IMPROVING LIVESTOCK TOLERANCE OF TOXICANTS IN KOCHIA TOWARD INCREASED USE AS A WATER-EFFICIENT CROP

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TECHNICAL COMPLETION REPORT
Project Number 1345650

January 1989

New Mexico Water Resources Research Institute

in cooperation with

Department of Animal and Range Sciences and
New Mexico Agricultural Experiment Station
New Mexico State University

The research on which this report is based was financed in part by the U. S. Department of the Interior, Geological Survey, through the New Mexico Water Resources Research Institute.

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ABSTRACT

Herbage of Kochia scoparia (L) Schrad (kochia) was collected at bud stage, dried and fed to rats, sheep and cattle to characterize the nature of early kochia toxicosis, to identify better the primary toxicant(s), and to evaluate certain treatments as ways to improve animal tolerance of toxicants, toward increased use of kochia as a water-efficient forage crop. In rats, severity of toxicosis was correlated with content of substances reactive to Dragendorff's reagent (presumably alkaloids) in fresh herbage. Clinical signs of toxicosis in rats, sheep and steers were consistent with alkaloids as the primary toxicant rather than oxalate or saponins. In rats, supplemental vitamins A and E or supplemental choline, inositol and folic acid tended to improve tolerance of dietary kochia, as did injected acetylcysteine; but for sheep, neither vitamins A and E nor injected acetylcysteine plus trans-stilbene oxide were effective, whereas supplemental zinc was detrimental. Earliest signs of kochia toxicosis in sheep and cattle were altered blood levels of metabolic hormones (insulin, prolactin, somatotropin) and impaired nitrogen retention, along with impaired bilirubin conjugation and leakage of hepatic enzymes. Findings suggest better ways to manage animal usage of kochia forage and suggest other treatments that might improve tolerance of toxicants in kochia.

Key words: Toxicity, Tolerance, Sheep, Cattle

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JUSTIFICATION OF WORK PERFORMED

Development of water efficient crops suitable for the High Plains of New Mexico has been deemed vital for the economic well-being of the area (High Plains Study Council 1982). Kochia scoparia (kochia), a vigorous annual of the Chenopodiaceae family, has become established in the area as a drought-resistant, opportunistic weed, and has received considerable attention as a prospective, water-efficient forage crop (Erickson and Moxon 1947; Bell et al. 1952; Finley and Sherrod 1971; Sherrod 1971, 1973; Durham and Durham 1979). The agronomic characteristics of the plant, as well as forage yields and nutritive value of forage, have justified major efforts to develop kochia as a viable alternate crop for New Mexico (Fuehring et al. 1985, 1986). But acceptance of kochia as a crop has been constrained by the fact that considerable numbers of livestock have been poisoned by ingestion of kochia herbage (Paulsen 1946; Galitzer and Oehme 1978; Dickie and Berryman 1979; Sprowls 1981; Dickie and James 1983; Boye 1983; Kiesling et al. 1984).

Prior to the 1980s, prebloom stages of kochia had not been implicated by investigators of kochia toxicity, but bloom and post-bloom plants were regarded as sometimes toxic. Toxicity was usually associated with drought, possibly because livestock consumed appreciable quantities of kochia herbage only during periods of feed scarcity. It has been known for a long time that kochia is an accumulator of oxalate under some conditions, and (perhaps because of this) oxalate was often implicated as the (suspected) toxicant in veterinary medical reports of kochia toxicosis (Galitzer and Oehme 1978; Sprowls 1981).

Photosensitization, which results from inability to excrete toxic products of heme degradation (bilirubin) and chlorophyll degradation

(porphyrins) and follows liver damage, and polyuria, which results from damage to the kidneys, often have been reported in cases of severe kochia toxicosis (Galitzer and Oehme 1978; Sprowls 1981; Kiesling et al. 1984). In addition to oxalate, which might cause or be implicated in these effects, various other toxicants have been incriminated such as saponins, tannins, nitrate and alkaloids (Dickie and Berryman 1979; Dickie and James 1983).

Because most reports of kochia toxicosis implicated mature plants involving stems and sometimes seed (Galitzer and Oehme 1978) and because most assessments of nutritive value dealt with herbage from immature plants and reported no toxicity (Erickson and Moxon 1947; Bell et al. 1952; Finley and Sherrod 1971; Sherrod 1971, 1973), the view that risk of toxicity from immature plants is negligible was widely publicized (Durham and Durham 1979). Moreover, even when toxicity of cropland kochia became evident (Sprowls 1981), the notion persisted that immature plants were not responsible and that oxalate accumulation in stems was the likely problem. Consequently, in the earlier research at the New Mexico State University Agricultural Science Center at Clovis, NM, primary attention was given to oxalate and efforts were directed toward selection for low oxalate as well as study of agronomic factors that might affect oxalate content (Fuehring et al. 1985). Meanwhile, Woldeghebriel (1983) fed kochia forage to rats and demonstrated negative calcium balance, suggesting that oxalates in forage was a primary concern.

However, grazing studies conducted at Tucumcari, NM, left no doubt that immature kochia on irrigated cultivated swards could be toxic when ingested by cattle: mortality occurred during grazing episodes in two of four years and clinical signs of toxicosis were observed in each of four consecutive years (Boye 1983; Kiesling et al. 1984). The clinical signs suggested that

early lesions were caused by some toxicant other than oxalate, although oxalate was probably involved in the later aspects of acute toxicosis (Kiesling et al. 1984).

In research conducted just prior to the work of this project, Dale Fuehring and Ralph Finkner directed attention to alkaloids content of kochia plants at the Agricultural Sciences Center at Clovis and demonstrated rather wide differences in plant content of substances reacting with Dragendorff's reagent (Fuehring et al. 1985, 1986) presumably alkaloids (Burns 1964). When plant juices were administered by gavage to rats, and when dried plant herbage was fed to rats, clinical signs of toxicosis were elicited that varied in direct correlation with herbage content of (presumed) alkaloids by Dragendorff's test (Smith et al. 1985, 1986a,b; Erickson 1986). It seemed likely that toxicity of kochia grazed or harvested as lush forage in pre-bloom to bud stages is related mainly to toxicant(s) other than oxalate or saponins, which may predominate in later stages of plant maturity. The (fragmentary) evidence available suggested alkaloids as a probable toxicant in pre-bloom kochia herbage in eastern New Mexico; although very low levels of alkaloids had been found in kochia harvested elsewhere (Drost-Karbowska et al. 1978). If an alkaloid is the primary toxicant in kochia there may be reason for hope that prophylactic or therapeutic measures can be found to increase animal tolerance of the toxicant.

Grazing of alfalfa, one of the world's most valuable forage crops, was constrained severely for many years by risk of bloat (and actually caused many thousands of livestock deaths) until the development of prophylactic treatments to prevent bloat. It seems probable that usage of kochia as a

water-efficient crop will be increased markedly if treatments or management systems can reduce the risk of kochia toxicity.

The objectives of the present research were to:

1. Produce and select plants with varied content of toxicants in quantities sufficient to support feeding experiments using rats, sheep and cattle;
2. Relate variations in plant content of toxicants to varied agronomic and(or) climatic conditions;
3. Further quantify, and identify, the alkaloids in kochia herbage that were regarded as toxicants;
4. Further characterize the biochemical and histological lesions attending early phases of kochia toxicosis; and,
5. Test certain treatments that promise enhanced tolerance by animals of the toxicants in kochia herbage.

OVERVIEW OF ANIMAL EXPERIMENTS

Research conducted under the present contract is best understood in relation to agronomic experimentation previously conducted at the NMSU - Agricultural Science Center at Clovis (Fuehring et al. 1985, 1986) and to livestock grazing studies already underway at the NMSU - Agricultural Science Center at Tucumcari (Kiesling et al. 1984) when the present series of studies was initiated. During the period 1984-86, rat experiments were initiated and conducted, in collaboration with these ongoing efforts, which led to the program of research conducted under the present project. An understanding of the research currently contracted can be facilitated by an overview of the entire program of animal experimentation within a single framework. Research to date includes:

A. Four-year study (1982-85) of irrigated kochia swards at Tucumcari and Clayton (NM) assessed kochia for nutritive value, was grazed by cattle and found to cause toxicosis (Kiesling et al. 1984; Hoefler et al. 1988; Thilstead et al. 1988);

B. Preliminary toxicological studies with rats (1984-86), conducted by G. S. Smith and M. K. Erickson, in collaboration with Fuehring and Kiesling, to determine whether rats may serve as laboratory species to model kochia toxicosis in livestock species.

Trial 1. Kochia herbage that had caused severe toxicosis when grazed by cattle (Kiesling et al. 1984) and milder toxicosis when consumed as hay by penned sheep (Hoefler et al. 1988) was fed to rats at dietary levels of 0, 12.5, 25 or 50% of diet for 6 weeks to ascertain toxicity and characterize toxicosis in rats;

Trial 2. Juice was squeezed from kochia herbage that tested "high" or "low" in alkaloids and dosed by gavage to rats to determine whether severity of toxicosis was related to plant content of alkaloids;

Trial 3. Frozen specimens of kochia herbage that tested "high" or "low" in alkaloids were dried and fed to rats at two dietary levels to study dose: response relationships;

C. Rat studies to further characterize kochia toxicosis and evaluate prospective treatments (1986, 1987), conducted by G. S. Smith, H. E. Kiesling and D. L. Rankins, Jr.:

Trial 4. Kochia herbage that tested "high" in alkaloids was fed to rats at 0, 15 or 30% of diet and treatments (supplementary vitamins A and E, or supplementary lipotropic factors or parenteral acetylcysteine) were imposed to further characterize kochia toxicosis and ascertain whether treatments modified animal tolerance of kochia;

Trial 5. Young rats were fed kochia herbage that tested "high", "medium" or "low" in alkaloids and treated with dietary vitamins or dietary zinc or parenteral acetylcysteine plus trans-stilbene oxide, to characterize further toxicosis and ascertain whether treatments modified animal tolerance of kochia;

D. Livestock experiments to further characterize early aspects of kochia toxicosis and test treatments to improve tolerance (1987, 1988), conducted by G. S. Smith, D. L. Rankins, Jr., D. M. Hallford and H. E. Kiesling.

Trial 6. To characterize the earliest aspects of kochia toxicosis in sheep and to evaluate dietary zinc or vitamins A and E or stimulators of hepatic glutathione metabolism as treatments to improve tolerance of kochia, 8 wethers and 4 rumen-cannulated ewes were fed kochia hay (35%) plus alfalfa hay (65%) for 4 weeks and then fed kochia hay (50%) plus alfalfa hay (50%) for 5 weeks, when treatments were imposed; feed intake, digestibility, rate of passage and serum chemistry were assessed;

Trial 7. To further characterize the earliest aspects of kochia toxicosis in sheep and to evaluate further ingested zinc and (or) acetylcysteine plus trans-stilbene oxide (as stimulators of hepatic glutathione metabolism), 12 wethers were fed kochia hay as total diet and drenched daily with aqueous $ZnSO_4$ and(or) injected intra-peritoneally (i.p.) with glutathione stimulators during 80 days in which feed intake, body weight, digestibility of dietary components, retention of nitrogen, blood clinical profiles and blood levels of metabolic hormones (insulin, prolactin and somatotropin) were monitored;

Trial 8. To evaluate earliest aspects of kochia toxicosis and separate effects of toxicants from effects of undernutrition, 8 steers were pair-fed kochia hay or alfalfa hay as total diet for 21 days during which alfalfa intake was restricted to levels equalling kochia hay intakes by pair-fed steers fed ad libitum; blood collected on days 0 and 21 was assessed for serum clinical profiles and for metabolic hormone levels;

Trial 9. To compare earliest aspects of kochia toxicosis in both cattle and sheep, and separate effects of toxicants from effects of under-nutrition, Trial 8 was replicated (roughly) using 10 wethers pair-fed alfalfa hay or kochia hay as total diet for 21 days; blood collected at day 0, 5, 10 and 21 was assessed for serum clinical profiles and for levels of metabolic hormones (insulin, prolactin and somatotropin).

Specific details of methods, materials and experimental procedures for each of these trials have been recorded in the Master's Theses of Mary Kay Erickson (Erickson 1986) and Darrell Lee Rankins, Jr. (Rankins 1987) and in the Doctoral Dissertation of Darrell Lee Rankins, Jr. (1989). Moreover, for Trials 1-7, specific details of experimentation are provided in published articles and manuscripts for publications that are included as appendices in this report.

METHODOLOGY (GENERAL)

Specific aspects of kochia cultivation for forage, selection based on plant content of oxalate and(or) alkaloids (as well as nutrients such as crude protein) and review of agronomic characteristics have been provided in previous Technical Completion Reports to WRRI (Fuehring et al. 1985, 1986).

Kochia herbage

For most of the experiments reported here, the kochia herbage that was fed to animals had been produced at the Agricultural Science Center at Clovis, New Mexico. For the rat experiments, alkaloids content of kochia herbage had been tested qualitatively using Dragendorff's reagent (Bismuth subnitrate, potassium iodide and glacial acetic acid; Burns 1964) and scored semiquantitatively by visual appraisal by experienced technicians at the Agricultural Science Center at Clovis. These tests were conducted using fresh juice at the time that the plants were harvested to provide herbage for the experiments. Plants were hand clipped to provide herbage specimens comprised of leaves and small stems, at pre-bloom (bud) stage of maturity. For the rat experiments, kochia herbage was allocated during harvest into batches identified as "high", "medium" or "low" in content of substances reactive to Dragendorff's reagent.

In experiments with sheep and cattle, kochia forage at bud stage was harvested as hay, from plots seeded to strains of "high alkaloid" kochia or to "wild type" seed stock, which did not differ significantly in alkaloid content from the strain selected as "high alkaloid" (Fuehring et al. 1986).

Oxalate

Oxalate content of kochia herbage was determined as soluble, insoluble and total oxalate by gas chromatography (Roughan and Slack 1973).

Animal experimentation

Specific protocols and details of animal experimentation have been

documented in the Master's Thesis of Mary Kay Erickson and the Master's Thesis and Doctoral Dissertation of Darrell Lee Rankins, Jr., sufficient to allow replication by interested investigators, and will not be presented here, except as noted within individual experiments. Various aspects of the research have been published in Proceedings articles (Smith et al. 1986b; Rankins and Smith 1987b; and Rankins et al. 1988b), and an additional manuscript intended for journal publication has been prepared. These are included in this report [Appendix 3 and 4], and they provide details for specific experiments.

