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METABOLIC ASPECTS OF MIMOSINE TOXICITY IN GOATS.

FINAL REPORT (1989-1990)

USAID Project No. DAN-5053-G-SS-8018-00.

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Summary

Two major experiments were conducted to investigate the effects of mimosine -- a natural, amino acid-like toxin found in the tropical legume Leucaena -- on goat health and productivity. The objectives were: (1) to determine mimosine-specific effects on hair fiber and milk production and quality in Angora (mohair) and Alpine (dairy) goats, respectively; and (2) to study the metabolic responses of the goats to subclinical mimosine toxicity. In the first study, short-term mimosine administration (i.v.) resulted in decreased mohair fiber production and plasma total protein concentrations in Angora wethers, suggesting a derangement in protein metabolism. Moreover, these studies demonstrated that mimosine can directly influence thyroid hormone levels and function. It appears that mimosine may affect hepatic and renal function as indicated by the elevated plasma bilirubin concentration and the development of uroschesis. In the second study, mimosine administration (i.v.) for 21 days in early lactating does did not affect milk yield or feed intake; however, the nitrogen content was higher in the milk from mimosine-treated does. Kids consumed 40% more milk when fed fresh milk from the mimosine-treated does as compared to the control; however, there were no consistent differences in growth rate or key blood metabolic indicators. Nevertheless, the effects of mimosine on

lactational performance and possible transfer into the milk are not conclusive and need further study.

Introduction

Leucaena, which is the common name for Leucaena leucocephala, is a leguminous tree forage ubiquitous to many tropical areas, and is considered to be a promising protein source for ruminants (National Research Council, 1984; Johnson et al., 1986). Leucaena contains between 20 to 30% crude protein and has a high digestibility (50 to 70%) (Jones, 1979; Johnson et al., 1986); however, a major limitation preventing it's widespread use is the existence of the toxic amino acid mimosine, also known as beta-[N-(3-hydroxy-4-pyridone)] -alpha-amino-propionic acid, which can comprise anywhere between 1 to 10% of the total plant dry matter (Jones, 1979, 1985; ter Meulen et al., 1979; ter Meulen and El-Harith, 1985; National Research Council, 1984). Mimosine has been associated with reduced feed intake, esophageal lesions, alopecia (hair loss) and derangements in thyroid hormone metabolism (ter Meulen et al., 1979; National Research Council, 1984; Jones, 1985). Moreover, microorganisms in the rumen breakdown mimosine to 3-hydroxy-4(1H)-pyridone or DHP, another toxin known to affect thyroid hormone function which has been suggested as the primary goitrogenic compound found in Leucaena (Hegarty et al., 1979; Jones, 1985). Most scientists suggest limiting Leucaena intake to 30% of the daily ration (Jones, 1985; ter Meulen and

El-Harith, 1985). For instance, in Thailand, goats can withstand a dietary mimosine level of approximately 0.18 g mimosine/kg BW/day, and thus, can consume 20 to 60% (depending on the plant variety and mimosine content) of their daily ration as Leucaena (Szyszka et al., 1984). Consequently, the development of mimosine and/or DHP toxicity can be a concern.

At the E (Kika) de la Garza Institute for Goat Research we have been studying the metabolic aspects of mimosine toxicity in goats for the last two years. Our overall objectives were: 1) to examine mimosine-specific effects on key carbohydrate, lipid and nitrogenous intermediates and regulatory hormone concentrations and function in goats; 2) to measure mimosine effects on mohair fiber yield and characteristics; and 3) to investigate the possible transfer and effects of mimosine in the milk of lactating does on kid performance and metabolic status.

Experiment 1

In the first study, we evaluated short-term, continuous mimosine administration (200 mg/kg$^{.75}$/day x 2 days) in five Angora goat wethers (avg. BW 51 kg) fitted with indwelling jugular vein catheters. The goats were kept in metabolism stalls and fed a complete mixed ration (12% CP and 2.4 Mcal ME/kg DM) ad libitum. Mimosine infusion (MIM) was preceded (PRE) and followed (POS) by 24 hr physiological saline (0.9% NaCl) infusions. Blood samples were taken at 4 to 8 hr intervals throughout the trial and analyzed for various plasma metabolites and hormones. In

addition, mohair fiber length, diameter and yield were determined.

