was thus hampered resulting in free Fe(III) which imparted a yellow color to the test media.

Ruminal fluid collected from steers grazing *L. leucocephala* made both sets of media turn black within 5 days, indicating that this ruminal fluid did indeed contain viable populations of DHP degrading bacteria. This inoculum most probably contained a larger mixed ruminal bacterial population and a greater amount of additional substrate thus allowing a greater production of sulphide for ferric sulphide production in the test vial.

CONCLUSIONS

The unexpected yellow color reaction of Fe-1 to a mixed ruminal population and *S. jonesii*, should be noted as a positive response to the presence of DHP-degrading bacteria in a mixed ruminal population. This test media probably does not supply sufficient substrate for sulphide producing bacteria in the mixed bacterial population. The deficient substrate results in a sulfide deficiency which causes a reduced formation of ferric sulphide and instead of the expected black color response readily obtained under similar conditions with Fe-2, free Fe(III) precipitates as a yellow color. Despite this, Fe-1 and Fe-2 media are effective tools in determining the viability of bacteria in an inoculant and also in establishing the success of inoculation. It should be remembered that both media produce the same result, so the usefulness of the one test above the other in determining DHP degradation is debatable. To reduce labor it is recommended that only one of these tests be used because both give the same result.

ACKNOWLEDGMENTS

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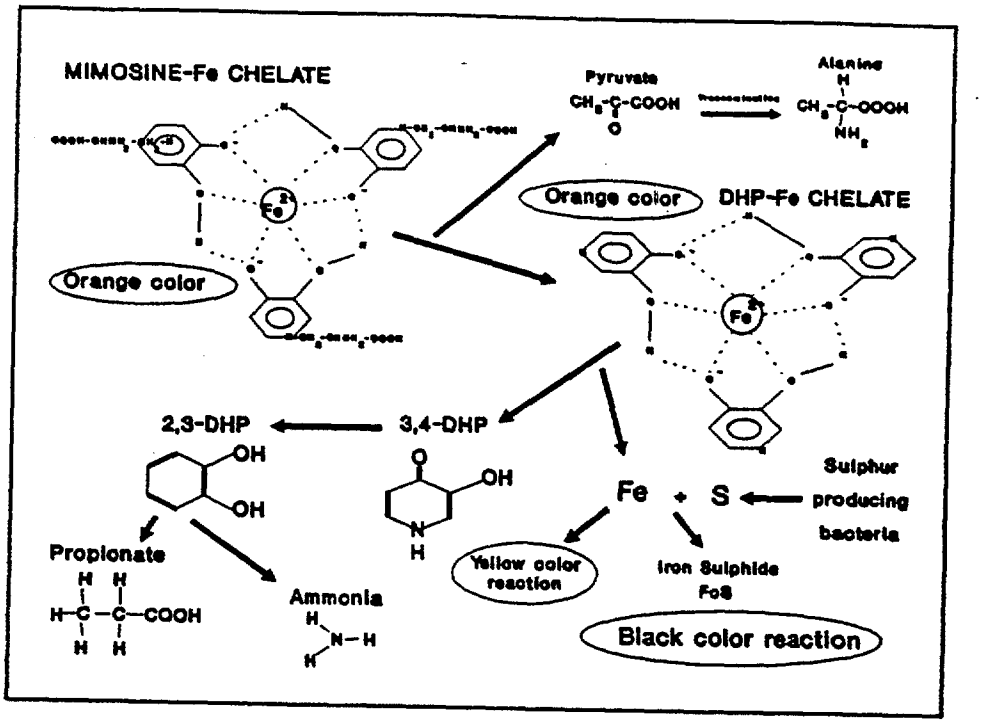

Figure 1. Degradation of DHP-Fe chelate in Fe-1 and mimosine-Fe chelate in Fe-2 media

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THE LEVEL OF SUCCESS OF TWO METHODS OF INOCULATION OF *Synergistes jonesii* INTO ANGORA GOATS FED *Leucaena leucocephala*

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SUMMARY

Two methods were used to inoculate *S. jonesii*, DHP-degrading bacteria, via stomach tubes into the rumen of goats consuming *L. leucocephala*. Methods were: inoculation of pure batch cultures of *S. jonesii* in growth media, and inoculation of *S. jonesii* already established in ruminal fluid from steers grazing *L. leucocephala*. Color reactions of test media Fe-1 and Fe-2, the optical density of the supernate or filtrate of ruminal fluid added to a ferric chloride solution read at 600 nm, and the ability of ruminal fluid to degrade DHP (measured by HPLC) were used to determine the success of inoculation. Inoculation of *S. jonesii* via ruminal fluid from steers grazing *L. leucocephala* was successful after one attempt, whereas two attempts to inoculate goats from pure batch cultures of *S. jonesii* in growth media were unsuccessful.

Keywords: Synergistes jonesii, Leucaena leucocephala, dihydroxypyridine, mimosine

INTRODUCTION

Mimosine, a toxic amino acid, which causes alopecia, reduced feed intake, esophageal lesions, renal and liver dysfunction and inhibits a number of biochemical reactions (Jones 1979; Jones and Hegarty 1984) is present in all parts of *Leucaena leucocephala*, a tropical leguminous shrub or tree. When *L. leucocephala* is ingested by ruminants, most mimosine is naturally degraded to a goitrogenic metabolite dihydroxypyridine (3-hydroxy-4[1H]-pyridine; DHP) by the mixed microbial population in the rumen (Tangendjaja et al. 1983; Jones 1985). If *L. leucocephala* is the sole diet it can successfully be used to improve the nutrition of ruminants if DHP-degrading bacteria are present in the ruminal fluid of these ruminants. This bacteria, now classified as *Synergistes jonesii* (Allison et al. 1992), is capable of degrading both the 3,4 and 2,3-isomers of DHP (Allison et al. 1987) into non-toxic compounds and, when transferred to ruminants that experience symptoms of mimosine toxicity, can result in the loss of all clinical signs of toxicity within 60 days (Jones and Megarrity 1986).