Rats. Albino rats of the Spraque-Dawley strain were used in all rat trials. They were purchased commercially from licensed dealers and housed in institutionally approved facilities at the NMSU-Primate Research Institute (Alamogordo, NM) (Trials 1 and 2) or at the NMSU-Animal Science Small Animal Research Laboratory on the main campus in Las Cruces, where temperature control, light cycles and restricted access allowed control of environmental conditions within acceptable guidelines.

Diets. Purina Certified Rodent Chow, #5001 or 5002 (Ralston Purina Company, St. Louis MO) was purchased commercially in batches assuring reasonable freshness for all trials. Rodent chow and kochia herbage were each freshly ground in a Wiley Mill to pass a 2-mm screen and mixed at onset of each trial (and at intervals thereafter if experimentation exceed four weeks duration). Mixed feeds were stored at room temperature during each experiment. Rats were observed daily. Feed and tap water were provided regularly for consumption ad libitum.

Blood sampling. Rats were bled by puncture of the infraorbital vasculature using heparinized, glass capillary tubes. Normally three or four ml of whole blood were collected from adult rats. Sheep and cattle were bled by jugular venipuncture. Whole blood for hematological evaluation

was collected into sterile, heparinized tubes and chilled until delivered to the hematology laboratory. Blood for serum analysis was collected into sterile tubes bearing serum separators, allowed to clot (30 minutes at room temperature) and centrifuged (2500 x g, 15 minutes). Serum was decanted into sterile containers, sealed and stored frozen until analyzed.

Blood analysis. For Trial 1, blood analysis was conducted by hematologists at the NMSU-Primate Research Institute (Holloman AFB, Alamogordo, NM). All other specimens were submitted to a commercial medical laboratory (Southwest Medical Laboratory, Las Cruces, NM) for routine hematology and(or) serum clinical profile analysis by Simultaneous Multichannel Analyzer with Computer (SMAC) or similar automated analysis. In most cases, serum clinical profiles included 25 or 30 items reported ("SMAC 25" or "SMAC 30").

Pathology and Histopathology. Except for Trial 1, in which rats were examined by pathologists at the NMSU-Primate Research Institute, all tissues examined were collected into neutral formalin and submitted to the New Mexico Veterinary Diagnostic Laboratory (Albuquerque, NM) where they were examined by John Thilstead and(or) Clair Hibbs, certified veterinary histopathologists.

Metabolic hormones. Sera from blood samples collected from sheep and cattle specifically for determination of metabolic hormone levels were analyzed in the NMSU-Animal Science Endocrinology Laboratory for concentrations of insulin, prolactin and(or) somatotropin. Insulin was quantified by double antibody radioimmunoassay, using anti-bovine insulin prepared from guinea pigs, as described by Sanson and Hallford (1984). Prolactin was likewise quantified by double antibody radioimmunoassay, as described by Spoon et al. (1988). Somatotropin ("growth hormone") was quantified by radioimmunoassay, as described by Hoefler and Hallford (1987).

RESULTS

The agronomic efforts at the NMSU-Agricultural Science Center at Clovis were successful in providing kochia herbage that tested "high", "medium" or "low" in alkaloids, as determined by Dragendorff's reagent (Burns 1964). At time of harvest, kochia was hand clipped during bud stage, in amounts that were sufficient to support the experiments conducted using rats. Likewise, kochia herbage testing "medium" to "high" in alkaloids were harvested at bud stage and processed as hay in amounts sufficient to exceed requirements for the experiments conducted with sheep and cattle, thereby accomplishing objective 1 (p. 4). Moreover, the efforts to relate variations in plant alkaloids and oxalate to varied agronomic and(or) climatic conditions, as reported by Fuehring et al. (1986), were sufficient to satisfy objective 2 (p. 4). Oxalate contents of kochia used in animal trials of this investigation ranged from 4.8 to 7.7% of dry matter, of which about 85% was soluble.

The results of experiments with rats, sheep and cattle, were sufficient to characterize the very early phases of kochia toxicosis and to confirm that the biochemical lesions attending early toxicosis resembled changes that accompanied early aspects of toxicosis from specific alkaloids, as reported by Lipham et al. (1988). Although specific alkaloids in kochia were not identified in relation to kochia toxicosis (objective 3), the results obtained were sufficient to characterize kochia toxicosis (objective 4) and to test some treatments that were deemed prospective for enhancing animal tolerance of kochia herbage (objective 5). Although the treatments tested in livestock species provided only slight benefit, they yielded data that are useful in predicting other treatments that may prove beneficial.

A. Previous work at NMSU

Research with lush kochia forage grazed by cattle or fed as hay to penned sheep, which confirmed the toxicity of kochia herbage under practical conditions in New Mexico, was published earlier (Kiesling et al. 1984; Hoefler et al. 1988). Copies of these articles are included herein [Appendix 1 and 2] because their data are directly related to the data from present experimentation, and ready access to their results greatly facilitates the interpretations of data reported herein.

B. Preliminary study with rats (Trials 1, 2 and 3)

Results from Trials 1, 2 and 3 were reported in detail in the Master's Thesis of Mary Kay Erickson (Erickson 1986), copies of which are on file at the NM WRRI, the Department of Animal and Range Sciences at NMSU, and the main library at NMSU. These experiments were summarized (Smith et al. 1986c) and published in Proceedings of the 1986 meeting of the Western Section, American Society of Animal Science (Smith et al. 1986b). A copy of this article is included herein [Appendix 3] as an appropriate summary of findings.

C. Rat studies to characterize toxicosis and evaluate prospective treatments (Trials 4 and 5)

Results from Trials 4 and 5 (also Trial 6, with sheep) were reported in the Master's Thesis of Darrell Lee Rankins, Jr. (Rankins 1987), copies of which are on file at NM WRRI, the Department of Animal and Range Sciences, NMSU, and the main library at NMSU. Trial 5 was summarized and published in Proceedings of the 1987 meeting of the Western Section, American Society of Animal Science (Rankins and Smith 1987b), a copy of which is included herein [Appendix 4].

Trials 3 (above), 4 and 5 have been summarized in a manuscript suitable to submit for publication in the Journal of Animal Science: "TOXICOLOGICAL EVALUATIONS OF KOCHIA HERBAGE FED TO YOUNG MALE ALBINO RATS" by D. L. Rankins, Jr., G. S. Smith, M. K. Erickson and H. D. Fuehring, New Mexico State University, Las Cruces 88003. Because data from both Trials 3 and 5 are presented in Appendices 3 and 4, it is not included herein; but because the data from Trial 4 are not otherwise presented in this report, a summary of Trial 4 has been excerpted from the Master's Thesis of Darrell Lee Rankins, Jr. (Rankins 1987) and is herein reported, as follows:

> [Trial 4] "...Immature male rats were fed diets with 0, 15 or 30% kochia herbage [with "high' alkaloids] and treated throughout 10 weeks with either: 1) no treatment (NONE; controls); 2) N-acetyl-L-cysteine (CYS; 100 mg/kg body weight, dosed i.p. in saline 3 times/week; 3) lipotropic factors (LTF; 3 g choline chloride plus 200 mg m-inositol plus 4 mg folic acid/kg diet); or 4) vitamins A and E (A + E; 80 mg retinyl palmitate plus 80 mg alpha-tocopherol per kg diet). Diets and treatments were arranged in a 3 x 4 factorial arrangement of treatments and administered to 6 rats/group. Body weight of rats fed 30% of kochia and untreated were depressed 16% below dietary controls ($P < .01$) at 13 weeks. Both LTF and A + E tended to improve body weight ($P < .10$). Changes in serum constituents reflecting early stages of mild toxicosis were evident at 4 weeks ($P < .05$). By 10 weeks, serum changes were less severe than at 4 weeks, but more components were affected: blood urea nitrogen (BUN), creatinine, globulin, total protein, alanine aminotransferase, alkaline phosphatase and lactic dehydrogenase values were increased ($P < .05$) by dietary kochia,

and calcium, inorganic phosphorus and uric acid were decreased ($P < .05$). At 10 weeks, LTF decreased BUN and creatinine ($P < .05$) while A + E increased calcium ($P < .01$)."

Results from Trials 3, 4 and 5 were summarized in the (unpublished) manuscript of Rankins, Smith, Erickson and Fuehring (mentioned above), as follows:

"...Pre-bloom kochia herbage was hand-clipped from agronomic plots in eastern New Mexico and fed to rats in three studies to characterize toxicosis and to evaluate treatments that might improve tolerance...Rats were fed diets with varied levels of dried, ground kochia herbage, which varied in alkaloids content as detected by Dragendorff's reagent. Treatments imposed were N-acetyl-L-cysteine, trans-stilbene oxide, lipotropic factors, vitamins A and E, and zinc. Serum enzyme activities changes reflecting mild, chronic toxicosis were evident and accentuated by depressed body weight gains ($P < .05$). In trial 2 serum calcium levels were depressed ($P < .05$) by high alkaloid herbage, but in the other trials Ca levels were unaffected ($P > .10$). Total bilirubin was increased ($P < .05$) as a result of dietary kochia in two of the three trials. Serum enzymes (alanine aminotransferase, aspartate aminotransferase, lactic dehydrogenase, alkaline phosphatase and gamma-glutamyl transpeptidase) were elevated ($P < .05$) by dietary kochia. Largest effects were associated with herbage that had "high alkaloid" rating. In [Trial 4] treatment with lipotropic factors or vitamins A and E were somewhat beneficial but N-acetyl-L-cysteine was detrimental. In [Trial 5] all treatments showed some benefits... Rats fed kochia herbage exhibited mild toxicosis... that varied in relation to herbage

content of alkaloids. Treatments imposed tended to alleviate some, but not all, adverse effects of ingested kochia."

D. Livestock experiments to characterize early kochia toxicosis and evaluate treatments (Trials 6-9)

Results from Trial 6 were reported in the Master's Thesis of Darrell Lee Rankins, Jr. (Rankins 1987), which is on file at NM WRRI, the Department of Animal and Range Sciences, NMSU, and the main library at NMSU. Findings were summarized in abstract form (Rankins and Smith 1987a) and reported at meetings of the Western Section, American Society of Animal Science (Rankins and Smith 1987b), but tabular data from this trial have not been published. However, this trial is described in a manuscript recently prepared for publication as a journal article, and it is presented herein with all the tabular data: "NUTRITIONAL AND TOXICOLOGICAL EVALUATIONS OF KOCHIA HERBAGE FED AS HAY TO FINE-WOOL LAMBS" by D. L. Rankins, Jr. and G. S. Smith, New Mexico State University, Las Cruces 88003 [see Appendix 5].

Results from Trial 7 were reported in abstract form and platform presentation (Rankins et al. 1988a), and published in Proceedings of the 1988 meeting of the Western Section, American Society of Animal Science (Rankins et al. 1988b), a copy of which is included herein [Appendix 6].

Trials 8 and 9. Results from these trials were reported at the 1988 meeting of Mountain West Chapter, Society of Toxicology (Sept. 22, 23, 1988) and published (Rankins et al. 1988c) in Abstracts of that meeting, as follows:

"METABOLIC HORMONE PROFILES IN SHEEP AND CATTLE FED KOCHIA SCOPARIA - D. L. Rankins, Jr., G. S. Smith and D. M. Hallford, Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003.

Kochia scoparia (L.) Schrad. is a rapidly growing, drought-resistant weed which has been considered as a valuable agronomic crop in dryland agriculture. However, acceptance of kochia as a water-efficient forage crop has been restricted because livestock grazing kochia have been poisoned. Two trials were conducted to evaluate hormonal changes associated with kochia toxicosis. Animals were pair-fed with alfalfa hay (91% organic matter [OM], 13% crude protein [CP] and 45% acid detergent fiber [ADF]) and kochia hay (85% OM, 13% CP and 42% ADF). TRIAL 1: Eight steers were pair-fed (4 alfalfa, 4 kochia) for 21 days and then bled hourly for 7 hours. Harvested serum was analyzed for insulin (INS), prolactin (PRL), growth hormone (GH) and serum clinical profile ("Chem-30"). Insulin and GH were not affected but PRL levels tended to be lower ($P = .14$) in kochia-fed steers (1.8 ng/ml vs 6.0 ng/ml in controls). Steers did not exhibit the characteristic hyperbilirubinemia associated with kochia toxicosis although lactic dehydrogenase and aspartate aminotransferase were elevated 1.3-fold ($P < .05$). TRIAL 2: Ten fine-wool wethers were pair-fed (5 alfalfa, 5 kochia) for 21 days and bled hourly for 7 hours on days 0, 5, 10 and 21. Prolactin was not affected and GH was suppressed ($P < .05$) on day 5 and remained somewhat lowered on days 10 and 21. Kochia-fed sheep exhibited hyperbilirubinemia (123% of control values; $P = .06$) related to increased indirect

(non-conjugated) bilirubin. In addition, aspartate- and alanine aminotransferase were elevated ($P < .05$). Metabolic hormonal changes associated with kochia toxicosis seem species dependent. Steers exhibited depressed PRL levels whereas lambs exhibited depressed GH levels and unchanged PRL levels."

Table 1 presents chemical composition data of the hays fed to steers in Trial 8 and to wethers in Trial 9.

These hays were closely matched for content of dry matter and crude protein but differed considerably in content of ash, organic matter, neutral detergent fiber and "remaining organic matter" (which represents soluble carbohydrates). Composition data suggest that the digestible energy intake of the kochia might have been considerably less than for the alfalfa.

Trial 8. Hay intakes by steers fed alfalfa hay were restricted to the intakes of kochia hay by the corresponding pair-fed steers, and amounted to 1.97 $\pm$ .51 kg per day during the 21 day trial. Body weights at onset of the trial and after 21 days are shown in table 2:

Hay intakes at approximately 2 kg daily represented less than 1% of steer body weight, and thus were insufficient to maintain body weight, even though highly nutritious. These low intakes reflect low palatability of the kochia hay. Body weight losses at 16 to 18 kg by day 21 reflect loss of gastrointestinal fill as well as catabolism of body components. Such loss reflects undernutrition mainly, because losses were no more drastic nor rapid for steers fed kochia than for those pair-fed alfalfa hay.