Table 1 shows the effects of mimosine infusion on daily feed intake and mohair production characteristics. Feed intake was reduced ($P < .05$) by nearly 30%, with little to no intake during the last 24 hr of mimosine administration. Mohair fiber length and diameter decreased ($P < .05$) by 33%and 6%, respectively, resulting in a 45% decline ($P < .05$) in total mohair yield. Furthermore, complete or partial alopecia was noted in three of the goats, while uroschesis (suppression of urinary output) was observed in all goats. The effects of mimosine on plasma metabolites and hormones are listed in Table 2. Plasma L-lactate (Figure 1), total bilirubin (Figure 2), cortisol and glucagon (Figure 6) concentrations increased ($.05 \leq P < .10$), while levels of total protein (Figure 3), triiodothyronine (Figure 5), thyroxine (Figure 5) and insulin (Figure 6) decreased ($P < .05$) with mimosine infusion. Neither plasma concentrations of glucose (not shown), urea N (Figure 3), nonesterified fatty acids (NEFA; Figure 4), nor the plasma enzyme gamma-glutamyl transferase (GGTP; Figure 2) were affected. This is surprising since the goats were off-feed and experiencing uroschesis during this time.

Experiment 2

Ten Alpine does (avg. BW 55 kg) 1 to 3 weeks postpartum were fed a complete mixed ration (18% CP, 70% TDN and 22% ADF) ad

libitum, blocked by milk yield and BW, and randomly assigned to one of two treatments: saline (SAL) or mimosine treatment (MIM; 60 mg/kg$^{.75}$/day). The treatments lasted 21 days and consisted of intrajugular administration of the experimental solutions in bolus doses at 12 hr intervals immediately after each milking (0600 and 1800). Milk from the saline-treated (SMK) and MIM-treated (MMK) does were fed twice-a-day (0800 and 1500) to 1 to 3 week old kids (n=5/treatment; avg. BW 5.5 kg) for 21 days. Jugular blood samples were obtained from the does and kids 2 hr after the AM dosing and feeding, respectively.

Tables 3 and 4 list the effects of mimosine administration on production and metabolic parameters, respectively, in the early-lactating does. Mimosine administration did not affect ($P > .10$) liveweight gain, dry matter intake, milk yield, milk fat or milk total solids, which averaged: 107 g/day, 2.7 kg/day, 2.75 kg/day, 3.6% and 12.1%, respectively. However, the non-casein N, true protein N, casein N and serum protein N fractions of the milk from MIM-treated does was greater ($P < .10$) resulting in an elevated total milk N content ($P < .10$). Plasma urea N, total protein, and non-esterified fatty acid concentrations were elevated ($P < .05$) in the MIM-treated does on day 7, while triiodothyronine level was 38% higher ($P < .10$) on day 14 of treatment (not shown). No consistent changes ($P > .10$) were observed in blood hemotcrit or plasma glucose, L-lactate, cortisol, insulin, thyroxine and triiodothyronine levels.

The results from the kid part of the trial are listed in Table 5. Kids fed the MMK consumed more milk ($P < .05$) than kids

fed the SMK; however, average liveweight gain (165 g/day) was not affected (P >.10). Plasma glucose and insulin were elevated, whereas total protein concentrations were depressed by day 21 in MMK-fed kids as compared to the SMK-fed kids (P < .05). No treatment differences (P >.10) were noted in plasma L-lactate, urea N, triiodothyronine and thyroxine levels which averaged: 20.3 mg/dl, 12.3 mg/dl, 2.6 ng/ml and 49.7 ng/ml, respectively.

Partial or complete alopecia were observed in neither the does nor kids within the 21 day scope of the trial. We are in the process of assessing possible mimosine-related pathological changes in tissue and cellular composition.

Conclusion

Mimosine administration resulted in decreased mohair fiber production and circulating total protein concentrations in Angora wethers, suggesting a derangement in protein metabolism (possibly via it's known tyrosine antagonist activities). Moreover, these studies demonstrated that mimosine can directly influence thyroid hormone (thyroxine and triiodothyronine) levels and function. It appears that mimosine may affect hepatic and renal function as indicated by the elevated plasma bilirubin concentration and the occurrence of uroschesis. In the limited context of this project, mimosine appears to exert a minor effect on milk production and quality, with the exception of the milk nitrogenous components. In addition, consumption of fresh milk collected from mimosine-treated does had little effect on growth

and metabolic parameters in young goat kids. It is important to note that the does and kids were sacrificed the day following the experiment, and thus, possible effects of mimosine on hair or physiological parameters over the following weeks was not studied. We believe, that at present, the effects of mimosine on lactational performance and possible transfer into the milk are not conclusive and is an area in need of further investigation before the widespread consumption of dairy products from Leucaena-fed ruminants (adapted or-nonadapted) is promoted by USAID or any other agency.