Jones and Megarrity (1986) were the first to successfully colonize ruminants, that had previously been prone to *L. leucocephala* toxicity, with DHP-degrading bacteria in the rumen. Since then, various techniques have been used to inoculate these bacteria into the rumen (Jones and Megarrity 1986; Quirk et al. 1988; Hammond et al. 1989; Hammond 1995). The objectives of this experiment were to inoculate a group of goats which would later be used to determine the influence of *S. jonesii* on the physiological characteristics of goats consuming *L. leucocephala*. It was decided to test two methods of *S. jonesii* inoculation, so that recommendations may be made to producers in the field who may wish to establish *S. jonesii* in their livestock.

MATERIALS AND METHODS

Experiment 1. Inoculation of batch cultures of S. jonesii in growth media

Experimental animals, housing and diets Eight adult Angora wethers (average BW = 42.5 kg SE .26) were weighed, fitted with fecal bags, housed in individual metabolic cages with head gates and adapted to a 20:80 *L. leucocephala* (CP = 17.2%): Alfalfa diet (CP = 18.5%) at 2% BW with free access to water for 1 week. Feed was offered at 0800 daily after feed refusals of the previous day had been collected and weighed. Four goats were randomly allocated to receive no DHP-degrading bacteria (CON1) and the remaining four goats were designated to receive DHP-degrading bacteria inoculation (INOC1). The two treatment groups were physically removed to two distinctly separate areas of the experimental building. Special care was taken to keep inoculated goats isolated from the control group to prevent cross-contamination of DHP-degrading bacteria. Daily, handling (feeding, cleaning and sampling) of the control goats was completed first, after which the control experimental room was sealed and not re-entered on the same working day. Separate sets of laboratory clothing were used for each treatment group of animals.

Inoculation. First attempt Three vials of pure *S. jonesii* cultures in growth media were obtained from Dr. M. J. Allison of the National Animal Disease Center, Ames, IA. These cultures had been

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maintained via twice weekly transfers as described by Allison et al. (1990). The growth media consisted of media 3,4-A as described by Allison et al. (1990) with 3% phytone peptone added to the media. The cultures of *S. jonesii* (90 mL) were added to 720 mL of strained ruminal fluid obtained from the four treatment Angora wethers. The mixture (MIX1) was bubbled with CO_2 and 180, 180, 180 and 270 mL, respectively was pulse-dosed into each of the four INOC1 goats via stomach tube.

Second attempt The first inoculation attempt was not successful (See *Tests to determine success of inoculation*), and prompted a second attempt to inoculate *S. jonesii* three weeks after the initial *S. jonesii* inoculation attempt. Ruminal fluid samples (250 mL) from each of the INOC1 goats were pooled and a new batch of *S. jonesii* bacteria in 3,4-A (Allison et al. 1990) growth media (30 mL; cultured from the initial batch obtained from Dr. Allison) was added to the pooled ruminal fluid and the mixture (MIX2) was pulse-dosed in equal portions (± 250 mL) into each INOC1 goat (n = 4) via stomach tube.

Experiment 2. Inoculation of S. jonesii via ruminal fluid from steers grazing L. leucocephala

Experimental animals, housing and diets Nine adult Angora wethers (average BW = 26.5 kg SE 1.3) were weighed, fitted with fecal bags, housed in individual metabolic cages with head gates and adapted to a 100% *L. leucocephala* diet (CP = 17.2%) offered *ad libitum* with free access to water for 1 week. Feed was offered at 0800 daily after feed refusals of the previous day had been collected and weighed. Four goats were randomly allocated to receive no DHP-degrading bacteria (CON2) and the remaining five goats were designated to receive DHP-degrading bacteria inoculation (INOC2). The goats were physically removed to two distinctly separate areas of the experimental building as before, with the same special care taken to keep inoculated goats isolated from control goats.

Inoculation Ruminal fluid (1 L) containing *S. jonesii* was obtained from steers grazing *L. leucocephala* from Dr. A. C. Hammond of the Department of Pharmacology and Toxicology of the USDA, ARS in Brooksville, FL. The ruminal fluid was shipped overnight in plastic bottles with screw-top lids. The five goats selected for inoculation with DHP-degrading bacteria were dosed with 200 mL of the ruminal fluid (MIX3) via stomach tube according to the technique of Jones and Megarrity (1986) within 1 hour of receiving the ruminal fluid.

Tests to determine success of inoculation

Experiment 1. First attempt One week after initial inoculation, ruminal fluid samples were collected from each of the eight goats via a stomach tube and inoculated into 8 separate vials of Fe-1 and Fe-2. Also, 17.5 mL of freshly strained ruminal fluid was collected via stomach tube from each of the inoculated goats and placed into each of four test tubes in a water bath at 38°C. To these test tubes were added .5 mL of 400 mM arginine (arginine/HCl - 2.11 g/25 mL) and 2.0 mL 20 mM mimosine (mimosine/water 39.6 mg/10 mL) and the mixtures were continuously gassed with CO_2. One milliliter of each mixture was removed to represent 0 h sampling time. The tubes were sealed with black rubber stoppers and hypodermic needles with cotton swabs were inserted through the stoppers to prevent gas build-up inside the test tubes. The test tubes were incubated at 38°C. Aliquots (1 mL) were removed at 24 h intervals while gassing (as above). Aliquots were centrifuged at 10,000 g in a microfuge for 5 min. An aliquot of 500 μL of supernate was added to 5 mL of a ferric chloride solution ($FeCl_3$-$6H_2O$/HCl - .6 g/2 mL made up to 1000 mL with H_2O) in a centrifuge tube and mixed well. The mixture was centrifuged at 10,000 g and the supernate decanted. The optical density of the supernate or filtrate was read at 600 nm. It was expected that if the mimosine added to the ruminal fluid (of INOC1 goats) was degraded by DHP-degrading bacteria, the optical density reading would decrease over time.