Blood serum constituents in yearling steers pair-fed alfalfa hay in relation to kochia hay for 21 days in Trial 8 are shown in table 3.

TABLE 1

Composition of kochia herbage fed as hay and of alfalfa hay pair-fed to steers in Trial 8 and to wethers in Trial 9

Component	Alfalfa hay	Kochia hay
	%	
Dry matter	94.0	94.0
	% of DM	% of DM
Organic matter	90.6	85.2
Ash	9.4	14.8
Crude protein (N x 6.25)	13.4	13.0
Acid detergent fiber	41.8	44.9
Neutral detergent fiber	54.5	68.2
(Remaining organic matter)	(22.7)	(4.0)
Oxalate, total	.8	6.3

TABLE 2

Body weights of steers fed kochia hay or alfalfa hay in Trial 8

Item	Day 0			Day 21		
	Alfalfa	Kochia	(SE)	Alfalfa	Kochia	(SE)
Number of steers	4	4		4	4	
Avg. body wt, kg	238	241	(2.3)	220	225	(3.2)
Body wt loss, kg	-	-		18	16	

Of 26 serum values reported only bicarbonate/CO_2, triglycerides, creatinine, lactic dehydrogenase (LDH) and aspartate aminotransferase (AST) values differed ($P < .05$) between steers fed kochia and those pair-fed alfalfa at day 21; but when compared with the corresponding values for the same animals on day 0 (table 3), none of these values reflect changes bearing biological significance. When data for all steers on day 21 are contrasted with comparable values for day 0 (see data marked ———*, table 3) there are increases ($P < .05$) at day 21 for phosphorus, cholesterol, and bilirubin (indirect = unconjugated), along with a decrease ($P < .05$) for BUN/creatinine. None of these changes are of sufficient magnitude to suggest tissue damage resulting from toxicants in the ingested kochia hay.

Table 4 shows serum prolactin levels in the steers pair-fed alfalfa hay and kochia hay for 21 days (Trial 8). Values represent data pooled across 7 hours. Prolactin levels were lower ($P = .14$) in steers that had ingested kochia hay (1.8 vs 6.0 ng/ml). Somatotropin levels did not differ ($P = .28$).

Table 5 shows serum insulin levels in growing steers pair-fed alfalfa hay or kochia hay for 21 days (Trial 8). Because there were interactions among diet and time of sampling, values are shown for each of the seven bleeding periods (0600 to 1200), which were started at feeding time (0600). For steers fed kochia hay, insulin levels were lower ($P = .15$) at 0600, markedly lower at 3 and 4 hours after feeding (.21 vs .54 and .18 vs .34), and similar at 5 and 6 hours after feeding.

Results confirmed, with cattle, that ingested kochia herbage disrupted metabolic hormones, before any changes in serum clinical profiles reflected tissue damage caused by kochia toxicosis.

TABLE 3

Blood serum constituents in yearling steers pair-fed alfalfa hay or kochia hay for 21 days in Trial 8

Constituent	Day 0			Day 21		
	Alfalfa	Kochia	SE[a]	Alfalfa	Kochia	SE[a]
Sodium, mEq/L	138	138	1.2	138	138	1.9
Potassium, mEq/L	4.4	4.3	.12	4.8	4.9	.07
Chloride, mEq/L	104	104	0.7	101[b]	104[c]	1.6
Bicarbonate/CO_2, mEq/L	29	30	1.0	33[b]	31[c]	0.5
Calcium, mg/dl	9.2	9.3	.20	9.2	9.2	.29
Phosphorus, mg/dl	6.2	6.5	.19	8.2 *	8.2 *	.18
Iron, mcg/dl	114	130	11.0	107	132	11.3
Glucose, mg/dl	72	68	3.0	70	63 *	2.4
Cholesterol, mg/dl	78	93	4.3	98[b]	116[c] *	8.0
Triglycerides, mg/dl	23	24	2.1	21[b]	26[c]	1.2
Urea nitrogen, mg/dl	18	20	1.0	16	17	0.8
Creatinine, mg/dl	1.2	1.3	.04	1.8[b]	2.0[c] *	.04
BUN/Creatinine	14	15	1.0	8	8	0.4
Uric acid, mg/dl	.55	.62	.049	.78	.85	.057
Albumin, g/dl	3.6	3.3	.11	3.8	3.6	.13
Globulin, g/dl	2.6	2.7	.08	2.8	3.0	.12
Total protein, g/dl	6.2	6.0	.12	6.5	6.6	.19
Total bilirubin, mg/dl	.28[b]	.21[c]	.020	.43 *	.34 *	.036
Direct bilirubin, mg/dl	.02	.01	.006	.05	.01	.012
Indirect bilirubin, mg/dl	.26	.20	.022	.38 *	.33 *	.035
ALK-P, U/L	108	102	13.8	108	80	13.0
CPK, U/L	263	303	22.2	292	410	158
GGTP, U/L	15	14	1.3	15	11	2.9
LDH, U/L	1128	900	183	951[b]	1202[c]	21
AST, U/L	72	55	13.6	54[b]	68[c]	2.6
ALT, U/L	22	26	2.6	20	20	1.8

[a]Standard error of the mean, N = 4.

[bc]Row values with different superscripts differ ($P < .05$).

*Indicates that (underlined) values for day 21 differ ($P < .05$) from corresponding values for day 0.

ALK-P = alkaline phosphatase.
CPK = creatine phosphokinase.
GGTP = gamma-glutamyl transpeptidase.
LDH = lactate dehydrogenase.
AST = aspartate aminotransferase.
ALT = alanine aminotransferase.

TABLE 4

Serum prolactin and somatotropin levels (ng/ml)[a] in growing steers pair-fed alfalfa hay and kochia hay for 21 days[a] (Trial 8)

Hormone	Diet		SE[b]	P[c]
	Alfalfa	Kochia		
Prolactin, ng/ml	6.0	1.8	1.8	.14
Somatotropin, ng/ml	7.4	11.4	2.4	.28

[a]Split-plot analysis of variance showed no diet x h interactions (P > .12). Therefore, means were pooled across h. Steers were bled hourly for 7 h.

[b]Standard error of the mean (n = 28).

[c]Probability value.

TABLE 5

Serum insulin (ng/ml) levels in growing steers pair-fed alfalfa hay and kochia hay for 21 days[a] (Trial 8)

Time (h)	Alfalfa	Kochia	SE[b]	P[c]
0600	.40	.26	.061	.15
0700	.35	.22	.077	.29
0800	.31	.42	.137	.58
0900	.54	.21	.104	.06
1000	.34	.18	.078	.21
1100	.24	.32	.063	.39
1200	.34	.31	.068	.78

[a]Split-plot analysis of variance showed a diet x h interaction ($P < .05$). Therefore, effect of diet was examined within h.

[b]Standard error of the mean (n = 4).

[c]Probability value.

Trial 9. Intakes of alfalfa hay were restricted to the levels of kochia hay consumed by the pair-fed sheep, and averaged 671 ± 125 g daily (mean ± standard deviation) during days 1 through 21, which amounted to 2.4% of live body weight. This level of ad libitum intake of kochia hay by sheep differed markedly from the level of this same kochia hay consumed ad libitum by steers (0.8% of body weight) in Trial 8. Body weight of the sheep at onset were 28.4 ± 3.4 and 29.2 ± 2.2 kg for sheep fed alfalfa and kochia hays, respectively. After 21 days, body weights were 28.1 ± 4.0 and 27.8 ± 4.0, respectively. Sheep fed alfalfa hay at the same level that kochia was consumed maintained body weight; but those fed kochia averaged 1.4 kg body weight loss during 21 days. These results show that alfalfa hay had typical nutritive value and that nutritive value of the kochia hay was slightly below that of the alfalfa hay.

Table 6 shows blood serum profiles for the sheep in Trial 9 at onset and after 21 days, in which alfalfa hay and kochia hay were pair-fed. Because so many of the values for day 21 are higher than the corresponding values for day 0 (e.g., all electrolytes, glucose, cholesterol, urea-N, albumin, globulin, etc.), it must be concluded that there was some degree of dehydration of all the sheep on day 21. The reason for such dehydration is not known at present; however this observation should not prevent comparisons and contrasts of values for sheep fed kochia versus alfalfa on day 21. The data (table 6) indicate that kochia hay elevated ($P < .10$) serum cholesterol, indirect (unconjugated) bilirubin, total bilirubin and also elevated ($P < .05$) albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) over corresponding values for sheep fed alfalfa. Somewhat

TABLE 6

Blood serum constituents in yearling sheep pair-fed alfalfa hay and kochia hay for 21 days (Trial 9)

Constituent	Day 0			Day 21		
	Alfalfa	Kochia	SE[a]	Alfalfa	Kochia	SE[a]
Sodium, mEq/L	128	131	5.7	160	169	6.4
Potassium, mEq/L	4.4	4.8	.33	5.9	6.0	.32
Chloride, mEq/L	89	91	4.8	123	133	6.3
Bicarbonate/CO_2, mEq/L	26	25	1.1	33	32	1.5
Calcium, mg/dl	7.5	7.0	.36	10.8	11.6	.49
Phosphorus, mg/dl	5.2	5.2	.42	7.3	7.6	.43
Iron, mcg/dl	165	141	20	155	134	12
Glucose, mg/dl	55	52	4.7	66	66	3.4
Cholesterol, mg/dl	30	27	3.8	46[b]	61[c]	4.6
Triglycerides, mg/dl	18	16	2.5	20	20	2.0
Urea Nitrogen, mg/dl	15	16	1.6	27	24	2.0
Creatinine, mg/dl	.7	.6	.04	1.1	1.0	.09
BUN/Creatinine	21	26	3.4	24	23	1.1
Uric acid, mg/dl	.24	.22	.022	.08	.10	.014
Albumin, g/dl	2.4	2.4	.17	3.7[d]	4.3[e]	.14
Globulin, g/dl	2.5	2.0	.23	3.7	3.5	.14
Total protein, g/dl	5.0	4.4	.37	7.3	7.8	.20
Total bilirubin, mg/dl	.22	.20	.018	.26[b]	.32[c]	.019
Direct bilirubin, mg/dl	.01	.01	0	.01	.01	0
Indirect bilirubin, mg/dl	.21	.19	.018	.25[b]	.31[c]	.019
ALK-P, U/L	93	110	16	179	111	27
CPK, U/L	236	167	38	338	226	96
GGTP, U/L	35	38	2.9	43[d]	34[e]	2.6
LDH, U/L	357	336	29	448	550	73
AST, U/L	68[b]	60[c]	2.8	72[d]	94[e]	6.1
ALT, U/L	6.2	7.8	.73	9.8[d]	17.6[e]	1.5

[a]Standard error of the mean (n = 5).

[bc]Row values with different superscripts differ ($P < .10$).

[de]Row values with different superscripts differ ($P < .05$).

ALK-P = alkaline phosphatase.
CPK = creatine phosphokinase.
GGTP = gamma-glutamyl transpeptidase.
LDH = lactate dehydrogenase.
AST = aspartate aminotransferase.
ALT = alanine aminotransferase.

surprisingly, values for gamma-glutamyl transpeptidase (GGTP) were lower ($P < .05$) in sheep fed kochia than in those fed alfalfa (34 vs 43 U/L). Similar trends were seen in the steers fed these same specimens of alfalfa hay and kochia hay (Trial 8; table 3), whereby not only GGTP but also alkaline phosphatase (ALK-P) values tended to be lower in animals fed kochia hay; whereas lactic dehydrogenase (LDH) values were higher ($P < .05$) in steers fed kochia (table 3) but only tended to be higher ($P > .10$) in the sheep fed kochia.

These relationships of serum enzymes in both steers (Trial 8) and sheep (Trial 9) fed the same specimens of kochia and alfalfa hays are important, because they verify that by day 21 slight leakage of enzymes from hepatic cells (ALT, AST, LDH) was evident, with no indication of cholestasis. Furthermore, serum levels of unconjugated (indirect) bilirubin were elevated ($P < .05$) in sheep (table 6), as expected from most other trials, even though no elevation had been seen in the steers (table 3).

Table 7 shows blood serum levels of prolactin and somatotropin in sheep pair-fed alfalfa hay and kochia hay at days 0, 5, 10 and 21 during Trial 9. Prolactin levels tended to be somewhat higher in the sheep fed kochia than in those fed alfalfa, a trend that differs from results in steers (table 4) where those fed kochia hay had lower values. (Note, however, that P values were high: .40 to .88) Somatotropin values were slightly lower in sheep fed kochia hay below those fed alfalfa (table 7); but in the steers fed these hays somatotropin levels were slightly higher for steers fed alfalfa (table 4). (Insulin values were not yet available from Trial 9 at the time this report was submitted.)

Results from Trials 8 and 9 must be interpreted with some caution.

Although the same specimens were fed in each trial, there were variables that affected the data in addition to species (sheep versus cattle). For example, feed intakes were considerably lower for the steers (< 1% of body weight daily) than for sheep (2.4% of body weight daily). Ambient temperature was cold (below freezing at night) when the steers were on trial and controlled (indoors) at 20° C when the sheep were on trial. The cattle were young and still growing when placed on trial, but the sheep were yearlings and somewhat more mature. These variables do not preclude usefulness of the comparisons; but they require that the data be interpreted accordingly, even as other variables must be considered when comparing results from the earlier experimentation reported herein.

The fact that serum bilirubin levels were elevated in the sheep (Trial 8) but not in the steers (Trial 9) fed kochia hay may be explained in terms of the large difference in kochia hay intakes (2.4% of body weight for sheep versus 0.8% for steers).

TABLE 7

Metabolic hormone levels[a] in blood serum of sheep pair-fed alfalfa hay and kochia hay for 21 days (Trial 9)

Hormone	Day of Sampling	Diet		SE	P
		Alfalfa	Kochia		
Number of sheep		5	5		
Prolactin, ng/ml	0	25	30	4.9	.49
	5	15	20	4.9	.50
	10	11	18	5.2	.40
	21	16	18	5.3	.88
Somatotropin, ng/ml	0	3.8	2.8	.61	.24
	5	5.0	2.5	.57	.01
	10	4.9	3.6	.94	.35
	21	3.9	3.0	.50	.25

[a]Values are means for samples collected at 0600, 0700, 0800, 0900, 1000, 1100 and 1200 hours.

