Acacia angustissima Intoxication of Menz Lambs Requires Two Components

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ABSTRACT

This study sought to determine if sheep suffer neurological symptoms when fed *Acacia angustissima* leaves, and whether an equivalent amount of 70% acetone extract would have the same effect. In addition, the study tried to determine if treatment of leaves with 70% acetone would destroy the activity of *A angustissima* toxins, and whether extraction with 70% aqueous acetone extract would separate two components of a toxic system. Twenty-five Menz lambs were randomly assigned to one of five treatments: (1) *A angustissima* leaves as half the diet, (2) dried extract (70% aqueous acetone) of the same quantity of leaves, (3) a corresponding amount of residue, (4) a recombination of the dried extract and dried residue, or (5) a control diet containing no *A angustissima* leaves or extract fractions. All animals fed the leaves and the recombined fractions died or were euthanized when they were observed to be dying of severe neurological derangement. None of the other animals showed any neurological signs of impairment. The results of this study indicate that healthy, well-fed sheep can be poisoned by *A angustissima*, that the toxins are not destroyed by acetone or oven drying, and that severe neurological intoxication requires two components, which can be separated by acetone extraction.

INTRODUCTION

Recently, research scientists in Australia, Zimbabwe, and Ethiopia have focused on the use of a neo-tropical leguminous tree, *Acacia angustissima,* as a multipurpose fodder protein supplement that could increase protein in livestock diets and possibly aid in environmental conservation.

Feeding trials and chemical analysis indicate that *A angustissima* leaves are a source of plentiful, high-quality protein (25.5% crude protein) and reasonable fiber concentrations (36.4% neutral detergent fiber).[1] The rapid growth and generous yields of the tree make it a potential conservation crop for depleted soils and eroded lands. *A* *angustissima* is adapted to acid soils, tolerant to drought, and retains green foliage even through dry seasons.[2] The plant seeds profusely, and can withstand regular defoliation.[3] The root structures of drought-tolerant acacias harbor rhizobia that can fix nitrogen, penetrate a variety of difficult soils, help to displace sodium, and normalize soil pH.[4]

The major obstacle to introducing *A angustissima* as a protein supplement is its observed toxicity to domestic sheep under certain conditions, primarily when high levels of supplementation begin abruptly. *A angustissima* supplements in amounts greater than 200 g dry matter per day have been reported to cause death in sheep in 9 to 21 days.[5] A potentially toxic agent, the non-protein amino acid 4-N-acetyl-2,4-diaminobutyric acid (ADAB) was isolated from *A angustissima* in 2001;[6] the neurological and hepatic symptoms of the sheep fed high levels of *A angustissima* are nearly identical to symptoms induced by a hydrolysate of ADAB (2,4-diaminobutyric acid; DABA).[7] Slow introduction of this supplement can apparently permit animals to adapt to the chemical factors present, probably aided by shifts in the microbiological makeup of the rumen ecosystem.[1]

Over the centuries, humans have learned to apply technology to render similar pest-resistant/toxic feeds and foods safe to eat yet able to retain their protection against insects, fungi, and other herbivores. *A angustissima* has such nutritional and ecological potential. Thus an examination of a safe strategy for its use is warranted.

It is clear from recent data published by Odenyo et al[8] that a compound (or compounds) with a high affinity for polyethylene glycol (PEG) is involved in some way in the toxicity of *A angustissima* to rats and rabbits. This factor may be a tannin of modest molecular weight, but it could be anything else sequestered by PEG as well. Furthermore, the same researchers have shown that acetyl diaminobutanoic acid (ADAB), *by itself,* is not toxic to rabbits, although it is structurally related to diaminobutanoic acid (DABA) and is plentiful (1.1%–2.5% of dry weight) in *A angustissima* foliage. These authors have also shown that although some rumen microbes degrade ADAB, DABA may not be a catabolite of this microbial process.[9] This does not exclude the possibilities that ADAB is converted to an active toxin other than DABA, or that ADAB or its catabolites might not be involved in poisoning along with a factor with an affinity for PEG. Nor does it preclude the conversion of ADAB to DABA by animal or plant enzymes.