Second attempt For the second inoculation attempt the degradation of DHP in ruminal fluid samples *in vitro* was tested. Approximately 50 mL ruminal fluid samples which were collected via stomach tube from each INOC1 goat and added to 2 mL of a 2,3-DHP/water solution (5.56 g 2,3-DHP/100mL H_2O) in test tubes. The mixtures were continuously gassed with CO_2. Three milliliters of each mixture was removed to represent 0 d sampling time. The tubes were sealed with black rubber stoppers and hypodermic needles with cotton swabs were inserted through the stoppers to prevent gas build-up inside the test tubes. The test tubes were incubated at 38°C. Aliquots (3 mL) were removed at 24 h intervals while gassing (as above). Disappearance of DHP from the sample was measured by HPLC according to the method of Tangendjaja and Wills (1980). This test is similar to the ferric chloride colorimetric test, but instead DHP is measured directly.

Experiment 2 Immediately before, 1 day after and 1 month after inoculation of MIX3, ruminal fluid samples (± 30 mL) were collected from INOC2 and CON2 goats via stomach tube. Aliquots (.5 mL) were injected into Fe-1 and Fe-2 media containers and incubated at 38°C.

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Statistical analyses

All variables were analyzed with the PROC REG and GLM procedures of SAS (1989). Treatment differences were tested using appropriate error terms and, when treatment effect was significant ($P < .10$), least square means were separated using the PDIFF procedure of SAS (1989) and pooled standard errors were calculated.

RESULTS

Tests to determine success of inoculation

Experiment 1. First attempt No color changes were observed in either the Fe-1 or Fe-2 vials of the CON1 or INOC1 goats for two weeks following inoculation of ruminal fluid into Fe-1 and Fe-2 test media. Also, no reduction in optical density was noticed during the 7 days following the incubation of ruminal fluid from inoculated animals in a ferric chloride solution (Figure 1).

Second attempt There was no decrease in the level of DHP, measured by HPLC, in incubated ruminal fluid from the inoculated and control animals over 4 days (Figure 2).

Experiment 2 When ruminal fluid samples were collected from INOC2 and CON2 goats and injected into Fe-1 and Fe-2 media containers, samples collected from inoculated and uninoculated goats prior to inoculation remained orange. Samples collected 1 day and 1 month after inoculation from inoculated goats produced a yellow color reaction in Fe-1 media and a black color reaction in the Fe-2 media within 5 days of incubation. Fe-1 and Fe-2 media of uninoculated goats remained orange.

DISCUSSION

The apparent inability of the pure cultures of *S. jonesii* in growth media to successfully establish a colony of *S. jonesii* intraruminally indicates that these cultures may not be a practical method to inoculate animals in the field. This is contrary to the results of Jones and Megarrity (1986) who inoculated cultures in media 98-5. Previously, Hammond (1995) used a scale-up technique to increase the volume of *S. jonesii* from a 10mL culture to approximately 1280 mL of inoculum, using repeated additions of coarsely strained ruminal fluid, *S. jonesii* cultures and L-arginine. This inoculum successfully established *S. jonesii* in inoculated animals after one attempt. This method was developed to eliminate the need for a specialized medium and the anaerobic technique used for the scale-up of pure *S. jonesii* cultures as was done in our experiment. Judging from the success of the inoculation of this media (Hammond 1995) it is recommended that his method rather than the one described in this experiment should be used.

The infusion of ruminal contents already containing established populations of the bacteria was more effective at producing successful inoculation. Quirk et al. (1988) were similarly successful in inoculating *S. jonesii* with ruminal contents through the rumen wall or via stomach tube. The greater success achieved here than when pure cultures were mixed with ruminal contents and then inoculated may be because the ruminal contents already contained all the elements of a healthy viable mixed ruminal population. The introduction of a foreign entity (in this case pure cultures of *S. jonesii* bacteria) to the rumen in small quantities may lead to the rapid demise of the introduced bacteria.

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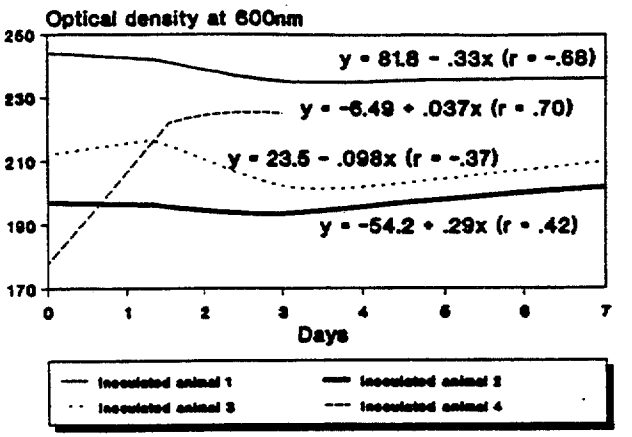

Figure 1. Change in optical densities (600nm) of rumen fluid from INOC1 goats inoculated with MIX1 over 7 days