DISCUSSION

Results reported by Kiesling et al. (1984) and included herein (Appendix 1) demonstrated conclusively that lush kochia herbage produced on irrigated cropland at Tucumcari, NM, was toxic when grazed by cattle. The symptoms (at late stages of toxicosis) were photosensitization and polyuria, as well as general unthriftiness, and histopathology confirmed both hepatic and renal involvement (Thilstead et al. 1988). When lush forage that caused mortality in steers was processed as hay and fed to sheep, milder toxicosis was elicited that implicated hepatotoxicosis in the early aspects, including elevated serum cholesterol and aspartate aminotransferase within one week, and, additionally, elevated gamma-glutamyl transpeptidase (GGTP) and elevated bilirubin (2x) by week 4. By week 8, lactic dehydrogenase levels were elevated (2x) along with GGT (2x). These observations support an hypothesis that early aspects of kochia toxicosis involves failure of hepatocytes to conjugate (and thus excrete) bilirubin (and presumably also the porphyrins derived from plant chlorophyll and other pigments). Subsequently, the changes suggest mild damage to the biliary tissues (e.g., increased GGTP), before the appearance of signs suggesting renal impairment. Few, if any, of the signs and symptoms seem consistent with the view that oxalate was the primary toxicant, although several observations (Thilstead et al. 1988) suggested that oxalates may have contributed to (subsequent) hepatic as well as renal damage.

Serum insulin values in sheep fed kochia for 10 weeks (Hoefler et al., 1988; Appendix 2, Table 6) were apparently deranged; but interpretations of the data were handicapped by obvious undernutrition of the sheep (6 kg weight loss in 10 weeks) as well as toxicosis. Moreover, the protein content of that hay, reported as 5.9% (Appendix 2; Table 1), seems highly questionable.

Our initial studies with rats (Appendix 3) demonstrated toxicosis when rats were fed kochia herbage that had caused toxicosis in both cattle and sheep. Furthermore, the signs were sufficiently similar to support usage of rats, as (tentative) models for kochia toxicosis in livestock. Signs and symptoms of kochia toxicosis in rats were more severe in those fed kochia that tested "high" versus "low" in content of substances reactive to Dragendorff's reagent (a presumptive test for alkaloids). Clinical signs of (mild) toxicosis in the early rat studies were consistent with the view that alkaloids (rather than oxalate or saponins) were the primary toxicant. Oxalate content of kochia was 5.7% of dry matter. Mild lesions elicited by dietary kochia for ten weeks were largely reversed by subsequent feeding of commercial rat feed for five weeks (Appendix 3; Table 3). Rats fed kochia recovered from ether anesthesia more quickly than controls, suggesting that substances in kochia had induced mixed-function oxygenase activity.

Our subsequent studies with rats (Trial 4; Appendix 4; and Trial 5, summarized on p. 15) further confirmed that severity of blood serum changes in rats fed kochia herbage was correlated directly with herbage content of substances reacting with Dragendorff's reagent. Oxalate contents of the kochia specimens were 5.8, 6.6 and 6.3% of dry matter for low-, medium- and high-alkaloid specimens. Changes in serum clinical profiles were poorly correlated with oxalate content of kochia and seemed inconsistent with typical oxalate toxicosis. The hypothesis that kochia herbage had induced hepatic mixed-function oxygenase activity was tested by measuring the pentobarbital sleeping times of rats that had received either commercial rat feed or 30% "high alkaloid" kochia herbage for 12 weeks. Although sleeping times were slightly shorter ($P < .05$) for rats fed kochia, critical evaluation of the data (Rankins 1987) revealed that there was an interaction whereby one of the treatments administered had prolonged sleeping times in

rats fed the control diet longer than in rats fed kochia. Thus, it was concluded that induction of mixed-function oxygenase activity did not characterize (mild) kochia toxicosis in rats.

The treatments tested in rat Trials 4 and 5, namely, vitamins A + E, lipotropic factors (choline, inositol, and folic acid), acetyl-cysteine, acetyl-cysteine + trans-stilbene oxide, or zinc were selected and administered at the dosages described (see Rankins 1987) in order to help characterize the nature of kochia toxicosis as well as to measure any benefits that might be observed. The theoretical reasons for selections of these particular treatments will be presented elsewhere (Rankins 1989, in preparation). Although none of the treatments fully alleviated the adverse effects of dietary kochia for rats, each of these treatments provided some benefits, in one form or another, which suggested that the tissue damage indicated by serum clinical profiles were non-specific in nature (Rankins et al. 1988d). Moreover, the time-course in which toxicosis developed in rats indicated that depressed appetite was a primary event in kochia toxicosis, preceding any serious change in serum clinical profiles. Verification whether this was true for ruminants, and determination whether derangement of metabolic hormones (such as prolactin, insulin and somatotropin) comprised the initial stage of kochia toxicosis thus became the focus of highest priority in further efforts to characterize kochia toxicosis (Smith et al. 1988a).

The importance of characterizing metabolic hormone profiles in the early stages of kochia toxicosis was illustrated by research reported concurrently, which showed that the toxicity of endophyte-infested tall fescue grass (Festuca arundinacea Schreb.) was related to peptide and clavine ergot alkaloids (Lyons et al. 1986). Reduction of feed intake accounted for major aspects of fescue toxicosis in cattle (Jackson et al.

1984), and early aspects of fescue toxicosis were related to drastic changes in serum levels of prolactin (Bond and Bolt 1986; Elsasser and Bolt 1987). These aspects of fescue toxicosis seem remarkably similar to the earliest aspects of kochia toxicosis. The importance of this similarity was further emphasized just recently when Lipham et al. (1988) alleviated toxicosis in steers grazing tall fescue by prophylactic treatment using the drug metoclopramide, which suggests that metoclopramide might similarly enhance the tolerance of kochia.

When kochia herbage that had tested "high" in alkaloids and had elicited toxicosis in rats was fed to sheep (Trial 7) as 35% of diet (plus 65% alfalfa) appetite was unaffected (dry matter intake averaged 3.4% of body weight daily for 4 weeks) and signs of toxicosis were negligible. Moreover, when this kochia (as hay) was increased to 50% of diet, intakes remained satisfactory at about 3.3% of body weight for 5 weeks and digestive functions (digestibility and rate of passage) were normal. However, when kochia was fed as 100% of diet for sheep (Trial 9), impaired bilirubin conjugation was evident within 21 days, even though prolactin levels were not yet affected and intake of kochia remained high through 21 days. These findings, together with minimal changes in clinical profiles (Appendix 5) showed that sheep had greater tolerance of kochia than rats and suggested that sheep may well be more tolerant than cattle. Effects of treatments (vitamins A + E, acetyl-cysteine plus trans-stilbene oxide, and zinc sulfate) were negligible because effects of kochia were slight. The results showed that studies of kochia toxicosis in sheep and cattle would likely require high levels of kochia intake in order to demonstrate clinical signs or effects of treatments, unless kochia feeding (and treatments) were continued for extended periods. These observations may explain why many livestock producers have grazed kochia for short durations with no evidence

of adverse effects.

Although the specific toxicant(s) in lush kochia herbage that caused toxicosis in rats, sheep and cattle has (have) not yet been identified, it has been demonstrated that severity of toxicosis in rats was correlated directly with content of substances (in the fresh herbage) reacting with Dragendorff's reagent and thus presumably alkaloidal in nature. Furthermore, the early signs and symptoms of kochia toxicosis seemed incompatible with oxalate or saponins as primary toxicant but quite compatible with alkaloids as toxicant. More importantly, perhaps, an early aspect of kochia toxicosis in both sheep and cattle was shown to be low serum levels of prolactin, which was accompanied by seriously impaired feed intake or by seriously impaired nitrogen retention even when feed intakes remained unimpaired. Derangement of metabolic hormone profiles, leading to impaired intermediary metabolism and seriously reduced feed intake is the primary characteristic of early fescue toxicosis in cattle, which is caused by peptide and clavine alkaloids associated with endophyte-infested fescue. Whether kochia toxicosis is likewise caused by similar alkaloids is not yet known, but is strongly suggested by the data currently available.

The information available suggests that (an) alkaloid(s) in kochia herbage alter metabolic hormone levels, notably prolactin, impair appetite, and alter intermediary metabolism as early aspects of kochia toxicosis, followed by impairment of bilirubin conjugation and, subsequently, by a cascade of tissue damage that seems to include hepatocytes initially. These signs suggest that kochia toxicosis may well be alleviated or prevented by the same types of drugs that recently were formed to alleviate or prevent fescue toxicosis.

Further research to test this possibility would seem to be of great importance not only to New Mexico but to many areas of the world where kochia is regarded as an important prospective forage crop.

CONCLUSIONS

Severity of kochia toxicosis in rats was related to herbage content of substances reactive with Dragendorff's reagent, presumably alkaloids. Kochia hay from lush forage testing "high" in (presumed) alkaloids invariably elicited signs of toxicosis in sheep and cattle when fed as 50% or more of total diet. Signs of toxicosis in rats, sheep and steers seemed inconsistent with oxalate or saponins as primary toxicant but resembled early aspects of fescue toxicosis, caused by certain peptide and clavine alkaloids. Treatments selected for well-known and well-characterized effects provided slight benefits for rats but were of little benefit for sheep fed kochia herbage; however, these treatments were useful in characterizing the tissue damage in kochia toxicosis as subsequent to metabolic derangement related to hormonal changes. Findings suggest that treatments which alleviate or reduce fescue toxicosis should likewise prove beneficial for livestock consuming herbage from lush kochia. Further work to develop such treatments seems highly important to New Mexico, and to water conservation in many areas of the world.

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Nutritive Value and Toxicity Problems of Kochia for Yearling Steers

Agricultural Experiment Station ● Research Report 546

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Summary

Nutrient composition of kochia forage collected by esophageal-fistulated cows during summers of 1982 and 1983 indicates kochia could be a good forage crop for yearling steers. However, weight loss, as well as other toxicity symptoms, occurred in cattle grazing the irrigated kochia pastures. Problems appeared in August and September 1982 and in July and August 1983. Affected steers appeared thin and lethargic, salivated excessively, had watery eyes, had difficulty breathing and isolated themselves from the rest of the herd. Weight loss was severe in some steers. Necropsy revealed damage to livers and kidneys.

Serum chemistry profiles showed some blood constituents (bilirubin, gamma glutamyl transpeptidase and serum glutamic oxaloacetic transaminase) to be consistently elevated in cattle grazing kochia. Blood urea nitrogen and lactic dehydrogenase were also elevated. These elevations indicate likely involvement of hepatotoxicants. These parameters returned to normal when cattle were removed from kochia. Most other blood constituents were within normal ranges for yearling steers.

Substances in kochia that cause these problems are not known, but several possibilities exist and need to be evaluated. Oxalate, however, does not appear to be the primary toxicant. Research is needed to determine toxic substances, and conditions that promote production of toxic substances, in kochia because of its potential value as a high quality forage crop.

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July 1984

Las Cruces, New Mexico

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Nutritive Value and Toxicity Problems of Kochia for Yearling Steers

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Kochia scoparia (L) Schrad, which belongs to the goosefoot family (Chenopodiaceae), is commonly called fireweed, summer cypress, burning bush, fireball, belvedere, Mexican fireweed or kochia. It is an early emerging, vigorous, rapidly growing plant that is extremely drought resistant. Yield of more than 11,000 kg/ha (5 ton/ac) under dryland condition was reported by Sherrod (1971). Baker (1974) reported yields of more than 360 kg (792 lb) of dry matter per 2.54 cm (1 in) of water used. Early work (Erickson and Moxon, 1947) with kochia indicated it was a palatable and nutritious forage, and generally comparable in digestibility to alfalfa. Finley and Sherrod (1971) reported digestibility of all nutrients decreased ($P < .01$) linearly from prebloom to full-bloom, but was comparable to alfalfa at early growth stages. However, in recent years, toxicity in livestock grazing kochia has been reported (Galitzer and Oehme, 1978; Dickie and Berryman, 1979; Sprowls, 1981; Dickie and James, 1983).

Few designed studies have been conducted on the potential value of kochia as a forage crop for grazing yearling steer calves. The objectives of this study were to (1) evaluate the nutritive value of kochia as indicated by performance of yearling steers on irrigated kochia pasture and (2) determine if toxicity would result from feeding kochia under controlled conditions.

Materials and Methods

A 4.8 ha (12 ac) plot on the Agricultural Science Center at Tucumcari was planted to kochia (*Kochia scoparia*) during spring 1982, using 91.5 cm (36 in) rows with a standard drill and a seeding rate of 4.5 kg/ha (4 lb/ac) and fertilized with 85 kg N/ha (76 lb/ac). The plot was divided into two pastures (east, west) and each pasture was subdivided into four .6 ha (1.5 ac) paddocks. All paddocks were watered initially plus one or two times during the summer. Each pasture received an average of 30 cm (12 in) of irrigation water. Three separate grazing trials were subsequently conducted to evaluate effects of kochia consumption on animal performance and health.

Trial one began June 3 when one paddock of each pasture was stocked with 18 medium to large-frame yearling steers of mixed beef breeding. Steers were rotated on a weekly basis so each paddock was grazed for 7 days and rested for 21 days. The animals were weighed initially and on days 28, 56, 84 and 105 of the grazing period. Jugular blood samples were collected from each steer initially and on days 56 and 105 after stocking. The serum separation tubes were transported in an ice chest to the Clayton Livestock Research Center (CLRC) where they were centrifuged at approximately 850 × g for 20 minutes. Serum was separated and stored frozen for serum chemistry profile analysis.

Two esophageal-fistulated cows were used to collect forage samples from one paddock before and after being grazed by steers every 28 days during the grazing period. All samples were transported to the CLRC where they were dried at 50 °C in a forced-air oven. Dried samples were transported to Las Cruces where they were ground through a 1-mm screen in a Wiley mill and analyzed for dry matter, ash, crude protein, acid detergent fiber and acid detergent lignin (A.O.A.C., 1980). Nutritive value and seasonal variation in composition of kochia were determined by analyzing the samples.