The objectives of the present study were fourfold: to determine if *A angustissima* leaves fed at 1.66% of body weight and at 50% of the total diet have the same previously observed neurological effects on sheep, even after the animals have been fed a well-balanced diet for several months prior to the experiment; to determine if a 70% aqueous acetone extract of *A angustissima* leaves contains any substances that affect the performance of Menz sheep; to determine if treatment of *A angustissima* leaves with 70% aqueous acetone destroys the activity of these anti-nutritional factors; and to determine if 70% aqueous acetone extract *of A angustissima* leaves separates two components of a chemical system that otherwise harms sheep.

MATERIALS AND METHODS

Extract and Residue Preparation

Acacia angustissima leaves (International Livestock Research Institute accession number 15132) were dried and ground (using a 1-mm screen) and then soaked in 3 kg water and 7 kg acetone per kilogram ground leaf for at least 1 hour in a large glass carboys. This extraction vessel was drained and the effluent filtered through 4 layers of cheesecloth into clean bottles, then through #4 filter paper into more clean bottles. The resulting leaf residue was re-extracted with 2.7 kg fresh water and 6.3 kg acetone per kilogram of original dried leaf. The filtration procedure was repeated. Excess acetone was pressed from the residue and the residue was allowed to dry completely in a hood until the acetone was gone, and then in a 50˚C oven until dry.

The extract was concentrated first by distilling off acetone slowly at 50˚C, with boiling chips, agitation, and/or stirring to prevent bumping. After all the acetone was gone, the extract was placed in a glass pan and water was removed by drying in an oven at 50˚C. The resulting yields of dried extract and extracted leaf residue were weighed and noted, and products were stored in doubled, sealed, heavy-gauge plastic bags or sealed large-mouth jars until mixed with the daily rations.

Animals and Diet

Twenty-five Menz lambs were fed 1.66% of body weight per day as Bermuda grass hay and 1.66% of body weight per day of supplement C (wheat bran, noug cake, and bone meal) for 2–5 months before being randomly assigned to one of 5 supplement groups. (See Table 1 for constituents of individual supplements.)

Controls: The 5 sheep in this group received 1.66% of body weight per day as grass hay, 1.66% of body weight per day as supplement C, plus 0.066% of body weight per day as molasses.

Acacia leaves (A angustissima): Because this feed varies from case to case in its content of putative toxic factors, the study design sought to ensure that the response to the extractives could be compared to the response to an equivalent amount of the intact plant. The 5 sheep involved in this treatment were fed 1.66% of body weight per day as grass hay, 1.66% of body weight per day as *A angustissima* leaves, and 0.066% of body weight as molasses.

70% acetone (Extract): This treatment tests the hypothesis that such an extract will affect the sheep to the same extent as an equivalent amount of the same plant. These 5 sheep were fed 1.66% of body weight per day as grass hay, 1.33% of body weight per day as supplement mixture X, 0.33% of body weight per day as acetone extract, and 0.066% of body weight per day as molasses.

Residue from extraction (Residue): Five lambs received 1.66% of body weight per day as grass hay, 0.61% of body weight per day as supplement mixture R, 1.05% of body weight per day as residue from acetone extraction, plus 0.066% of body weight per day as molasses.

Recombination: The 5 sheep receiving this treatment were given 1.66% of body weight per day as grass hay, 0.22% of body weight per day as supplement mixture X plus R, 1.44% of body weight per day as a proportional recombination mixture of extract and residue, and 0.066% of body weight per day as molasses.

The resulting balanced and isonitrogenous rations, including all ingredients—hay, supplements, experimental material, and molasses—each provided 12.88 g nitrogen (80.5 g crude protein) per day per 15 kg of lamb and met all nutritional requirements as specified by the National Research Council.[10] The amounts of extract and residue in the 3 latter treatments were the same as that which would have been taken by extraction process from the amount of leaves fed in the *A angustissima* treatment.