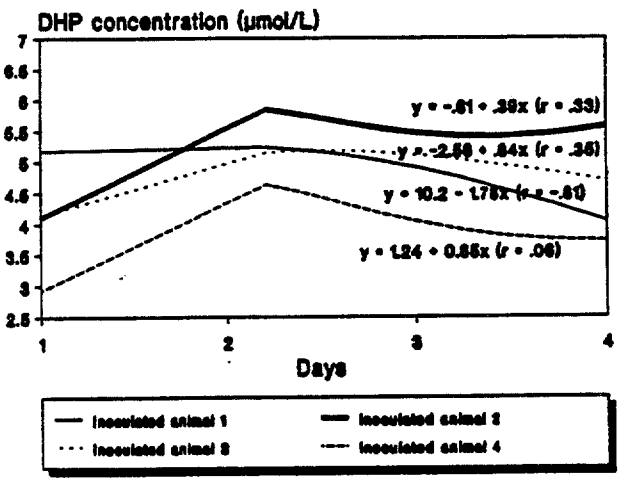

Figure 2. DHP degradation in rumen fluid of INOC1 goats inoculated with MIX2 over 4 days

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EFFECT OF CHELATING OF 2,3-DIHYDROXYPYRIDINE ON ITS TOXICITY IN ANGORA GOATS

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SUMMARY

To study the effect of mineral supplementation on 2,3-DHP toxicity, 16 mature Angora wethers were randomly allocated to four treatment groups (M, DM, D, C). Two hours after the morning feeding; group M received a mineral mixture of 2 g of $Fe_2(SO_4)_3 \bullet 7H_2O$, 2 g of $MgSO_4 \bullet 7H_2O$ and 2 g of $ZnSO_4 \bullet 7H_2O$ intraruminally in 80 mL of water; group DM received a similar mineral mixture plus 6 g of 2,3-DHP (DHP chelated with metal ions), group D received 6 g of 2,3-DHP in 80 mL of water and group C received 80 mL of water (control). In group D, two animals died within 24 h of receiving DHP and the other two animals were sacrificed to prevent suffering. Ruminal concentration of DHP was approximately 18 times higher in group D than in group DM (2.8 vs 0.16 mM; $P<0.05$) 4 h following treatment. Plasma DHP concentration, 6 h post treatment, was lower in group DM than in group D (15.2 vs 85.9 μM; $P<0.05$). Urinary excretion of 2,3-DHP was much more efficient in group DM than in group D (3.95 vs 1.8; $P<0.05$). Plasma triiodothyronine in group D was lower (110 vs 211 ng dL^{-1}; $P<0.05$) than in group DM. Mineral supplementation prevented 2,3-DHP toxicity. More efficient urinary excretion of chelated 2,3-DHP suggests that 2,3-DHP must be chelated to be efficiently transported and cleared by the kidney.

Keywords: Angora goats, 2,3-dihydroxypyridine, zinc, copper, magnesium.

INTRODUCTION

Mimosine is a naturally occurring toxic amino acid found in the seeds and leaves of *Leucaena leucocephala*. Its relatively high protein content (220 to 330 g kg^{-1} DM) makes it an attractive feedstuff for animals; however, its value is limited due to its high mimosine (10 to 40 g kg^{-1} DM). The chelating property of mimosine was the basis for it being administered as a drug for treating iron-overloading in humans (Brock et al. 1988). It is believed that mimosine forms soluble iron, zinc, magnesium and copper complexes (Schmid 1988; Kontoghiorghes 1990). In the presence of mimosine, these metal ions are chelated and excreted via the kidney in amounts proportional to the dose of mimosine (Schmid 1988). Mimosine ingested by ruminants is converted to the toxic compounds, 3,4-dihydroxypyridine (3,4-DHP) and 2,3-dihydroxypyridine (2,3-DHP) in the rumen. The toxic action of 2,3-DHP and 3,4-DHP has not yet been fully elucidated. Li (1987) assumed that it is mainly due to their goitrogenic action (Li 1987). The similarity in structure of 2,3-DHP and 3,4-DHP to mimosine suggested that 2,3-DHP and 3,4-DHP can also form chelates with ions and, therefore, it is possible that their toxic effect may be attributed to creating mineral deficiencies in the body. The objectives of this study were to determine if mineral supplementation to a diet containing 2,3-DHP will reduce or prevent DHP toxicity and to compare changes in blood and ruminal metabolites in animals fed diets supplemented with chelated and non-chelated 2,3-DHP.

MATERIALS AND METHODS

Animals, diets and experimental design

Sixteen Angora wethers (35 ± 2 kg) were fitted with venous jugular silicon catheters (i.d. 0.51 mm, o.d. 0.94 mm) under local anaesthetic. Animals were transported to individual cages for recovery for 2 d. During the experimental period the animals had ad libitum access to a complete mixed diet (11.0% CP and 9.99 MJ ME kg^{-1} of DM), fed once daily at 0800 h, and free access to water. Following 14 days of adaptation to the diet, animals were randomly allocated to four treatment groups. The experimental period lasted 3 days. Blood samples were taken from the jugular vein at 1000 h (2 h after feeding) on the first day. Immediately after sampling, animals received their assigned treatment intraruminally via a rumen tube. Group M received a mineral mixture of 2 g of $Fe_2(SO_4)_3 \bullet 7H_2O$, 2 g of $MgSO_4 \bullet 7H_2O$ and 2 g of $ZnSO_4 \bullet 7H_2O$ in 80 mL of water. Group DM received the same mineral mixture plus 6 g of 2,3-DHP. Group D received 6 g of 2,3-DHP in 80 mL of water. Group C received 80 ml of water. All solutions were adjusted to pH 6.5 with NaOH. Blood samples were taken from the jugular vein 2, 4, 6, 28 and 52 h after dosing. Urine and faeces were collected daily for a period of 3 d. Ruminal samples were taken daily 4 h after treatment.