For 1983 (trial two), the kochia pastures were allowed to reseed with volunteer seed from the 1982 planting. Before seedling emergence, new water furrows were established to thin the potential crop and to facilitate irrigation. Pastures

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were fertilized with 195 kg/ha (174 lbs/ac) of N before grazing. The plot (4.8 ha, 12 ac) was divided into four pastures with each pasture subdivided into four .3 ha paddocks. All paddocks were irrigated early in the season and either one or two times during the summer. Each pasture received 53 cm (21 in) of irrigation water.

On June 30, 60 medium to large-frame yearling steers of mixed beef breeding were randomly divided into four groups. Each group was assigned to one of the kochia pastures and one of four supplemental feeding treatments. The supplemental treatments were: (1) no supplement; (2) a 15% calcium, 10% phosphorus free-choice mineral supplement; (3) a grain supplement of .7 kg (1.5 lb) of cracked corn per head per day; and (4) a combination of the free-choice mineral plus grain. Steers were weighed and jugular blood samples were collected; serum was separated and stored frozen for later serum chemistry profile determination. As in 1982, steers were allowed to graze a paddock for 7 days, then were moved to the next paddock. Because of excessive weight loss of steers, the grazing trial was terminated in 3 weeks. Steers were allowed to graze native grass for the next 3 weeks. Jugular blood samples were withdrawn upon termination of kochia grazing and grass grazing.

Thirty steers of similar size and breeding which had been grazing native grass during the June 30-July 21 grazing trial were randomly allotted to one of three pastures on July 28 (trial three): native grass only, kochia only or kochia plus native grass. Because of excessive weight loss of the steers in the kochia-only pasture, this study was terminated after 2 weeks. At termination, jugular blood samples were withdrawn from all steers.

Esophageal-fistula forage samples were collected by two cows at the beginning and end of trial two in all four pastures. At the end of trial three, esophageal-fistula forage samples were collected from the two experimental kochia pastures and from another plot where kochia was growing without being grazed. A final sample was collected 33 days later from the kochia pasture and the other plot of kochia. All samples were analyzed as described for those collected in trial one.

All serum samples were sent to the New Mexico Department of Agriculture Veterinary Diagnostic Services in Albuquerque for serum chemistry profile analysis using a simultaneous multi-channel analyzer with computer (SMAC). Twenty-four serum constituents were subsequently quantified in each sample.

The primary objective in trial one was to evaluate changes in serum chemistry profiles with time on kochia. Data were, therefore, subjected to analyses of variance appropriate for randomized block designs using pasture as the block and bleeding date as the other source of variation. When date effects were detected, means were separated using the least significant difference method (LSD, Snedecor and Cochran, 1967). Also, when pasture (east, west) × date interactions were observed, effects of bleeding date were evaluated within pasture. Serum chemistry data collected during trials two and three were examined by analyses of variance for completely randomized designs using time (trial two) or pasture type (trial three) as sources of variation. Significant mean differences were separated in trial three by LSD.

Results

Trial One. Nutrient composition of kochia forage collected by esophageal-fistulated cows during the grazing period for 1982 is shown in table 1. Crude protein, acid detergent fiber (ADF) and acid detergent lignin (ADL) were not different ($P>.10$) among dates, either before grazing or after grazing. All values, however, differ ($P<.01$) between grazing times within date. Ox-

Table 1. Nutrient composition (organic matter basis) of esophageal fistula forage samples before and after grazing a paddock on the Agricultural Science Center at Tucumcari during summer 1982 (trial one)[1]

Item	June 3	July 1	July 29	Sept. 16	SE[2]
Before grazing					
Crude protein, %	18.6	20.1	19.1	20.5	1.24
Acid detergent fiber, %	29.1	26.3	27.8	26.5	2.11
Acid detergent lignin, %	5.4	5.4	4.8	5.6	.48
Oxalate, %	2.1	1.4	1.1		.14
Nitrate, ppm	21.2	21.7	32.5	54.5	53.97
After grazing					
Crude protein, %	17.4	16.0	11.6	12.5	1.52
Acid detergent fiber, %	42.8	33.2	35.5	35.1	2.59
Acid detergent lignin, %	5.9	6.3	6.3	7.1	.58
Oxalate, %	2.1	1.3	1.4		.14
Nitrate, ppm	70.5	484.0	32.2	21.2	66.10

[1]Means with four observations each.
[2]Standard error.

alate concentration was higher (P<.01) in June for before and after grazing than in either July collection, but all values were very low. Nitrate concentration was higher in samples collected after grazing on July 1 than all other samples. All values were very low. These levels of oxalate and nitrate should not be toxic for yearling steers grazing kochia. Levels for protein, ADF and ADL were adequate, but not excessive, and would indicate kochia could be a good forage crop for yearling steers.

Weight and gain data for cattle grazing kochia forage for 105 days during 1982 (trial one) are shown in table 2. All steers gained weight for 56 days, after which some gained and others lost weight, so the average gain was 0 from 56 to 84 days in the grazing season. During the last 21 days, steers lost an average of 6 kg with those in the west pasture losing more (11 kg) than those in the east pasture (1 kg). Average daily gain was .51 kg for 84 days, but only .35 kg for the 105-day grazing period. During the last 49 days, more steers lost (20) than gained (16) weight. Sprowls (1981) reported similar problems when animals had grazed kochia for a minimum of 40 days. At termination of the trial, one steer was severely emaciated and was sacrificed for pathological examination. The post-mortem exam revealed severe subacute to chronic nephrosis. Several steers were severely emaciated, lethargic and had staggered gaits. These steers were allowed to graze native pasture and three died within 10 days after removal from kochia pasture.

Serum chemistry profile data for steers grazing kochia during 1982 are shown in tables 3 and 4. Certain nutritional or metabolic constituents (calcium, protein, albumin, globulin, creatinine) increased in concentration from first (day 0) to third (day 105) bleeding times; however, all values were within or near normal ranges (table 10) as reported by Galyean and Hallford (1983) or furnished by Vet-Path (cited by Galyean and Hallford, 1983). Potassium and chloride decreased from first to second (day 56) bleeding times and remained unchanged at the third bleeding period but, again, all values were within normal ranges. Gamma glutamyl transpeptidase (GGT) increased (P<.01) from day 0 to day 56 and from day 56 to day 105. Serum glutamic oxaloacetic transaminase (SGOT) increased (P<.05) from day 0 to day 105 with day 56 being intermediate and not different from either day 0 or 105.

Pasture × day interactions (table 4) were detected (P<.05) for several constituents. Values for the east pasture were within or near normal ranges. For the west pasture, blood urea nitrogen (BUN) and total, direct and indirect bilirubin

Table 2. Weights of steers grazing kochia on the Agricultural Science Center at Tucumcari, 1982 (trial one)[1]

Item	Pasture		
	East	West	Average
Number of steers	18	18	
Initial weight, kg	182	184	183
Weight at 56 days, kg	229	222	226
Weight at 84 days, kg	225	227	226
Weight at 105 days, kg	224	216	220
Total gain, kg	42	32	37
Last 49 days			
Number losing weight	10	10	
Number gaining weight	8	8	

[1]Mean values with 18 steers per pasture.

Table 3. Serum chemistry profiles of steers grazing kochia, 1982 (trial one)[1]

Item	Days on pasture			
	0	56	105	SE[2]
Calcium, mg/dl[4]	10.1 i	11.1 ij	11.9 j	.4
Potassium, mM/l[3]	6.9 f	5.4 g	5.4 g	.2
Sodium, mM/l[6]	145.1	144.5	141.9	1.8
Chloride, mM/l[4]	99.2 i	94.8 j	91.0 j	1.5
Carbon dioxide[6]	23.3	23.1	24.9	1.1
Electrolyte balance[6]	22.6	26.6	26.1	1.7
Total protein, g/dl[4]	7.0 i	7.4 j	8.4 k	.2
Albumin, g/dl[4]	3.6 i	3.9 j	4.3 k	.1
Globulin, g/dl[3]	3.3 f	3.5 f	4.1 g	.1
Alb/Glob (ratio)[6]	1.1	1.1	1.1	.04
Creatinine, mg/dl[5]	1.2 l	1.2 l	1.7 m	.2
Uric acid, mg/dl[6]	1.1	.9	1.0	.2
Triglyceride, mg/dl[6]	21.1	17.0	18.7	3.4
Cholesterol, mg/dl[3]	99.2 f	68.6 g	72.7 g	5.3
Alkaline phosphatase, Units/l[6]	150.2	150.8	128.6	16.0
Gamma glutamyl transpeptidase, units/l[3]	26.0 f	86.1 g	140.4 h	13.1
Serum glutamic oxaloacetic transaminase, units/l[4]	114.4 i	149.1 ij	201.9 j	24.3
Lactate dehydrogenase, units/l[4]	901.1 i	1256.1 j	906.1 i	104.2

[1]Mean of 14 steers for each date. Pasture × day interactions were not detected (P > .05).
[2]Standard error.
[3]Means within row followed by different letters are statistically different (P < .01).
[4]Means within row followed by different letters are statistically different (P < .05).
[5]Means within row followed by different letters are statistically different (P < .10).
[6]Row means do not differ statistically (P > .20).

Table 4. Serum chemistry profiles of steers grazing kochia in two pastures, 1982 (trial one)[1]

Item	Pasture							
	Days on East Pasture[3]				Days on West Pasture[3]			
	0	56	105	SE[2]	0	56	105	SE[2]
Phosphorus, mg/dl	8.5 e	7.0 f	5.8 g	.2	8.5 e	5.0 f	6.8 g	.6
Blood urea nitrogen, mg/dl	16.7 e	13.3 fg	12.7 g	.8	16.2 e	10.0 e	40.3 f	6.6
Glucose, mg/dl	79.1 e	58.0 fg	65.0 g	4.5	69.0[4]	69.7	61.1	3.8
Total bilirubin, mg/dl	.08 e	.26 fg	.31 g	.05	.11 e	.21 e	4.08 f	1.1
Direct bilirubin, mg/dl	0.0 e	.01 e	.06 f	.01	0.0 e	0.0 e	.78 f	.21
Indirect bilirubin, mg/dl	.08 e	.17 ef	.26 f	.04	.12 e	.21 e	3.2 gf	.88

[1]Mean of 7 steers per pasture for each date. Pasture × day interactions were detected ($P < .05$).
[2]Standard error.
[3]Row means within pasture with different letters are statistically different ($P < .05$).
[4]Row means within pasture do not statistically differ ($P > .10$).

were high ($P<.05$) after 105 days grazing kochia. Serum values at the last bleeding period were pooled across pastures and examined between animals that gained and those that lost weight throughout the experiment (table 5). This procedure revealed that most of the high BUN and bilirubin values were registered by animals that lost weight during the trial. All other values were within normal ranges. Elevated SGOT and bilirubin in cattle grazing kochia was also reported by Dickie and James (1983).

Table 5. Serum constituents that were different ($P<.10$) between steers gaining and losing weight after grazing kochia pasture, 1982 trial one)[1]

Item	Gain		Loss	
	Mean	SE	Mean	SE
Phosphorus, mg/dl	5.3	.53	7.0	.47
Blood Urea nitrogen, mg/dl	13.3	8.5	36.4	7.3
Total bilirubin, mg/dl	.18	1.3	3.7	1.2
Direct bilirubin, mg/dl	.02	.26	.72	.22
Indirect bilirubin, mg/dl	.17	1.1	2.98	.94
Lactate dehydrogenase, units/l	1164.0	143.3	807.2	124.1

[1]Means and standard error (SE) of 6 steers in the gain group and 8 steers in the loss group for serum samples collected after 105 days on kochia.

Trial Two. Nutrient composition of kochia forage collected by esophageal-fistulated cows during summer 1983 is shown in table 6. Protein decreased while fiber increased from beginning to end of trial two, but differences were not significant. Crude protein collected from the experimental pastures for trial three was lower ($P<.01$) than that collected from kochia growing freely. There was no difference ($P>.10$) for ADF or ADL, although ADL tended to be higher in the free growing kochia. Values for the final collection were similar to those for trial three and crude protein was lower ($P<.05$) for kochia collected from the experimental pasture than for that growing freely. As in 1982, levels of protein, ADF and ADL were adequate but not excessive, so kochia contains sufficient nutrients for yearling steers.

Weight gain and loss data for cattle receiving supplement while grazing kochia pastures during 1983 are shown in table 7. The trial was terminated after only 3 weeks because all steers were losing weight. Average weight loss was 14 kg, although some steers lost as much as 40 kg. Several steers showed classical signs of photosensitization (photophobia, skin lesions,

Table 6. Nutrient composition in percent (organic matter basis) of esophageal fistula forage samples collected on the Agricultural Science Center at Tucumcari during summer 1983[1]

Nutrient	Trial Two		Trial Three		Final	
	Beginning	End	Experimental	Other area	Experimental	Other area
Crude protein	17.7	13.1	8.8	29.0	12.3	25.6
Acid detergent fiber	21.1	25.8	22.3	22.5	22.5	23.7
Acid detergent lignin	7.5	5.5	5.5	9.0	6.5	10.9

[1]Means; trial two = 8 observations each, trial three = 4 observations for experimental and 2 for other area, final = 2 observations each.

Table 7. Weights of steers grazing kochia, grass or combination pastures on the Agricultural Science Center at Tucumcari, 1983 (trials two and three)[1]

Item	Trial Two[2] (kochia pasture + supplemental feed)					Trial Three[2] (pasture only)			
	Control	Grain	Mineral	Mineral + grain	$\bar{X}$	Kochia	Grass	Kochia + grass	$\bar{X}$
Number of steers	15	14	15	15		10	10	10	
Initial weight, kg	226	236	226	234	230	312	307	298	306
Final weight, kg[2]	210	228	210	214	216	268	318	301	296
Total change, kg	− 16	− 8	− 16	− 20	+ 14	− 44	+ 11	+ 3	− 10

[1]A total of 6 steers were sacrificed or died between 7-21 and 9-8.
[2]Trial two terminated after 3 weeks; trial three terminated after 2 weeks.

skin necrosis, enlarged head). Photosensitization appears to occur frequently in cattle grazing kochia (Galitzer and Oehme, 1978; Dickie and Berryman, 1979: Sprowls, 1981). Two steers were sacrificed for pathological examination and post-mortem evaluations were done on two other steers that died. Reports on all four steers indicated nephrosis and liver damage. All other steers were allowed to graze native pasture for the next 7 weeks. During this time two steers died. Tissues from one of these steers were examined histologically. No significant lesions were found. Dickie and Berryman (1979) reported sickness and death in cattle grazing kochia and indicated the problems stopped when cattle were removed from kochia pasture.