Table 1. Supplement Mixtures Used to Balance Rations in 25 Menz Lambs Assigned to 1 of 5 Supplement Groups

Treatment	Wheat bran	Noug cake	Bone meal
C	38.5%	57.7%	3.8%
X	38.5%	56.7%	4.8%
R	36.8%	52.6%	10.5%
X+R	30.2%	46.5%	23.3%

Preliminary Trial

The first sheep assigned to each of 4 treatment groups (excluding only the residue treatment group) began consuming the supplements dry in feeders, but refusals to eat acacia leaf meal and other supplements resulted in the necessity of inventing a method to administer the entire desired dose by mouth. A slurry drench was discovered to be feasible and was applied for the last 2 feedings of the 5-day period before euthanasia.

Table 2. Chemical Analyses of Forage Ration Components (g/kg) Administered to 25 Menz Sheep

Component	Dry matter (%)	Ash (% DMB)*	Organic matter (% DMB)	Nitrogen (% DMB)	Phosphorus (% DMB)	Calcium (% DMB)
Acetone extract	91.13	3.87	96.14	2.10	0.04	0.04
Acetone residue	90.72	7.82	92.19	10.08	0.41	1.77
Acacia angustissima leaves	90.59	6.02	93.98	4.94	0.28	1.45
Recombination of extract + residue	91.45	6.20	93.80	5.35	0.31	1.24
Supplement for controls	91.35	12.76	87.25	4.37	1.37	1.75
Supplement for extract group	92.84	13.08	86.92	4.22	1.49	2.09
Supplement for residue group	91.46	16.69	83.65	4.33	1.93	3.34
Supplement for recombination group	91.47	22.95	77.05	4.38	2.73	0.07
Bermuda grass hay	91.86	11.77	88.23	0.72	0.24	0.46

*DMB, dry matter basis.

Main Trial

The remaining 4 sheep per treatment group (and all 4 residue sheep) were tested simultaneously. Refused portions of experimental materials (leaves, extract, residue, and recombination material, previously ground through a 0.5-mm screen) were mixed into a thick water slurry and administered into the esophagus with a drench gun. Care was taken to ensure no feed entered the lungs during this process. Data from both, essentially identical, trials were pooled.

Standard Daily Operating Procedures for Both Trials

All animals were weighed on the first day to set dosages, and then at 8 AM each day thereafter. Refused portions were weighed, concentrate supplements or leaf meal were fed, molasses (0.066% of body weight) was added to each bucket (~10 g), and diets and leaves were sampled (~50 g/d and combined for each diet) for analysis to check rations for adherence to formulated chemical composition (Table 2).

RESULTS

The 10 sheep that died succumbed to a toxic reaction that included neurological signs (unsteady gait, sawhorse stance, difficulty walking, and eventually failure to stand) when they consumed the unextracted *A angustissima* leaves (1.66% of body weight) or equivalent amounts of extracted leaves (residue) with the dried extract mixed back in. There were no indications of fever or microbial disease, although in the last stages, some of the prostrate and dying lambs aspirated some rumen fluid into their lungs (Table 3).

The amount of material extractable from the same dose of leaves by 70% aqueous acetone did not have this effect on the sheep, nor did the same amount of residue left behind by such an extraction. The sheep that consumed these materials appeared healthy, as did the controls, although the feed intake of the sheep fed extract was somewhat reduced (Table 4), but not as much as the sheep that would later show signs of intoxication. Since refused experimental materials were added to the rumen as a drench, the variation in total feed intake observed tended to be entirely in the grass-hay fraction of the diet.