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Analytical and statistical methods

Triiodothyronine (T_3) and thyroxine (T_4) were analyzed using commercially available kits from ICN Biomedicals, Inc. (Costa Mesa, CA). The intra-assay coefficient of variation averaged 5.4% for T_3 and 3.7% for T_4. Amino acid analysis were performed using an AminoQuant amino acid analyzer (Hewlett Packard, San Fernando, CA) with pre-column derivatization using o-phthalaldehyde, 9-fluorenylmethylchloroformate and UV detection. Concentrations of 2,3-DHP in plasma, rumen fluid and urine were estimated using a HPLC method described by Tangendjaja and Wills (1980). Plasma glucose concentrations were analyzed colorimetrically using a Sigma diagnostic kit (Sigma, St. Louis, MO). Plasma urea N was determined as described by Chaney and Marbach (1962). The volatile fatty acids were analyzed using gas chromatography (Hewlett-Packard Co., Avondale, PA) utilizing a 1.98 x 4 mm i.d. glass column packed with 15% SP-1200 plus 1% H_3PO_4 on 100/120 Chromosorb W AW (Supelco Inc., Bellefonte, PA).

Mortality rate was analyzed by a Chi squared contingency table. The blood data were analyzed as a split-plot in time using SAS (SAS 1985). The main plot was treatment tested by the error term, animal within treatment. The sub-plot designated as time of sampling and the interaction of time of sampling and treatment was tested against the residual error. Least-square means for each parameter were compared using a protected least-significant difference technique (Steel and Torrie 1980).

RESULTS

The 2,3-DHP given to group D was lethal. Two animals from this group died within 24 h following treatment. The symptoms proceeding death were frequent urination, foaming at the mouth and severe muscular trembling. The remaining two animals from this group were sacrificed when these symptoms became apparent. Signs of discomfort in group D were observed 6 h after dosing. No changes in behavior or feed consumption were observed in the other groups. The ruminal concentration of 2,3-DHP 4 h after treatment was approximately 18 times higher in group D than in group DM (2.8 vs 0.16 mM; $P<0.05$). Plasma 2,3-DHP concentration was lower in group DM than group D (Table 1). Treatments had no influence on pH or volatile fatty acid concentrations in the rumen fluid samples taken 4 h after dosing (values not shown).

Table 1. The effect of DHP and mineral supplementation on concentration of 2,3-DHP in plasma (μM) in Angora goats

	Treatment				
Time, h	C	M	DM	D	SEM
0	0	0	0	0	0
2	0c	0c	160.2b	767.7a	30.0
4	0c	0c	40.6b	363.0a	30.0
6	0c	0c	15.2b	85.9a	30.0
28	0c	0c	4.0b	20.8a	2.3
52	0	0	0	–[y]	0

[y] missing observation due to death of animals.
a-c Values in the same row with different letters differ significantly ($P<0.05$).
SEM Standard error of the mean.

Although total volume of urine excreted was highest in group D, urinary 2,3-DHP excretion was highest in group DM (Table 2). About 67% of intraruminally injected 2,3-DHP was excreted during the first 24 h by DM goats compared to 30% excreted by D goats. Treatment had no effect on plasma urea N, however, plasma glucose was lowest in group D (Table 3). Plasma T_3 from samples taken 28 h after treatment was lower in D goats than DM goats (110.3 vs 206.5 ng dL^{-1}; $P<0.05$), however, there were no effect of treatment on T_4 plasma concentration (Table 3).

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Table 2. The effect of DHP and mineral supplementation on urine volume and urinary excretion of 2,3-DHP in Angora goats

Items	Treatment C	M	DM	D	SEM
Urine, mL d^{-1}	566b	605b	542b	1405a	102
Urinary 2,3-DHP, g d^{-1}					
Day 1	0c	0c	3.95a	1.8b	0.1
Day 2	0	0	1.05	–[y]	0.1
Day 3	0	0	0.20	–[y]	0.1

[y] missing observation due to death of animals.
a-c Values in the same row with different letters differ significantly ($P<0.05$).
SEM Standard error of the mean.

Table 3. The effect of DHP and mineral supplementation on concentration of plasma metabolites in Angora goats

Items	Treatment C	M	DM	D	SEM
Glucose, mg dL^{-1}	52.2a	52.3a	52.2a	44.7b	1.0
Urea N, mg dL^{-1}	7.2	6.8	7.7	6.6	0.5
T_3, ng dL^{-1}	183.1a	206.5a	211.2a	110.3b	12.3
T_4, μg dL^{-1}	6.33	5.10	5.34	5.62	0.35

a-c Values in the same row with different letters differ significantly ($P<0.05$).
SEM Standard error of the mean.

DISCUSSION

Some nutritional studies have shown that mimosine can form chelates with metals or cause mineral deficiencies. For example, the mimosine content of Leucaena meal was reduced following treatment with a solution of $FeSO_4$ (12.6 g/kg leucaena meal) (Sunaria and Vidya-Sagar 1989). Prasad (1989) fed goats a diet containing 50 to 75% Leucaena leucocephala and observed that they became anorexic, weak, developed frothing and erosive lesions in the mouth and had a rough hair coat. Clinical improvement, without a change of diet, was achieved by adding 2.0 g/d of a mineral mixture (equal parts of zinc, copper and iron) to the diet.