Serum chemistry profiles for 10 steers taken before grazing kochia, after 3 weeks of kochia grazing and again after 3 weeks of grazing native grass after being removed from kochia pasture are shown in table 8 (trial two). Total, direct and indirect bilirubin ($P<.05$), GGT ($P<.10$) and SGOT ($P<.05$) values were elevated after grazing kochia, but returned to normal levels after grazing native grass for 3 weeks. Most other values were within or near normal ranges shown in table 10.

Trial Three. Steers grazing native grass only or a combination of grass and kochia gained weight while those grazing only kochia lost weight during a subsequent 2-week period (table 7). Weight loss was severe (44 kg) for steers grazing kochia only. Serum chemistry profiles (table 9) show elevated ($P<.01$ or $<.05$) levels of total and indirect bilirubin, GGT, SGOT and lactate dehydrogenase (LDH) for steers grazing kochia versus those grazing native grass. Although differences ($P<.10$) were detected for other constituents, most values were within or near normal ranges (table 10).

Discussion

In the three trials reported herein, animals grazing kochia had elevated bilirubin, GGT and SGOT, which is indicative of liver damage. In addition, histologic evidence of mild liver disease was found in most cattle examined post-mortem. Interestingly, most of the bilirubin was indirect (unconjugated). Elevations in indirect bilirubin are usually associated with hemolytic disease, but a decrease in conjugating capacity of hepatocytes (due to liver damage) could also be responsible. Reduction in conjugating capacity of the liver might explain the photosensitivity observed in some animals because photoreactive compounds are normally conjugated and removed from circulation by the liver.

In addition to hepatotoxic effects of kochia, nephrotoxic effects were also apparent in some animals. One of the four steers that died in the first trial was necropsied and severe nephrosis was evident. Necropsies on five animals from trial two revealed nephrosis in four steers. Results of serum constituent analysis and histopathologic examinations, therefore, suggest grazing kochia under conditions used in this experiment resulted in hepatotoxicity and renal damage.

Kochia could provide the nutrients needed by cattle. The nutritive value of kochia is similar to alfalfa. However, weight loss and other toxicity symptoms do occur, so kochia should be grazed with caution. Careful and frequent observation of cattle grazing kochia might minimize potential problems. Grazing kochia in combination with other forage might be advisable. Considerable potential for usage of nutrients in kochia appears to exist if toxicity can be identified and managed.

Table 8. Serum chemistry profiles of steers before grazing kochia, after grazing kochia for 3 weeks, and after grazing native grass for 3 weeks, 1983 (trial two)[1]

Item	Before Kochia	Kochia 3 weeks	Grass 3 weeks	SE[2]
Calcium, mg/dl[3]	10.4	11.3	10.9	.3
Phosphorus, mg/dl[3]	5.7	5.4	5.4	.4
Potassium, mM/l[4]	5.2 i	5.7 ij	6.2 j	.3
Sodium, mM/l[4]	138.3 d	138.3 d	145.3 e	.7
Chloride, mM/l[3]	99.3 g	96.8 gh	93.8 h	1.4
Carbon dioxide[4]	24.5 d	22.0 e	26.3 d	.9
Electrolyte balance[4]	14.5 d	19.5 e	23.3 e	1.9
Total protein, g/dl[3]	7.8	8.6	8.1	.4
Albumin, g/dl[4]	3.4 d	4.0 e	3.7 f	.1
Globulin, g/dl[3]	4.4	4.6	4.4	.4
Alb/Glob (ratio)[3]	.8	1.0	.9	.1
Blood urea nitrogen, mg/dl[5]	11.7 g	13.7 g	4.8 h	2.3
Creatinine, mg/dl[6]	1.3 g	1.9 h	1.5 g	.2
Uric acid, mg/dl[3]	1.4	1.2	1.9	.4
Glucose, mg/dl[4]	97.8 d	84.5 d	13.5 e	8.4
Triglyceride, mg/dl[6]	13.2 i	16.0 i	27.5 j	4.6
Cholesterol, mg/dl[3]	102.0	112.8	87.7	13.4
Total bilirubin, mg/dl[5]	.5 g	5.1 h	.25 g	1.3
Direct bilirubin, mg/dl[6]	.05 i	1.5 j	.05 i	.5
Indirect bilirubin, mg/dl[6]	.4 g	3.6 h	.2 g	.8
Alkaline phosphatase, units/l[3]	230.3	233.2	265.7	38.1
Gamma glulamyl transpeptidase, units/l[6]	21.3 i	109.7 j	52.7 ij	25.6
Serum glutamic oxaloacetic, transaminase, units/l[3]	130.0 g	237.8 h	111.5 g	33.0
Lactate dehydrogenase, units/l[3]	1483.3	1605.8	1235.7	159.0

[1]Mean of 10 steers.
[2]Standard error.
[3]Row means do not differ ($P > .10$).
[4]Row means with different letters are statistically different ($P < .01$).
[5]Row means with different letters are statistically different ($P < .05$).
[6]Row means with different letters are statistically different ($P < .10$).

Table 9. Serum chemistry profiles of steers grazing native grass or kochia pastures, 1983 (trial three)[1]

Item	Pasture		
	Grass	Kochia	SE[2]
Calcium, mg/dl[6]	10.4 h	11.4 i	.3
Phosphorus, mg/dl[3]	6.6	6.9	.4
Potassium, mM/l[3]	4.9	5.1	.2
Sodium, mM/l[3]	143.7	143.3	1.6
Chloride, mM/l[3]	94.9	95.2	.9
Carbon dioxide[6]	24.3 h	20.3 i	1.0
Electrolyte balance[3]	24.5	27.8	3.0
Total protein, g/dl[6]	7.0 h	8.5 i	.1
Albumin, g/dl[6]	3.8 h	4.1 i	.1
Globulin, g/dl[6]	3.2 h	4.4 i	.1
Alb/Glob, ratio[6]	1.2 h	.9 i	.05
Blood urea nitrogen, mg/dl[5]	5.9 f	12.8 g	2.3
Creatinine, mg/dl[3]	1.6	2.0	.2
Uric acid, mg/dl[3]	1.1	1.0	.2
Glucose, mg/dl[3]	46.6	40.9	6.9
Triglyceride, mg/dl[3]	24.3	29.3	4.3
Cholesterol, mg/dl[6]	94.8 h	125.6 i	4.3
Total bilirubin, mg/dl[5]	.2 f	2.8 g	.8
Direct bilirubin, mg/dl[4]	.1 d	.8 e	.3
Indirect bilirubin, mg/dl[5]	.1 f	2.0 g	.5
Alkaline phosphatase, units/l[3]	274.0	281.7	35.4
Gamma glutamyl transpeptidase, units/l[6]	18.8 h	153.4 i	19.6
Serum glutamic oxaloacetic transaminase, units/l[6]	108.6 h	334.4 i	31.8
Lactate dehydrogenase, units/l[6]	1367.8 h	2338.0 i	195.3

[1]Mean of 6 steers per pasture.
[2]Standard error
[3]Row means are not statistically different ($P > .10$).
[4]Row means with different letters are statistically different ($P < .10$).
[5]Row means with different letters are statistically different ($P < .05$).
[6]Row means with different letters are statistically different ($P < .01$).

Table 10. Serum chemistry profile reference values

Item	Galyean and Hallford	Vet-Path[2]
Calcium, mg/dl	9.5 - 10.4	9.9- 2.5
Phosphorus, mg/dl	5.0 - 7.0	3.4 - 6.7
Potassium, mM/l	4.3 - 5.2	2.8 - 5.6
Sodium, mM/l	127.8 - 140.1	133 - 143
Chloride, mM/l	92.4 - 97.6	91 - 105
Total protein, g/dl	5.9 - 6.8	4.9 - 7.7
Albumin, g/dl	3.1 - 3.4	2.4 - 3.8
Globulin, g/dl	2.5 - 3.6	1.8 - 4.6
Alb/Glob, ratio	.9 - 1.4	.5 - 1.5
Blood urea nitrogen, mg/dl	8.0 - 21.8	5 - 21
Creatinine, mg/dl	1.3 - 1.9	.9 - 1.9
Uric acid, mg/dl	.6 - 1.3	0 - 1.6
Glucose, mg/dl	94.5 - 114.8	73 - 123
Triglyceride, mg/dl	46.0 - 89.4	14 - 56
Cholesterol, mg/dl	81.9 - 119.8	20 - 147
Total bilirubin, mg/dl	.1 - .5	0 - .8
Direct bilirubin, mg/dl	.04 - .1	0 - .3
Alkaline phosphatase, units/l	35.4 - 57.1	18 - 97
Gamma glutamyl, units/l	8.6 - 14.3	8 - 28
Serum glutamic oxaloacetic transaminase, units/l	80.4 - 137.0	9 - 67
Lactate dehydrogenase, units/l	899.0 - 1404.4	357 - 756

[1]Ranges reported by Galyean and Hallford (1983).
[2]Reference ranges according to Vet-Path, Teterboro, New Jersey.

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AGRI-PRACTICE — TOXICOLOGY/NUTRITION

Six fine-wool wether lambs received ground alfalfa hay and six were fed ground kochia twice daily during a 69-day trial. Digestibility of the two diets was determined twice during the treatment period; serum chemistry profiles (including insulin) were examined regularly. Kidney and liver samples were evaluated from two kochia-fed lambs killed at the end of the 69-day trial and two that were killed after 45 days of post-trial alfalfa feeding. Nutrient digestibility, feed intake, animal weight, serum chemistry and pathology data are discussed in relation to use of kochia as a forage for ruminants.

VOL. 9, NO. 3, MAY/JUNE 1988

Feedlot Characteristics, Serum Constituents and Histopathology of Lambs Fed Kochia

Submitted for publication January 30, 1987.

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Introduction

Kochia scoparia (L) Schrad, which is also called summer cypress, burning bush, fireweed, kochia, fireball, belvedere and Mexican fireweed, belongs to the family Chenopodiacea. Kochia can be grazed in its immature state or it may be ensiled before going to seed. Some ranchers bale kochia for use as winter feed. Forage from kochia has comparatively high nutritive value, particularly in the early vegetative stages and has a crude protein content and digestibility value comparable to alfalfa hay.[1] Kochia also improved digestibility of energy components when mixed with alfalfa hay,[2] which suggests that it could be an acceptable forage if harvested early. However, in recent years, toxicity in livestock grazing kochia has been reported.[3-7] Few studies have evaluated kochia as a forage for livestock. The objectives of this study were to examine the nutritive value of kochia as indicated by performance of young lambs and to determine if toxicity would result from feeding kochia under controlled conditions.

Materials and Methods

Twelve uniform fine-wool wether lambs approximately 70 days of age were transferred to individual feeding pens (1.5 x 3.0 meter) inside the Nutrition Physiology Research Complex at New Mexico State University. A light-dark cycle of 14 and 10 hours, respectively, was maintained throughout the trial. All lambs had similar access to fresh water, salt and mineral; each received 500,000 IU Vitamin A and 75,000 IU Vitamin-D_3 IM on the day before the trial began.

Lambs were randomly assigned to one of two treatments. Six lambs (average weight = 31.0 kg [68.2 lb]) received ground alfalfa; six lambs

Continued

TABLE 1
Chemical Analysis of Diets Fed to Lambs

Composition*	Percentage in Diet	
	Alfalfa	Kochia
Dry matter	93.8	93.6
Ash	9.9	10.0
Fiber, neutral detergent	42.1	53.8
Fiber, acid detergent	33.2	28.5
Lignin, acid detergent	7.2	4.9
Protein, crude	17.5	5.9

* Dry matter basis.

Journal Article 1290 of the New Mexico Agricultural Experiment Station.

TABLE 2
Nutrient Digestibilities (%) Determined at Two Time Periods in Lambs Fed Alfalfa or Kochia Diets

Item	Time after Initiating Feeding: 3 Weeks Alfalfa (n = 3)	3 Weeks Kochia (n = 3)	3 Weeks Standard Error	6 Weeks Alfalfa (n = 3)	6 Weeks Kochia (n = 3)	6 Weeks Standard Error
Dry matter	62.6[a]	54.6[b]	0.9	67.1[a]	61.0[b]	0.9
Organic matter	64.4[a]	55.9[b]	0.8	68.4[a]	61.4[b]	0.8
Crude protein	76.4[a]	42.5[b]	0.2	80.1[a]	62.9[b]	1.6
Acid detergent fiber	45.0[a]	24.5[b]	2.2	48.6[a]	33.2[b]	1.8
Neutral detergent fiber[c]	44.6	43.4	1.6	48.8	50.1	1.0

[a,b] Row means within time with different superscripts differ ($P < 0.01$).
[c] Row means within time do not differ ($P < 0.40$).

(average weight = 30.8 kg [67.7 lb]) received ground kochia (late bloom stage). Feed was offered twice daily at 6 a.m. and 6 p.m. Animals were individually fed in this manner throughout the 69-day period and daily feed consumption was recorded. Animal weights were monitored at 2-week intervals.

Fourteen days after initiating treatment, three lambs from each group were used to determine digestibilities of the two diets. Lambs were placed in crates, and after a 7-day adjustment period, feces were collected for 7 days. Lambs were subsequently removed from crates and maintained on their respective diets for 28 days, at which time a second digestibility trial was conducted.

During the collection periods, animals were fed 90% of their *ad libitum* intake. All orts were weighed and sampled for analysis.[8] Feces were collected daily, weighed and sampled (10%) for analysis. Samples of feed and feces were oven-dried for 3 days at 55°C, ground through a 2-mm screen in a Wiley mill and analyzed for dry matter, ash, crude protein, neutral detergent fiber, acid detergent fiber and acid detergent lignin.[8] Chemical analysis of diets is shown in Table 1.