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Tables

Table 1. Mimosine administration and mohair production in Angora goats (Experiment 1).

	Predose	Mimosine	P <
DM Intake, g/day	503.6	348.2	.10
Clean mohair yield, g/cm^2	1.63	.84	.05
Mohair fiber length, cm	1.28	.82	.05
Mohair fiber diameter, um	39.43	36.98	.05

Table 2. Mimosine administration and metabolic status of Angora goats (Experiment 1).

	Predose	Mimosine	Postdose	P <
Urea N, mg/dl	12.3	13.8	13.7	NS
Glucose, mg/dl	76.8	75.6	75.7	NS
L-Lactate, mg/dl	6.8	12.5	10.7	.05
NEFA, ueq/l	287.2	367.5	355.2	NS
Total protein, g/l	66.4	61.3	60.9	.05
Bilirubin, mg/dl	.10	.17	.18	.10
GGTP, U/ml	25.7	25.8	24.1	NS
Insulin, uU/ml	14.2	11.8	15.5	.05
Glucagon, pg/ml	79.7	108	118.6	.05
Thyroxine, ng/ml	62	60	57	.05
Triiodothyronine, ng/ml	1.42	1.14	1.08	.05

Table 3. Effects of saline and mimosine administration on production parameters in lactating Alpine does (Experiment 2).

	Saline	Mimosine	P <
DM intake, kg/day	2.83	2.64	NS
BW gain, g/day	119	95	NS
Milk yield, kg/day	2.53	3.02	NS
4% FCM yield, kg/day	2.34	2.84	NS
Milk fat, %	3.50	3.57	NS
Milk total solids, %	12.0	12.2	NS
Milk total N, %	.453	.529	.10
Milk casein N, %	.377	.442	.10
Milk non-casein N, %	.076	.087	.10
Milk non-protein N, %	.025	.025	NS
Milk ash, %	.82	.80	NS

Table 4. Effects of saline and mimosine administration on blood hematocrit and key plasma parameters in lactating Alpine does on day 7 (Experiment 2).

	Saline	Mimosine	P <
Hematocrit, %	23.6	27.2	.10
Urea N, mg/dl	24.8	27.9	.05
Glucose, mg/dl	82.8	86.0	NS
L-lactate, mg/dl	4.0	4.4	NS
NEFA, meq/l	.18	.36	.05
Total protein, g/l	66.0	73.2	.05
Insulin, uU/ml	22.6	20.5	NS
Thyroxine, ng/ml	48.1	51.1	NS
Triiodothyronine, ng/ml	2.4	2.8	NS
Cortisol, ng/ml	8.2	14.7	NS

Table 5. Effect of feeding milk from saline- or mimosine-treated does on kid growth and plasma metabolite and hormones on day 21 (Experiment 2).

	Saline	Mimosine	P <
Milk intake, kg/day	1.34	1.72	.05
BW gain, g/day	143	187	NS
Urea N, mg/dl	13.8	12.9	NS
Glucose, mg/dl	149.8	172.5	.05
Total protein, g/l	54.8	51.8	.05
Insulin, uU/ml	12.8	44.2	.05
Thyroxine, ng/ml	53.9	47.6	NS
Triiodothyronine, ng/ml	2.51	2.50	NS