The results of this experiment clearly showed that mineral supplementation played a significant role in overcoming 2,3-DHP toxicity. The group given minerals plus 2,3-DHP did not display any signs of toxicity while the goats given DHP alone died. The dose of DHP was based on what was considered to be an acceptable daily intake of mimosine in sheep (0.14 g kg^{-1} BW; Szyszka and Meulen, 1985). It was assumed that purified 2,3-DHP could be absorbed much faster than that from Leucaena leaves. For this reason 2,3-DHP was given 2 h after feeding at which time there should be elevated levels of rumen metabolites which may aid in protecting against DHP toxicity (Dominguez-Bello et al. 1993). Lack of changes in rumen volatile fatty acids, ammonia and pH indicated that 2,3-DHP was not affecting the microorganisms responsible for rumen fermentation. Brock et al. (1988) showed that mimosine can enhance growth of some microorganisms. In another study, Dominguez-Bello et al. (1993) showed that mimosine increased the number of protozoa by 25% in fermentors inoculated with rumen liquor.

2,3-DHP metabolism was different in the D and DM group. Group D had much higher concentrations of 2,3-DHP in the rumen and plasma, while the urinary excretion was only 50% of group

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DM (Table 2). This indicates that 2,3-DHP must be chelated in order to be effectively removed from the body. In a previous study (Puchala et al. 1995) mimosine caused a decrease in plasma concentrations of zinc, magnesium and iron. Decreased plasma concentration of minerals was due to chelation of minerals with Leucaena toxins. The lower concentration of 2,3-DHP in the plasma and the higher urinary excretion of 2,3-DHP in group DM suggests that kidney clearance of 2,3-DHP was very efficient when 2,3-DHP was in the chelated form. In contrast, the higher plasma 2,3-DHP concentration and lower urinary excretion in group D, suggests problems in clearance of 2,3-DHP by the kidney. It is possible that filtration of free 2,3-DHP by the nephrons is either very slow/or does not occur or that 2,3-DHP is reabsorbed from the tubular portion of the nephron.

In the current experiment a decrease in plasma triiodothyronine in group D occurred 28 h after treatment, with no changes in thyroxine (Table 3). This may indicate that the goitrogenic effect of 2,3-DHP is not a primary effect of 2,3-DHP toxicity. Many studies have concluded that 2,3-DHP is a potent goitrogen and responsible for the thyroid gland enlargement and decreases in blood thyroid hormone levels (Prasad 1989; Li 1987). However, Megarrity and Jones (1983) concluded that the goitrogenicity of 3,4-DHP is only partly responsible for the toxicity of Leucaena to ruminants, and that low feed intake and low liveweight gain is related to other effects of DHP. McSweeney et al. (1984) demonstrated that in cattle the 2,3-DHP goitrogenic effect was overcome with thyroxine therapy but that other toxic effects remained. This suggests that the goitrogenic effect of 2,3-DHP is only one of the many changes occurring as a result of 2,3-DHP treatment. Iron and zinc deficiencies depress thyroid function (Lukaski et al. 1992) and it is possible that 2,3-DHP indirectly affects thyroid function by chelating necessary minerals.

It is suspected that the primary mimosine/2,3-DHP toxic effect is due to depletion of metallic trace elements in the body. Our results indicated that the chelated form of DHP was excreted faster via urine.

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396 Influence of mineral supplementation on 2,3-dihydroxypyridine toxicity in Angora goats. R. Puchala, T. Sahlu, J. J. Davis, S. P. Hart, and M. Smuts*, E (Kika) de la Garza Institute for Goat Research, Langston University, OK.

To study the effect of mineral supplementation on 2,3-DHP toxicity, 16 mature Angora wethers were randomly allocated to four treatment groups (M, DM, D and C). Two hours after the morning feeding; group M received a mineral mixture of 2 g of $Fe_2(SO_4)_3 \cdot 7H_2O$, 2 g of $MgSO_4 \cdot 7H_2O$ and 2 g of $ZnSO_4 \cdot 7H_2O$ intraruminally in 80 mL of water; group DM received a similar mineral mixture plus 6 g of 2,3-DHP (DHP chelated with metal ions), group D received 6 g of 2,3-DHP in 80 mL of water and group C received 80 mL of water. All solutions were adjusted to pH 6.5 with NaOH. In group D, two animals died within 24 h of receiving DHP thus the other two animals were also sacrificed at this time. Ruminal concentration of DHP was approximately 18 times higher in group D than in group DM (2.8 vs 0.16 mmol L^{-1}; $P<0.05$) furthermore, only 47% of ruminal DHP in group D was chelated 4 h following treatment. Plasma DHP concentration, 6 h post-treatment, was lower in group DM than in group D (15.2 vs 85.9 nmol mL^{-1}, $P<0.05$). Sixty-seven percent of the intraruminally injected DHP was excreted via urine during the first 24 h of the experiment by group DM compared with 30% by group D ($P<0.05$). Treatment had no effect on plasma urea N, however, plasma glucose was lower in group D (44.7 vs 52.2 mg dL^{-1}). Plasma triiodothyronine in group D was lower (110 vs 211 ng dL^{-1}; $P<0.05$) when compared with group DM. It appears from the results of this experiment that mineral supplementation prevented 2,3-DHP toxicity. It is possible that 2,3-DHP causes mineral deficiencies in the body due to its strong chelating properties. Higher rates of urinary excretion of chelated DHP suggests that DHP must be chelated to be efficiently transported and cleared by the kidney.

This study was partially supported by USAID Grant #PCE-5053-G-00-3066-00 and DAN-5053-G-00-1033.