At 1 and 69 days after initiating treatment, lambs were subjected to an intensive sampling period. After a 12-hour fast, feed was then offered to the lambs. After 1 hour, all feed was again removed, and animals were fasted during the ensuing 6 hours.

Blood samples were collected via jugular venipuncture one-half hour before feeding and then each hour for 6 hours after feeding. Blood was allowed to clot at room temperature for 30 minutes and serum harvested by centrifugation at 2,300 x g at 4°C. Serum was then stored at -20°C until analysis could be accomplished. Serum insulin was quantified in all samples by double antibody radioimmunoassay.[9] Twenty-four additional serum constituents representing a clinical chemistry profile were determined in samples obtained 1, 4 and 8 weeks after treatment began.

Animal weights, digestibility and serum constituents were evaluated by analysis of variance for completely randomized designs.[10] Insulin values were subjected to split-plot analysis of variance for repeated measurements on animals as described by Gill and Hafs.[11]

TABLE 3
Weekly Feed Intake (kg) of Lambs Fed Alfalfa or Kochia Diets

Number of weeks after beginning treatment	Diet: Alfalfa (n = 6)	Diet: Kochia (n = 6)	Standard Error
1	7.5	3.5	0.6
2	9.1	4.8	0.7
3	7.4	3.2	0.7
4	6.9	3.2	0.6
5	6.8	3.4	0.6
6	7.0	2.7	0.3
7	6.9	3.3	0.2
8	6.9	4.3	0.2
9	6.9	3.9	0.4
10	5.9	3.7	0.3
Overall Mean[c]	7.2[a]	3.6[b]	0.3

[a,b] Row means within time with different superscripts differ ($P < 0.01$).
[c] Diet-x-time interactions were not detected ($P > 0.15$). Therefore, only differences between overall means were tested.

Results and Discussion

NUTRIENT DIGESTIBILITY

Both alfalfa and kochia diets were similar in percentage dry matter and ash (Table 1). Kochia was slightly higher in neutral detergent fiber (NDF); however, alfalfa was higher in acid detergent fiber (ADF) and acid detergent lignin (ADL). Crude protein (CP) was almost 3 times high-

Continued

TABLE 4
Body Weights (kg) Obtained at Two-Week Intervals During the Ten-Week Trial[a]

Number of Weeks after beginning Treatment	Diet		Standard Error
	Alfalfa (n = 6)	Kochia (n = 6)	
0[b]	31.0	30.8	2.2
2	32.2[c]	27.1[d]	2.5
4	31.3[e]	23.4[f]	2.3
6	31.6[e]	24.1[f]	3.7
8	32.3[e]	23.9[f]	3.4
10	31.3[e]	23.0[f]	2.9

[a] Split plot analysis of variance revealed a diet x time interaction ($P < 0.01$). Diet means were, therefore, examined within time.
[b] Row means within time do not differ ($P > 0.20$).
[c,d] Row means within time with different superscripts differ ($P < 0.05$).
[e,f] Row means within time with different superscripts differ ($P < 0.01$).

er in alfalfa than in kochia. Fiber and lignin values are similar to those reported by Kiesling and others.[7] However, these authors reported crude protein values as high as 20.5%, which is much higher than values shown in Table 1.

Nutrient digestibilities determined at 3 and 6 weeks after treatment began are presented in Table 2. Dry matter (DM), organic matter (OM), CP and ADF digestibilities were higher ($P < 0.01$) for alfalfa-fed than kochia-fed lambs during the initial trial and 6 weeks after diet consumption. However, digestibility of DM, OM, CP and ADF increased after 6 weeks of treatment in kochia-fed lambs, which indicated that lambs may adapt to kochia. Neutral detergent fiber did not differ ($P > 0.40$) among diets during either digestion trial.

FEED INTAKE AND LAMB WEIGHTS

Feed intake was higher ($P < 0.01$) in alfalfa-fed lambs (Table 3). Average weekly intake was 7.2 and 3.6 kg (15.8 and 7.9 lb) of alfalfa and kochia, respectively, over the 10-week period. Lower nutrient digestibility and the decreased protein content of kochia could account for low feed intake.

Lamb weights did not differ ($P > 0.20$) between diets before treatment (Table 4). Two weeks after initiating the trial, lambs fed kochia weighed less ($P < 0.05$) than lambs fed alfalfa. This difference remained apparent throughout the trial. Lambs fed kochia appeared to maintain body weight by 4 weeks into the trial. Initial weight loss in kochia-fed lambs may be due to low feed intake and CP content of kochia.

SERUM CONSTITUENTS

Jugular blood samples were collected from five lambs in each treatment group at 1, 4 and 8 weeks after trial initiation. Each sample was analyzed for 24 serum constituents routinely monitored by automated chemistry analyzers. Table 5 presents means for each constituent that differed between the treatment groups. Serum constituents were used to provide insight into metabolic effects of kochia feeding. Serum urea/nitrogen/creatinine ratio was lower ($P < 0.05$) in kochia-fed lambs throughout the trial, reflecting a low plane of protein nutrition. Serum cholesterol and aspartate transaminase (SAST) were higher ($P < 0.05$) at 1 and 4 weeks after diet consumption in kochia-fed lambs; however, by 8 weeks, no treatment differences ($P > 0.10$) were evident. Four weeks after treatment began, carbon dioxide (CO_2) and total bilirubin were slightly higher ($P < 0.05$) in lambs fed kochia; but after 8 weeks of diet consumption, no treat-

Continued

TABLE 5
Serum Constituents that Differed Between Lambs Fed Kochia and Those Fed Alfalfa

Item	Treatment		Standard Error
	Alfalfa (n = 5)	Kochia (n = 5)	
One week after initiation of trial			
SUN/creatinine, mg/dl	24.0[c]	7.0[d]	1.20
Cholesterol, mg/dl	32.6[c]	54.8[d]	4.10
Serum aspartate transaminase, U/L	81.0[c]	108.6[d]	3.60
Four weeks after intiation of trial			
SUN/creatinine, mg/dl	21.2[c]	12.6[d]	1.20
Cholesterol, mg/dl	51.4[a]	74.2[b]	6.60
Serum aspartate transaminase, U/L	97.6[a]	156.0[b]	20.40
Total carbon dioxide, meq/L	29.4[a]	32.0[b]	0.70
Gamma glutamyl transpeptidase, (GGT), U/L	68.6[c]	158.4[d]	13.90
Total bilirubin, mg/dl	0.1[a]	0.3[b]	0.04
Eight weeks after initiation of trial			
SUN/creatinine, mg/dl	24.6[a]	14.0[b]	2.30
GGT, U/L	81.2[a]	197.2[b]	30.40
Cl, meq/L	110.4[a]	104.6[b]	1.60
Albumin, g/dl	3.9[a]	3.5[b]	0.10
Globulin, g/dl	2.6[a]	3.4[b]	0.20
Alb/glob	1.5[c]	1.1[d]	0.70
Lactate dehydrogenase, U/L	334.2[a]	635.0[b]	77.30

[a,b] Row means with different superscripts differ ($P < 0.05$).
[c,d] Row means with different superscripts differ ($P < 0.01$).

ment differences ($P > 0.20$) were noted. Gamma glutamyl transpeptidase (GGT) was higher ($P < 0.05$) in lambs fed kochia 4 and 8 weeks post-treatment. After 8 weeks of diet consumption, serum chloride, albumin and albumin/globulin ratio were lower ($P < 0.05$) in kochia-fed lambs, while globulin and lactate dehydrogenase (LDH) were higher ($P < 0.05$) than in controls. These trends reflect changing phases of mild toxicosis (see Pathology, below) in lambs fed kochia herbage, even though some constituents reflect changes related mainly to protein and energy deficiency.

SERUM INSULIN

Jugular blood samples were collected from six lambs in each treatment group at 0 and 10 weeks after initiating feeding. Samples were analyzed for serum insulin; the means are reported in Table 6. Since time-x-diet interactions were not detected ($P > 0.10$) within weeks, only differences in overall means were tested. No differences ($P > 0.10$) in serum insulin were detected between treatment groups before initiating feeding. However, by 10 weeks after diet consumption, serum insulin was greatly reduced ($P < 0.01$) in kochia-fed lambs. This reduction in serum insulin may be partially attributed to low feed intake throughout the trial and/or the low protein content of the kochia.

PATHOLOGY

Two lambs were killed after being fed kochia for 70 days. Liver and kidneys were removed and sent to a veterinary laboratory for pathological examination. Two additional lambs that were fed kochia throughout the trial were placed on alfalfa for an additional 45 days, at which time they were killed. Liver and kidneys were removed and sent for pathological examination.

In both lambs fed kochia for 70 days, a mild degenerative hepatopathy was present. In addition, mild nonsuppurative inflammation of the bile duct was evident. Crystalline structures that were found in the liver of both lambs resembled oxalate crystals. Vacuolar changes were observed in the kidney, which is suggestive of a mild nephrotoxicity. Recent results from New Mexico State University[12] demonstrated with rats that toxicity of kochia herbage may be correlated with plant content of substances reactive to Dragendorff's reagent (presumably alkaloids) and poorly related to oxalate content.

When lambs were fed alfalfa for an additional 45 days, mild liver lesions were found in both lambs. However, these lesions were not severe enough to have caused clinical abnormalities. Microscopic examination revealed no significant lesions in the kidney. This finding suggests that although feeding a protein-deficient kochia to lambs may cause some pathological effects, these effects were not permanent and may be reversed by feeding alfalfa.

TABLE 6
Serum Insulin (pg/ml) in Lambs Fed Diets Containing Ground Alfalfa or Ground Kochia[a]

Time after feeding (hours)	Weeks after initiating feeding: 0[b] Alfalfa	0[b] Kochia	0[b] Standard Error	10[b] Alfalfa	10[b] Kochia	10[b] Standard Error
-0.5	479	311	63	223	120	45
1.0	418	403	136	456	121	70
2.0	254	345	54	456	64	85
3.0	467	299	80	350	51	39
4.0	332	292	79	214	99	62
5.0	266	418	71	220	32	46
6.0	347	414	105	289	74	51
Overall	366	355	148	316[c]	80[d]	63

[a] Time x diet interactions were not detected ($P > 0.10$) within weeks. Therefore, only differences between overall means were tested.
[b] Six samples per time mean, 42 samples per overall mean.
[c,d] Row means differ ($P < 0.01$).

ACKNOWLEDGEMENTS

The authors wish to thank M. Metzler, G. Armas, D. Sanson, M. Anderson, J. Blackwell, S. O'Neill and P. Ross for their assistance and Dr. J.P. Thilsted, Veterinary Pathologist in the New Mexico Department of Agriculture for laboratory and histopathological evaluations.

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Proceedings, Western Section, American Society of Animal Science

Vol. 37, 1986

TOXICITY OF KOCHIA HERBAGE RELATED TO ALKALOIDS CONTENT: RAT STUDIES[1]

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Summary

Kochia herbage that poisoned cattle and sheep was fed to young, adult female rats as 0, 12.5, 25 or 50% of diet, replacing commercial rat feed for 7 wk, to characterize kochia toxicosis in rats. Growth depression (78% of controls) and increased (P<.05) serum bilirubin, cholesterol, triglycerides and serum enzymes at 6 wk in rats fed higher levels of kochia resembled some aspects of mild toxicosis in cattle and sheep. Juice from lush kochia plants testing high in alkaloids with Dragendorff's reagent was more toxic than juice testing low when dosed by gavage in fasted, immature rats. Dried herbage from plants testing low and high in alkaloids was fed to immature, male albino rats (6 per diet) as 0, 12.5 or 25% of diet, replacing commercial rat feed for 9 wk. Body weight was depressed only 5% below controls by kochia, but serum enzyme (AST, ALT, CPK, LDH and ALK-P) activities were elevated 1.5- to 3-fold (P<.05), roughly in proportion to kochia alkaloids in diets. Rats fed kochia recovered from ether anesthesia quicker than controls, indicating induced mixed-function oxidase activity. Rats fed kochia for 9 wk reverted to normal when fed commercial rat feed. Results show rats might serve to model kochia toxicosis, and toxicity of lush kochia herbage parallels plant content of alkaloids.

Introduction

Kochia scoparia L. Schrad (kochia, fireweed) is a valuable forage plant on arid and semiarid land (Erickson and Moxon, 1947). It has potential as a valuable agronomic crop in dryland agriculture (Finley and Sherrod, 1971; Sherrod, 1971; 1973), but acceptance is constrained by occasional poisoning of livestock grazing kochia (Sprowls, 1981). Kochia toxicosis in livestock has been characterized (Galitzer and Oehme, 1978; Dickie and Berryman, 1979; Dickie and James, 1983), and oxalate has been identified as a primary toxicant in drouth-stricken, mature or overgrazed kochia. Other toxicants have been implicated, especially in toxicity of lush forage (Kiesling et al., 1984). Identification of toxicant(s) and better characterization of toxicosis is necessary for agronomic development of kochia as a forage crop. Use of laboratory animals to model kochia toxicosis in livestock seems desirable. This study attempted to characterize kochia toxicosis in albino rats, and related changes in rat serum constituents during mild chronic toxicosis to levels of alkaloids in lush kochia herbage.

Methods and Materials

Trial 1 - Characterization of Kochia Toxicosis in Rats

Herbage was collected as hay from mature kochia on agronomic plots that poisoned cattle grazing lush forage in 1983 at Tucumcari, NM (Kiesling et al., 1984). This hay elicited less severe toxicosis when fed as total diet to penned sheep (H. E. Kiesling, unpublished data). It was ground to pass a 2-mm screen and mixed with ground commercial rat feed[5] (PCRC 5002) as 0, 12.5, 25 and 50% of mixtures. These mixtures were fed as total diet to 24 female albino (Sprague-Dawley) rats (body wt 232 ± 20 g) allocated randomly (6 per group) to the four diets. They were caged in wire-bottom metal cages with feed provided ad libitum in stainless steel feeders and water ad libitum in glass bottles fitted with ball-and-tube fountains. Feed intake and body weight were measured weekly through 5 wk. At week 6, all six rats from each treatment were anesthetized with diethyl ether and bled by puncture of infraorbital vasculature, using heparinized glass capillary tubes (2 mm x 30 mm), into sterile serum separator tubes. Clotted blood was centrifuged (2,500 x g) and serum was analyzed for 22 constituents [SMAC-22 profile: Na, K, Cl, CO_2, Ca, inorganic P, serum Fe, glucose, blood urea nitrogen (BUN), uric acid, total bilirubin, creatinine, cholesterol, triglycerides, serum enzyme activities (aspartate aminotransferase, AST; alanine aminotransferase, ALT; creatine phosphokinase, CPK; lactic dehydrogenase, LDH; and alkaline phosphatase, ALK-P), total protein, albumin, globulin and albumin/globulin]. These were determined by experienced technicians of the NMSU-Primate Research Institute, using automated analyzers. At week 7, all rats were ether anesthetized, exsanguinated and autopsied. Livers, kidneys, hearts and intestines were collected into formalin, fixed, processed and examined microscopically by a veterinary pathologist.