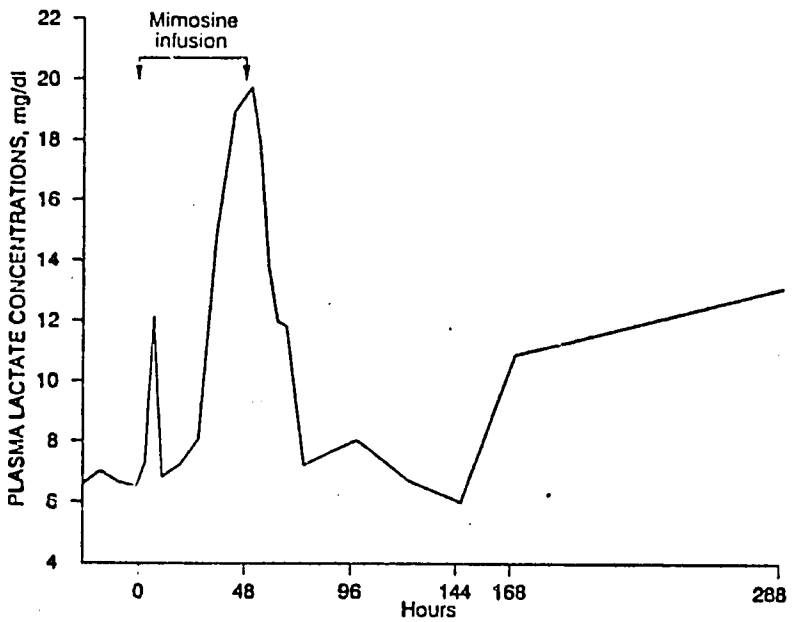

Figure 1. Effects of mimosine administration on mean plasma L-lactate concentration (Experiment 1).

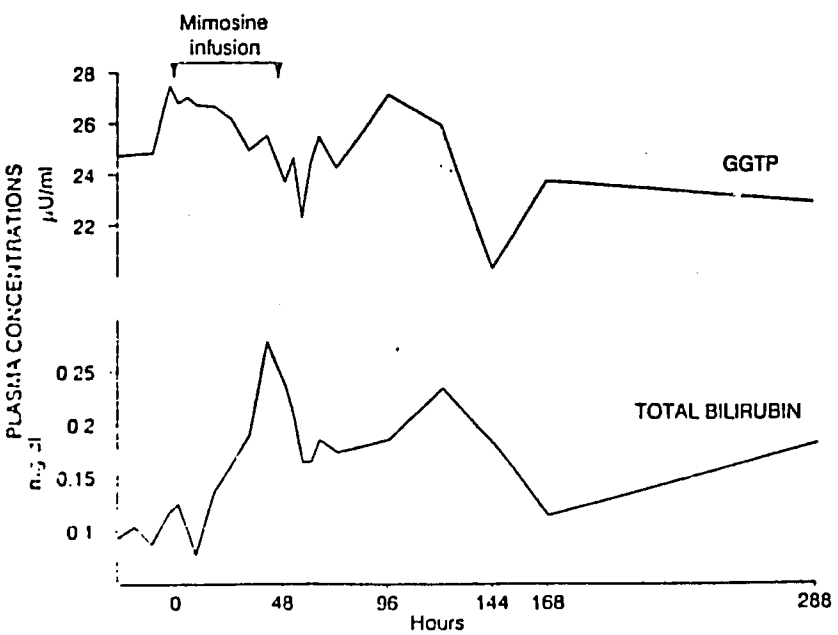

Figure 2. Effects of mimosine administration on mean plasma gamma-glutamyl transferase (GGTP) and total bilirubin concentrations (Experiment 1).

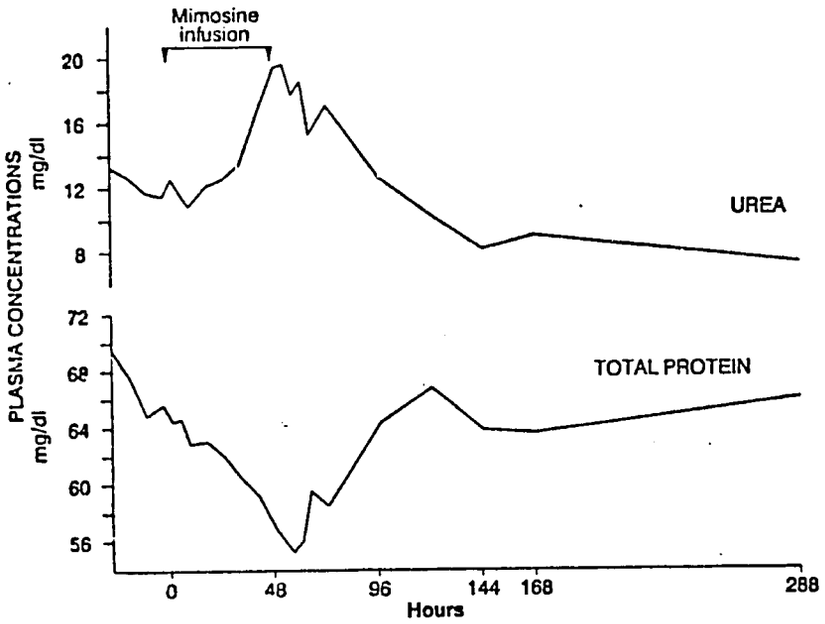

Figure 3. Effects of mimosine administration on mean plasma urea N and total protein concentrations (Experiment 1).