Key Words: Angora goats, 2,3-dihydroxypyridine, mineral

PHYSIOLOGY AND ENDOCRINOLOGY

397 The effect of propylthiouracil(PTU) on number of primordial follicles in rats. G. Adamska-Jarecka*, A. Stewart, D. Doolin, S. Meredith, Lincoln University, Jefferson City, MO.

The objective of the present experiment was to determine the effect of transient hypothyroidism, using PTU, on number of primordial follicles. Pregnant Sprague-Dawley rats were housed at a temperature of 20-22°C with 12h light and 12h dark (lights on at 0630h). Pregnant females were divided into three groups: Group 1 rats (n=6) received no PTU, group 2 rats (n=5) received PTU in their drinking water at a concentration of 0.1% from d 12 of gestation until pupping, and group 3 rats (n=6) received PTU in a final concentration of 0.05 % in water, from pupping until postpartum d 15. Water intake was measured daily from 8 d before pupping until 18 d after birth. On the day of birth, the litters were weighed, then adjusted within treatment, to between 8 and 10 per mother. Female pups were killed on d 15 (n=19), 25, (n=21), and 40 (n=20). Weights were taken and ovaries were collected from pups at each kill date. PTU treatment during lactation resulted in decreased ($P<.01$) pup weights on d 40. The number of primordial follicles in pups were not different ($P>.05$) among groups and are shown below (Means+SEM).

Days	Group 1	Group 2	Group 3
15	2124±231	1805±484	1819±385
25	1563±245	1903±309	2237±289
40	2176±206	1549±155	1610±146

In conclusion, thiouracil did not affect number of primordial follicles.

Key Words: Rats, thiouracil, primordial follicles.

398 **Polyclonal antibodies to rat adipocyte plasma membrane expressed tissue and species specificity.** C. B. Choi* and S. Y. Lee, *Yeungnam University, Gyeongsan, Korea.*

The objective of this study was to develop polyclonal antibodies to rat adipocyte plasma membrane (APM) and to investigate their tissue and species specificity. APM from female Sprague-Dawley rats (150-200g) was prepared using a self-forming Percoll gradient. Mature Corriedale male sheep (40-50kg) were passively immunized at 3-week interval with the purified APM. Blood was taken from immunized sheep 10-20 days after the third and each subsequent booster injection. Serum was stored for determination of antibody titres and immunoblotting. Sheep serum with the highest titre of antibodies against APM were used for all studies. Plasma membrane proteins from adipocyte, liver, heart, and kidney were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. Nitrocellulose membranes containing plasma membrane proteins were incubated with sheep antiserum against rat APM followed by incubation with anti-sheep immunoglobulin G-horseradish peroxidase conjugate. Color development was arrested by washing nitrocellulose membranes with peroxidase substrate and distilled water. Antibodies developed in sheep to rat APM reacted most strongly to rat APM proteins. And immunoblotting of the same antibodies with APM from rat, pig, and chicken showed the strongest reactivity to rat APM. This study suggests the existence of APM specific proteins. And we are undertaking further studies to investigate molecular sizes and components of APM specific proteins.

Key Words: Rat, Adipocyte, Antibodies

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177 Effect of *Leucaena leucocephala* as a protein source in a cottonseed hull diet on body weight gain, mohair production and rumen parameters of Angora goats. J. J. Davis[1], T. Sahlu[1], R. Puchala[1], and T. Shenkoru[2,*], [1]E (Kika) de la Garza Institute for Goat Research, Langston University, OK, [2]Alemaya University of Agriculture, Ethiopia.

Thirty Angora goats (19 wethers, 11 doelings) 8 mo of age and 17 kg BW were used in a 85-d study to determine the potential of *Leucaena leucocephala* (10.21% CP) as a supplement for low quality roughages such as cottonseed hulls (6% CP) used as rations for goats in developing countries. The five treatments (Trt 1-5) were: Control (CSH + 10% alfalfa); and CSH plus 10; 20; 30 or 40% leucaena. The leucaena, CSH and added vitamins and minerals were mixed together and fed as a TMR. The animals were offered feed at 0830 ad libitum to ensure 10% orts. Water was available at all times. Goats were weighed before feeding weekly and ruminal fluid samples were taken before and 2 h after feeding at the middle and end of the trial. Apparent DM digestibility of the rations was measured at the end of the trial. Dry matter intake was highest in Trt 4 and 5 and lowest in Trt 3 (.563, .593, .528, .742 and .709 kg/d for Trt 1-5, respectively; $P<.08$). Apparent DM digestibility of the ration was not different between treatments (46.7, 43.3, 43.2, 39.5 and 44.3%; $P>.38$). Goats fed the 40% leucaena ration had a higher ADG than goats on the other treatments (-20, -30, -33, -6 and 2 g/d for Trt 1-5, respectively; $P<.002$). No differences were found in fiber quality (diameter, med and kemp) or in clean fleece production (.57, .58, .61, .66, .70 kg/hd; $P>.30$). Total ruminal VFA concentration was highest for Trt 4 and 5 and lowest for Trt 2 (45.3, 40.6 38.9, 51.0 and 50.0 mmol/L, Trt 1-5, respectively; $P<.02$). Molar proportions of individual acids were not different between treatments and the fermentation pattern was 78:16:6 for acetate:propionate:butyrate. Rumen pH was similar for all groups. Leucaena could be used at 30-40% of the DM in a low quality roughage diet to maintain goats in developing countries.