Pathology

All animals fed the acacia leaves and 2 of the sheep fed the recombination material

Table 3. Morbidity and Mortality of Experimental Animals*

Animal ID	Treatment	Days survived	Adverse signs	Death
112	Acacia leaves	3	Very weak	Euthanized
92	Acacia leaves	3	Very weak	Spontaneous
125	Acacia leaves	5	Weak	Euthanized
144	Acacia leaves	4	Dead	Spontaneous
148	Acacia leaves	4	Very weak, circled	Euthanized
142	Recombination	4	Weak	Euthanized
91	Recombination	4	Very weak	Euthanized
151	Recombination	4	Very weak, circled	Euthanized
136	Recombination	4	Dead	Spontaneous
149	Recombination	5	Died on 5th day	Euthanized
138	Control	5	None	Euthanized
152	Control	5	None	Euthanized
116	Control	5	None	Euthanized
115	Control	5	None	Euthanized
117	Control	5	None	Euthanized
104	Extract	5	None	Euthanized
96	Extract	5	None	Euthanized
88	Extract	5	None	Euthanized
124	Extract	5	None	Euthanized
154	Extract	5	None	Euthanized
150	Residue	5	None	Euthanized
130	Residue	5	None	Euthanized
132	Residue	5	None	Euthanized
107	Residue	5	None	Euthanized
95	Residue	5	None	Euthanized

*Animals in the acacia and recombination groups were intoxicated and died at a greater-than-expected rate ($P < 0.005$).

displayed severe congestion of the brain or neuronal degeneration. These signs were mild in the other groups and absent entirely from the control animals. In addition, several of the acacia-treated animals showed signs of aspiration pneumonia, but this was not a factor for the other groups, except for 1 animal in the recombination group. Atrophy of the rumen papillae was observed in sheep in the acacia, extract, and recombination groups, much less with the residue group, and none at all with the controls.

DISCUSSION

A angustissima leaves fed at 1.66% of body weight and at 50% of the total diet had the same previously observed lethal neurological effects[1] even after the animals have been fed a well-balanced diet for several months prior to the experiment. Toxicity has been previously observed in sheep of unrecorded nutritional backgrounds that may or may not have been well-nourished before joining the trials. The present results show that even sheep maintained on diets designed to meet all known nutrient requirements are susceptible to *A angustissima* intoxication.

The 70% aqueous acetone extract of *A angustissima* leaves did not contain the substances necessary to cause morbidity or mortality in these animals (although there may have been a slight reduction in feed intake), nor did the residue left behind by the extraction cause any significant problems. But

Table 4. Body Weight and Total Feed Dry Matter Intake*

	Body weight (kg)	Feed dry matter intake (% body weight)
Controls	19.5 (1.2)	3.02 (0.37)†
Acacia angustissima leaves	19.7 (1.2)	2.08 (0.29)‡
Extract	18.6 (2.4)	2.29 (0.43)‡
Residue	17.9 (1.8)	3.02 (0.23)†
Extract plus residue	18.2 (1.5)	2.11 (0.12)‡

*Mean (SD); n = 5 for all treatment groups.
†,‡Means with different superscripts within the same column are significantly different ($P < .05$).

together, the observed toxicity was similar to the intact plant. For this reason we believe that extraction of *A angustissima* leaves with 70% aqueous acetone does not destroy the activity of anti-nutritional factors, suggesting that the plant protection system used by *A angustissima* may have two components, one extractable in acetone, and one that is not.

For example, the binary system might consist of ADAB and an enzyme that converts it to a toxic form. This would explain the apparent independence between ADAB concentrations and toxicity of various *A angustissima* accessions.[11] If the second factor is absent or deficient or not induced for some reason, then high ADAB, low toxicity accessions could exist. This is particularly important information for plant breeders and forage resource screeners, since it means that single trait selection against ADAB may not be an entirely adequate strategy if there is a second (or third) factor present in the toxicological complex presented by *A angustissima*.

An alternative explanation for these data would be that perhaps the same single toxic factor, such as condensed tannin, could exist in a soluble and in an insoluble bound state. If a specific dose is required and such a single toxin were divided by extraction between soluble and bound, but active, forms, then perhaps the separate extract and separate residue treatments merely lacked a sufficient dose to cause apparent intoxication symptoms in these sheep.

Future work should include further fractionation of both the acetone extract and residue and testing of these subfractions to clearly identify the nature of the plant protection system. Then reliable recommendations could be made on the rate of introduction of *A angustissima* to ensure adaptation and suggestions made for processing *A angustissima* leaves to eliminate toxic reactions entirely.

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