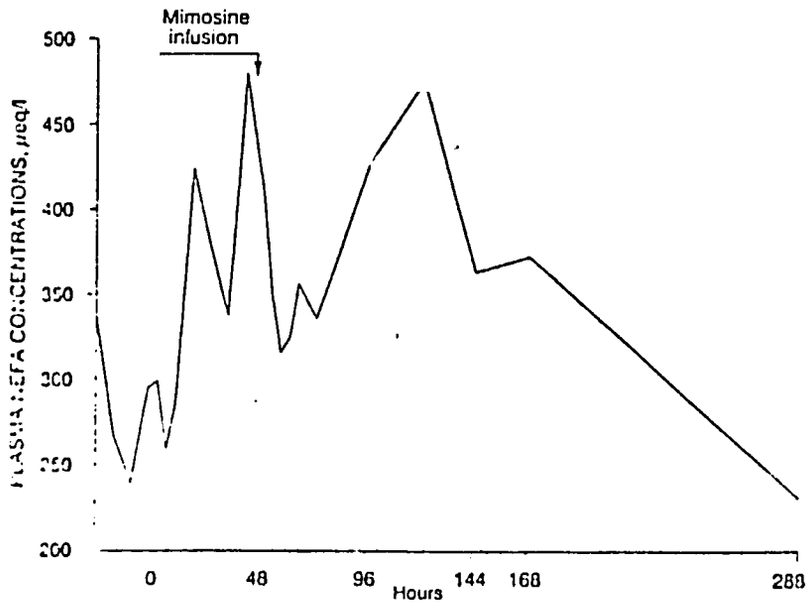

Figure 4. Effects of mimosine administration on mean plasma non-esterified fatty acid (NEFA) concentration (Experiment 1).

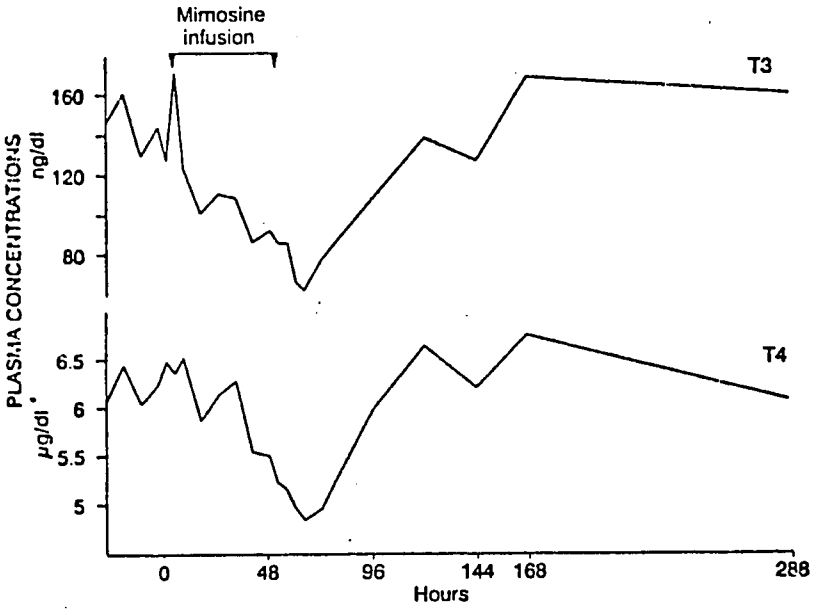

Figure 5. Effects of mimosine administration on mean plasma thyroxine (T4) and triiodothyronine (T3) concentrations (Experiment 1).