This study was supported by USAID Grant #PCE-5053-G-00-3066-00

Key Words: Leucaena, mohair growth, Angora goats

178 The effect of *Gliricidia sepium* supplement on dairy cows. T. Clavero*, O. Obando, and R. Van Praag, *University of Zulia, Maracaibo, Venezuela.*

A field experiment was carried out at tropical dry forest in Zulia State, Venezuela with the objective to evaluate the effect of three diets (0 kg of *Gliricidia sepium* leaf meal + 3 kg of commercial supplement, 1/2 kg of *Gliricidia sepium* leaf meal + 2 1/2 kg of commercial supplement and 1 kg of *Gliricidia sepium* leaf meal + 2 kg commercial supplement) on milk production (MP), fat (F), ash concentration (AC) and acid levels (AL). A complete random design with six replications was used. A total of 18 dairy cows were selected homogeneous in milk production and genotype. The milk production was recorded every week and samples were taken for lab analysis. Differences were observed ($P<0.001$) in MP and F due to *Gliricidia sepium* leaf meal supplement. The best treatments on production parameters included *Gliricidia sepium* leaf meal on diet.

Key Words: *Gliricidia sepium*, Milk Production

179 Growth performance of indigenous Sanga cattle in South Africa. G.J. Taylor[1,*], F.J.C. Swanepoel[1], and A.J. Bothma[2], [1]University of the Orange Free State, Bloemfontein, South Africa, [2]Department of Agriculture, Far North Province, South Africa.

An investigation into growth performance of two Sanga (Nguni) stud herds in different agro-ecological regions in South Africa were conducted. The term Sanga is generally applied to all types of African cattle having a cervico-thoracic hump. Nguni cattle are neck-humped Sanga cattle, derived from Zebu and humpless long-horn parents. Sanga cattle originated in Ethiopia and the Sudan from where they migrated west and southward. The two agro-ecological environments can be described as follows: location one is partly rocky and mainly sandy with an average rainfall of 350 mm per year occurring mostly from November to February. This location is classified as arid sweet bushveld, while location two is reasonably homogeneous with an average rainfall of 600 mm per annum. This area is classified as sour, mixed bushveld. Data where analysed using the General Linear Models (GLM) procedure of SAS. The corrected weight measurements were analysed for the effects of locality, sex, month of birth, year of birth, cow weight, age of cow; productive status of cow and sire. Statistically highly significant ($P<0.01$) and significant ($P<0.05$) effects were established for all these sources except age of cow. Highly positive correlations between different weights on the growth curve from $r = 0.13$ ($P<0.05$) between birth weight and 100 day weight and $r = 0.83$ ($P<0.01$) between weaning weight and yearling weight were reported. The average weight for the growth parameters compared favourably with the averages reported by the National Beef Cattle Performance and Progeny Testing Scheme in South Africa.

Birth weight	= 23.85 kg	-	National average	= 27.0 kg
100 day weight	= 91.96 kg	-	National average	= 99.0 kg
Weaning weight	= 148.40 kg	-	National average	= 158.5 kg
Year weight	= 174.70 kg	-	National average	= 196.5 kg
18 month weight	= 255.40 kg	-	National average	= 255.0 kg
Cow efficiency	= 43.30 kg	-	National average	= 41.6 %

It can be concluded that different eco-types have developed within the Nguni in South Africa. The performance of the two stud herds investigated compare favourably with the national South African average. A target weight of 200 kg for first mating is recommended for the Nguni in South Africa.

Key Words: Sanga cattle, growth performance, South Africa.

180 Inbreeding and environmental factors affect litter performance at weaning in Botucatu rabbits. A.S. Moura [1*], R. Polastre [2] and W. R. Lamberson[3], [1]Unesp, Botucatu - Brazil, supported by CNPq, [2]Embrapa, Concordia - Brazil, supported by CNPq, [3] Department of Animal Sciences, University of Missouri, Columbia 65211.

The purpose of this study was to evaluate the effects of the inbreeding coefficient of the doe (F), of parity (1 to 6 and 7 or more) and of the month and year of parturition on the number of rabbits weaned per litter (NW), total litter weaning weight (WW), mean litter weaning weight (MW) and litter mortality rate from birth to weaning (LM) in Botucatu rabbits. It was conducted at the Unesp rabbitry located in Botucatu - SP, Brazil, from January, 1992 to May, 1994. Data on 1,380 litters were analyzed by the least-squares method. The litters were uniformly weaned at 30 days of age. The values of F ranged from zero to 0.33. Most of the females were usually culled before the seventh parturition, either due to decreased productivity, or to some alteration in their health status, so that the number of data on seventh and further parities were small and they were grouped together in a single class. The overall means and standard errors for NW, WW, MW and LM were, respectively: 5.19 ± 0.08, 3141 ± 44g, 543.6 ± 6.2g and 0.30 ± 0.01. Month of parturition influenced NW, WW, LM ($P<0.01$) and also MW ($P = 0.04$). This effect could be mainly attributed to seasonal variation in temperature and photoperiod. Year of parturition affected WW ($P = 0.01$) and also MW ($P = 0.04$). Parity was the most important source of variance for all traits ($P<0.001$). The first litter of a female presented significantly ($P<0.05$) lower NW (4.13) and also WW (2524g) when individually compared to each one of the other parity classes, except for the sixth. The other parity classes did not differ each other for these two traits and averaged 5.57 and 3437g for NW and WW respectively. The inbreeding coefficient had a significant effect on NW and WW ($P<0.01$), but not on MW ($P = 0.16$) or LM ($P = 0.58$). For each increment of 10% in F, a corresponding reduction of 0.66 rabbits in NW and of 247g in WW was observed. The simultaneous decreases in NW and WW could partially explain the constancy of MW and LM as F increased.

Key Words: Rabbits, inbreeding, litter performance