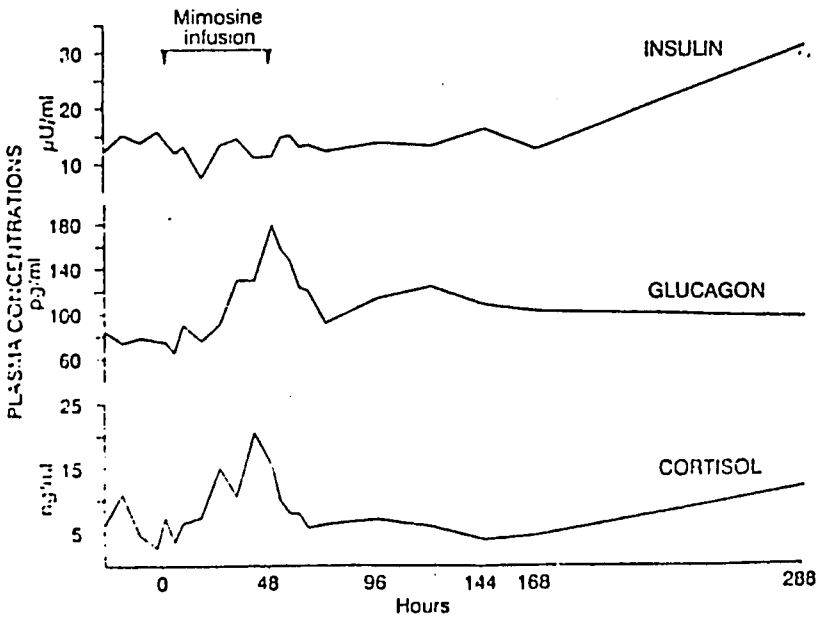

Figure 6. Effects of mimosine administration on mean plasma insulin, glucagon and cortisol concentrations (Experiment 1).

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2. Mimosine administration in goats: Effects on lactational performance and metabolism in early lactating Alpine does. 1991. J.M. Fernandez, T. Sahlu, C.D. Lu and A.O. Akinsoyinu. Journal of Animal Science 69(Supplement 1), (abstr.) (Accepted).

3. Mimosine administration in goats: Performance and metabolism in kids fed milk from mimosine-treated does. 1991. J.M. Fernandez, T. Sahlu, C.D. Lu and H.M. El Shaer. Journal of Animal Science 69(Supplement 1), (abstr.) (Accepted).

4. Mimosine effects on in vitro fermentation patterns and in sacco release of mineral elements from Leucaena in the goat. A.O. Akinsoyinu, N. Jacquemet, H. Carneiro, T. Sahlu, C.D. Lu and J.M. Fernandez. Journal of Animal Science 68(Supplement 1), 406 (abstr.).

5. Mohair quality and metabolic profile of Angora goats during acute mimosine toxicity. N. Jacquemet, J.M. Fernandez, T. Sahlu and C.D. Lu. Journal of Animal Science 68(Supplement 1), 400 (abstr.).

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Mimosine administration in goats: Effects on lactational performance and metabolism in early lactating Alpine does. J.M. Fernandez, T. Sahlu, C.D. Lu and A.O. Akinsoyinu*, E. (Kika) de la Garza Institute For Goat Research, Langston University, OK.

In order to study the effects of mimosine (MIM), a toxic amino acid found in the tropical legume Leucaena, 10 Alpine does (avg. BW 55 kg) 1 to 3 wk postpartum were blocked by milk yield (MY) and BW and randomly assigned to one of two treatments: Saline (CON) and MIM treatment (60 mg/kg$^{.75}$ BW/d). The treatment lasted 21 d and consisted of intrajugular administration of the experimental solutions (pH 8.5) in bolus doses at 12 h intervals immediately after milking (0600 and 1800). Does were fed ad libitum a ration containing 18% CP, 70% TDN and 22% ADF. Though non-significant (P > .10), mean DM intake (kg/d), daily gain (g/d), MY (kg/d), milk fat (%) and milk total solids (%) for the CON and MIM groups, respectively, were: 2.8, 119, 2.5, 3.5 and 12; and 2.6, 95, 3, 3.6 and 12.2. However, total N (.53 vs .45%), true protein N (.50 vs .43%), casein N (.44 vs .38%) and non-casein N (.09 vs 0.08%) contents were greater (P < .10) in milk from the MIM-treated does. Jugular blood samples were obtained 2 h after the morning dosing on d 0, 7, 14 and 21. Blood hematocrit (26.9 vs 24.4%) and plasma urea-N (27.9 vs 24.8 mg/dl), total protein (73.2 vs 66 g/l) and free fatty acid (.36 vs .18 meq/l) concentrations were elevated in the MIM does on d 7 (P < .05). No changes (P > .10) were noted in plasma glucose, L-lactate, cortisol, insulin and thyroxine, though triiodothyronine concentration was 38% higher (P < .10) in the MIM-treated does on d 14. In conclusion, i.v. administration of MIM at the level used in the present study did not result in alopecia nor appear to affect the overall lactational performance of the early [illegible]

[illegible] This project was supported by USAID Grant No. DAN-5053-G-SS-8018-00.

KEY WORDS: Mimosine, Goats, Lactation, Metabolites, Hormones, Leucaena.

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Mimosine administration in goats: Performance and metabolic status of kids fed milk from mimosine-treated does. J.M. Fernandez*, T. Sahlu, C.D. Lu and H.M. El Shaer, E. (Kika) de la Garza Institute For Goat Research, Langston University, OK.

To investigate possible detrimental effects of mimosine (MIM) appearance in milk, 10 Alpine kids (6 M and 4 F, avg. BW 5.5 kg) 6 to 14 d of age were blocked by sex and BW and fed al libitum raw milk from either saline-treated (SMK) or MIM-treated (MMK; 60 mg/kg $^{.75}$ BW/d; i.v.) does. The treatment lasted 21 d and consisted of twice-a-day feeding (0800 and 1500). Composition of the milk from the two groups was similar ($P > .10$) and averaged 3.6% fat, 4.6% lactose and 12.1% total solids; nevertheless, milk from the MIM-treated does had higher ($P < .07$) levels of protein (3.4 vs 2.9%). Average liveweight gain and milk intake for the SMK and MMK kids, respectively, were: 143 and 187 g/d (NS, $P > .10$); and 1.34 and 1.72 kg/d ($P < .05$). Jugular blood samples were taken via venipuncture on d 0, 7, 14 and 21 of the trial 2 h after the AM feeding. No treatment differences ($P > .10$) were noted in plasma concentrations of urea-N (mean 12.3 mg/dl), L-lactate (mean 20.3 mg/dl), non-esterified fatty acids (mean .3 meq/l), triiodothyronine (mean 2.64 ng/ml) and thyroxine (mean 49.7 ng/ml). Plasma glucose (172.5 vs 149.8 mg/dl) and insulin (44.2 vs 12.8 µU/ml) concentrations were elevated and total protein levels depressed (51.8 vs 54.8 g/l) by d 21 in the MMK kids as compared to the SMK kids ($P < .05$). Lastly, within the 21 d scope of this trial, MMK kids did not exhibit the characteristic alopecia that is generally associated with MIM or Leucaena toxicity.

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KEY WORDS: Mimosine, Goats, Milk, Metabolites, Hormones, Leucaena.

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and the in vivo concentration of testosterone was not influenced by gossypol administration. Gossypol is known to inhibit oxidative phosphorylation and studies indicate an inhibition of the lactate dehydrogenase (LDH-X) enzyme specific to mammalian testes. Recent results from with bovine males fed whole cottonseed, meal and(or) hulls indicated few persistent problems with spermatozoa collected in ejaculates, however, germinal epithelium of the seminiferous tubules was damaged. Damage at these sites may indicate an inhibition of normal spermatogenesis. Data from the ram also support these findings, but germinal damage was not as severe but Sertoli as well as Leydig cell morphology appeared affected. Data is limited concerning the effects of gossypol on female reproduction. Normal estrous cycle activity and pregnancy were inhibited in rodents fed gossypol. Intrauterine administration of gossypol prevented pregnancy in only the treated horn. The inclusion of gossypol to early stage bovine embryos inhibited in vitro development in a dose dependent fashion. Further studies are required to elucidate the mechanisms of gossypol induced infertility and to determine at which concentration of free gossypol per feed/day these problems begin to be of practical significance.

KEY WORDS: Gossypol, Toxic, Reproduction, Livestock

420 Effects of certain poisonous plants on uterine function and fetal development in livestock. T.D. Bunch*, K.E. Panter and L.F. James, Utah State University, Logan.

Ingestion of locoweed (Astragalus spp. and Oxytropis spp.) by pregnant livestock may result in fetal malformations, delayed placentation, reduced placental and uterine vascular development, hydrops amnii, abnormal placentome development, interruption of fetal fluid balance and abortion. Ultrasonography of pregnant sheep showed that abortion was first preceded by changes in fetal heart rate and fetal heart action, structural changes of the placentomes followed by increased accumulation of fetal fluid within the placental membranes and death of the fetus. Similar changes in cardiac function and output occur in calves grazing locoweed at high elevation resulting in right heart failure and fluid accumulation in the jaws, brisket and visceral cavities of the body. The toxin in locoweed (swainsonine) passes through the milk to the calves and evidently passes through the placental barrier to the fetal lamb. Plants containing piperidine alkaloids with a specific molecular configuration that includes a piperidine ring with a side chain alpha to the N of at least 3 carbons are teratogenic. Fetal malformations include multiple congenital contractures (MCC) resulting in fixed extension or flexure of the carpal, tarsal or fetlock joints, enlargement of these joints, lateral deviation of the forelimbs, scoliosis, torticullis, lordosis and cleft palate. Ultrasonography of pregnant sheep and goats gavaged with Conium maculatum and Nicotiana glauca 30-60 days of the gestation period suggests that the primary cause of MCC and cleft palate is the degree and the duration of the alkaloid-induced fetal immobilization.

KEY WORDS: livestock, poisonous plants, fetus, malformations, ultrasound

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Mimosine effects on in vitro fermentation patterns and in sacco release of mineral elements from Leucaena in the goat. A. Akinsoyinu*, N. Jacquemet, H. Carneiro, T. Sahlu, C.D. Lu and J.M. Fernandez, American Institute for Goat Research, Langston University, Oklahoma.

The objective of the first study was to determine the effects of mimosine (MIM), a toxic amino acid found in the tropical legume Leucaena (LEU), on in vitro volatile fatty acid and ammonia production. The study was conducted as a 3x3 Factorial arrangement, with 3 levels of forage (Bluestem hay) to concentrate (88% corn + 10% SBM) ratios (100:0, 75:25 and 50:50) and 3 levels of MIM (0, 2 and 4 mg/ml). Rumen fluid was obtained from a rumen-fistulated Alpine wether maintained on the 50:50 diet. Acetate, propionate and butyrate concentrations (umole/ml) for 100:0, 75:25 and 50:50 rations, respectively, were: 22.4, 25.9 and 28.9; 8.2, 10.4 and 12.5; and 2.3, 3.6 and 3.6. There was a diet effect ($P < .01$), but MIM or MIM*diet interactions were not significant. Ammonia N for 0, 2, and 4 mg MIM, respectively, was 8.4, 9.5 and 10.6 mg/dl ($P < .01$). In the second study, LEU was incubated in sacco for up to 48 hr using 5 um pore size dacron bags to determine the extent and rate that key mineral elements (Ca, Cu, K, Mg, Na, P, Zn) are released. The experiment was repeated 3 times. Release of minerals was faster than that of dry matter, and peaked at 2 hr. Variation in mineral release during this early phase was great, but the rate of mineral release was Na > K > Cu > Ca > Zn > P. This project was supported by USAID Grant No. DAN-5051-G-SS-8018-00.

Jacquemet

Last name of first author

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ABSTRACT AND ABSTRACT HEADING (see instruction sheet for example)

Mohair quality and metabolic profile of Angora goats during acute mimosine toxicity. N. Jacquemet*, J.M. Fernandez, T. Sahlu and C.D. Lu, American Institute for Goat Research, Langston University, Oklahoma.

The effects of continuous intravenous infusion of mimosine (MIM), a toxic amino acid found in the tropical legume Leucaena, on mohair fiber yield and key blood parameters was investigated in 5 Angora wethers (avg. BW 51 kg). The wethers were kept in metabolism crates and fed a complete mixed ration (12% CP, 2.4 Mcal ME/kg DM) ad libitum. Wethers were fitted with indwelling jugular vein catheters, and infused with a MIM-saline solution (~8.33 mg/kg $BW^{.75}$/hr) for 48 hr. This was preceeded (PRE) and followed (POST) by 24 hr saline infusions. Jugular blood samples were obtained at various intervals. Feed intake was reduced 25% by MIM, and uroschesis coupled with submandibular and ventral abdominal edema was observed. Two wk following MIM infusion, mohair yield, and fiber length and diameter decreased 52, 33, and 6%, respectively. Complete or partial alopecia was observed in 3 of the wethers within 10 days. As compared to PRE, MIM infusion decreased ($P<.05$) plasma triiodothyronine, thyroxine, insulin, and total protein levels by 3, 20, 17, and 8%, respectively, whereas total bilirubin, cortisol, and glucagon concentrations increased ($.01<P<.10$) by 70, 77, and 36%, respectively. No changes were observed in plasma glucose, urea, or nonesterified fatty acid concentrations. It remains to be determined if mimosine effects on fiber production are primary or secondary in nature. This project was supported by USAID Grant No. DAN-5053-G-SS-8018-00.

KEY WORDS: Mimosine, Leucaena, Angora Goats, Mohair, Metabolism, Hormones

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