Trial 2 - Acute Toxicity of Kochia Press Juice

Kochia was grown on irrigated plots at the NMSU Agricultural Science Center (Clovis, NM) in 1984, and tested semi-quantitatively for level of alkaloids in juice from leaves and upper stems of pre-bloom plants, using Dragendorff's reagent (Burns, 1964). Herbage from these plants was composited into samples identified as low or high in reactive substances, presumably alkaloids. They were stored frozen until thawed and squeezed to yield juice for toxicity testing. Four healthy, young, adult female rats were maintained on commercial rat feed and fasted overnight in preparation for dosing. Kochia press juice, either low or high in alkaloids, was administered by gavage to two rats per sample. Each rat

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[1] Scientific paper no. 264, New Mexico Agr. Exp. Sta., Las Cruces, NM. Supported by New Mexico Water Resources Research Institute. Data are from the M.S. Thesis of Mary K. Erickson.

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[5] Purina Certified Rodent Chow 5002, Ralston-Purina Co., Inc., St. Louis, MO.

first received 10 ml; after 2 h another 5 ml was given; and 2 h later another 5 ml was administered, totalling 20 ml per rat in 4 h. One rat died. Survivors were provided commercial rat feed ad libitum and observed for 5 d. On day 6, they were dosed with 10 ml (non-fasted) and subsequently dosed with 8 ml daily (non-fasted) for 7 d. On day 10 after the initial dose, they were decapitated and examined grossly. Organs were fixed, sectioned and examined microscopically by a veterinary pathologist.

Trial 3 - Rat Feeding Trial with Low- and High-Alkaloid Kochia

Samples of frozen kochia herbage identified as low or high in level of alkaloids (as described above) were dried, ground to pass a 2-mm screen and mixed with commercial rat feed (PCRC 5002) as 0, 12.5 and 25% of mixtures. Weanling male albino rats of the Sprague Dawley strain were purchased (Charles River, Inc., Wilmington, MA), caged in wire-bottom metal cages and fed the mixtures ad libitum as total diet in glass feeders with water in glass bottles with ball-and-tube fountains. Thirty healthy rats (183 ± 9 g) were assigned randomly, six rats per diet, to each of the five mixtures as total diet for 9 wk. Feed intake and body weights were monitored. At week 3 and 9, blood was collected and processed as described above and sera were analyzed for 22 constituents described above. At week 9, three rats from each group were ether anesthetized, exsanguinated and autopsied. Livers, kidneys and hearts were fixed, sectioned and examined microscopically.

To monitor any residual effects of high-alkaloid kochia, remaining rats were fed PCRC 5002 as total diet for 6 wk, then bled, sacrificed and examined. Rats that had received low-alkaloid kochia (as 12.5 or 25% of diet) were given low-alkaloid kochia as 25% of diet for 6 wk, then bled, sacrificed and examined.

Blood sera were analyzed for serum constituents as described under trial 2, above. Data by weeks were analyzed for variance, using a program for 2 x 3 factorial design and tests of means for main effects (diets and levels) and interaction. Within levels, contrasts were determined for linear and quadratic effects.

Results and Discussion

Trial 1. Kochia that had poisoned cattle and elicited clinical toxicosis in sheep depressed ($P<.05$) body wt of female rats (to 78% of wt for controls) when fed as 50% of diet for 5 wk (table 1). High levels of kochia caused moist, soft fecal pellets more suggestive of undigested fiber than diarrhea. Serum constituents that differed ($P<.05$) at 6 wk (table 1) included depressed Na, creatinine, and globulin, and increased BUN (1.8-fold), bilirubin (1.3-fold), cholesterol (1.5-fold), triglycerides (1.5-fold), albumin/globulin (1.2-fold), and serum enzymes: AST (1.15-fold), ALT (1.5-fold) and ALK-P (2.5-fold), as well as increased ($P<.10$) Ca. Sheep fed this same hay as total diet had decreased ($P<.05$) BUN/creatinine at 2, 4 and 8 wk; increased ($P<.05$) cholesterol and AST at 2 and 4 wk; increased ($P<.05$) bilirubin and CO_2 at 4 wk; and increased ($P<.05$) LDH and gamma-glutamyl transpeptidase (GGT) at 8 wk, when compared with controls fed alfalfa hay (H. E. Kiesling, unpublished data). Cattle poisoned while grazing green forage had increased serum Ca, cholesterol, total protein, GGT, LDH and AST (Kiesling et al., 1984). Photosensitization and extremely high levels of indirect (unconjugated) bilirubin characterized the main lesions in cattle, although histopathology revealed nephrosis in some steers with extreme toxicosis.

Although the mild toxicosis elicited by kochia in rats of this trial caused no increase in the incidence of histopathology in livers, kidneys, hearts or intestinal tracts, results suggest kochia toxicosis in rats resembles kochia toxicosis in livestock species.

Trial 2. Rats given juice from low-alkaloid kochia by gavage showed moderate distress and mild diarrhea for 1 d, but rats given juice from high-alkaloid kochia had extreme distress and severe diarrhea, and one rat died 6 h after the initial dose. Pulmonary edema was the main abnormality seen at autopsy of that rat. In surviving rats, pilo-erection, humped stance, mild lethargy and diarrhea were evident for 6 d after the initial dose, and these symptoms were more pronounced in the one survivor given juice from high-alkaloid kochia. Symptoms abated by day 10 after initial dosing when survivors were sacrificed. No gross or microscopic lesions where found.

Trial 3. Rats fed low or high-alkaloid kochia as 12.5 or 25% of diet, increased water consumption 1.3-fold over controls, and excreted feces notably more moist than those of controls. Rough hair coat was evident by week 2 and continued through week 9. Body weights (g) at week 9 were: controls, 355; 12.5% low-alkaloid kochia, 331; 25% low-alkaloid kochia, 337; 12.5% high-alkaloid kochia, 252; and 25 high-alkaloid kochia, 341 ($P = .13$ for linear effect of levels), reflecting only 4 to 5% reduction in bod weight. Feed intakes were increased about 7% over controls for rats fed 12.5% kochia, and increased about 15% over controls for rats fed 25% kochia.

Table 2 shows serum constituents affected by diets at week 3 and 9. Probability values are shown for main effects and interactions (diets x levels). Significant interactions occurred in only two cases (CPK and LDH in week 9). At week 3, chloride (Cl) was marginally affected, uric acid was decreased (bu the control value, 2.50, is questionable), BUN was increased slightly and enzyme activities (AST and CPK) were elevated. By week 9, numerous changes wer evident, most notable of which are decreased chloride, Ca, glucose, triglycerides, total protein, albumin and globulin, and increased inorganic phosphorus, uric acid, cholesterol, serum enzymes (AST, ALT, CPK, LDH and ALK-P) and albumin/globulin. In general, severity of changes in serum constituent tended to parallel the level of kochia alkaloids in the diet, with some exceptions. Serum enzyme activities, which tend to reflect tissue damage when level are increased markedly, consistently paralleled kochia alkaloid levels in diets, reflecting increase over controls as follows: AST, 1.7-fold; ALT, 1.3-fold; CPK, 2.7-fold; LDH, 3.6-fold; and ALK-P, 1.4-fold. These changes are especially notable because histopathology of organs from these rats was unremarkable.

Carry-over Effects. Rats fed low-alkaloid kochia as 12.5 or 25% of diet for 9 wk, then fed low alkaloid kochia as 25% of diet for 6 wk, weighed about 7% less than controls fed PCRC 5002 as total diet throughout. Rats fed high-alkaloid kochia for

wk, then fed PCRC 5002 as total diet, had body weight equal to controls. Rats that continued on low-alkaloid kochia as 25% of diet through weeks 10 to 15 had serum constituents (K, inorganic phosphate, uric acid, BUN, ALK-P, globulin and albumin/globulin) that differed (P<.05) from controls (table 3), but rats fed PCRC 5002 as sole diet during weeks 10 to 15, after having received high-alkaloid kochia for weeks 1 to 9, had serum values that did not differ from controls at week 15. Thus, they seemed to recover rapidly and completely from mild kochia toxicosis that was evident at week 9.

Discussion

There seem to be few, if any, reports in the literature describing kochia toxicosis in rats. The reports describing toxicosis in livestock provide widely varying clinical characterizations, caused (it seems) largely by varied maturity of kochia ingested and varied stages in the progression of toxicosis. This study shows rather clearly that rats are susceptible to toxic kochia herbage, and that mild toxicosis bears clinical resemblance to the early stages of toxicosis in cattle and sheep. Furthermore, the severity of early, mild toxicosis in rats fed herbage from lush kochia tended to parallel rather closely the level of substances reactive to Dragendorff's reagent, presumably alkaloids. Further work is needed to identify these substances and to characterize animal toxicosis from these (isolated) substances.

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TABLE 1. SERUM CONSTITUENTS THAT DIFFERED IN YOUNG ADULT FEMALE ALBINO RATS FED DRIED KOCHIA HERBAGE AS 0, 12.5, 25 OR 50 PERCENT OF DIET, REPLACING COMMERCIAL RAT FEED, THROUGH SIX WEEKS

Item	Kochia levels				SELSM[d]	Probability		
	0%	12.5%	25%	50%		Levels	Linear	Quadr.
Body wt, g	252[a]	244[a]	250[a]	201[b]	9	.001	.001	.064
Na, mEq/liter	141[a]	140[a]	140[a]	148[b]	1.8	.018	.007	.065
Ca, mg/dl	10.2	10.2	10.8[h]	10.8[h]	.24	.154	.067	.49
BUN[e], mg/dl	17.7[a]	20.7[a]	21.8[a]	31.0[b]	1.7	.001	.001	.32
T. bilirubin, mg/dl	.217[ab]	.200[a]	.300[c]	.283[bc]	.025	.027	.023	.40
Creatinine, mg/dl	.783[a]	.733[ab]	.700[ab]	.650[b]	.034	.069	.010	.64
Cholesterol, mg/dl	62[a]	77[b]	78[b]	92[c]	4.2	.001	.001	.46
Triglycerides, mg/dl	43[a]	45[ab]	42[a]	66[b]	7.6	.112	.037	.24
ALT[f], U/liter	28[a]	26[a]	35[ab]	42[b]	2.6	.001	.001	.48
ALK-P[g], U/liter	47[a]	52[a]	65[a]	117[b]	11	.001	.001	.22
Globulin, g/dl	2.23	2.08	2.32[h]	1.93[h]	.13	.21	.18	.35
Alb/Glob	1.52[a]	1.68[ab]	1.62[a]	1.85[b]	.07	.021	.005	.82

[abc] Means in the same row with different superscripts differ (P<.05).
[d] Standard error of least squares means, based on six rats per group.
[e] Blood urea nitrogen.
[f] Alanine aminotransferase (serum glutamic-pyruvic transaminase, SGPT).
[g] Alkaline phosphatase.
[h] Values differ from each other, or from controls (0% kochia, P<.10).

TABLE 2. SERUM CONSTITUENTS THAT DIFFERED IN YOUNG ADULT MALE ALBINO RATS FED DRIED KOCHIA HERBAGE, EITHER LOW OR HIGH IN ALKALOIDS, AS 0, 12.5 OR 25 PERCENT OF DIET THROUGH NINE WEEKS

Item	Group means					SELSM[d]	Probability		
Diet (D)	Control	Low alk. kochia		High alk. kochia			Main effect		
Level (L)	0%	12.5%	25%	12.5%	25%		Diet	Level	D x L
Third week (n = 3 per group)									
Cl, mEq/liter	101.0[a]	101.0[a]	99.3[b]	102.3[a]	100.7[a]	.47	.04	.01[f]	.30
Uric acid, mg/dl	2.50[a]	.90[ab]	.73[b]	1.07[ab]	.77[b]	.53	.88	.01[e]	.18
BUN, mg/dl	15.7[a]	18.3[ab]	19.7[b]	20.0[b]	16.3[ab]	1.31	.61	.05[f]	.19
AST, U/liter	60.3[a]	75.0[ab]	63.7[ab]	86.3[b]	68.7[ab]	7.8	.41	.02[f]	.77
CPK, U/liter	187[a]	317[ab]	211[ab]	366[b]	299[ab]	60	.37	.04[f]	.77
Ninth week (n = 6 per group)									
Cl, mEq/liter	103.7[a]	100.3[b]	102.2[ab]	101.2[b]	102.2[ab]	.84	.69	.01[f]	.85
Ca, mg/dl	10.15[a]	10.12[a]	9.63[b]	9.75[b]	9.90[a]	.14	.78	.04[e]	.10
Inorg. P, mg/dl	6.77[a]	7.60[b]	7.43[ab]	7.20[ab]	7.55[b]	.26	.66	.02[e]	.59
Uric acid, mg/dl	.65[a]	.88[ab]	.88[ab]	1.05[b]	1.15[b]	.13	.19	.02[e]	.59
Glucose, mg/dl	164[a]	177[a]	179[a]	153[ab]	136[b]	11	.02	.73	.15
Chol., mg/dl	80[a]	84[ab]	84[ab]	87[ab]	92[b]	3.5	.20	.03[e]	.80
Trigly., mg/dl	73[a]	60[a]	41[b]	71[a]	63[a]	6.9	.06	.01[e]	.28
AST, U/liter	58[a]	70[ab]	76[b]	85[b]	96[c]	4.8	.01	.01[e]	.12
ALT, U/liter	46[a]	48[a]	56[ab]	58[b]	62[b]	3.8	.10	.01[e]	.46
CPK, U/liter	112[a]	150[a]	166[a]	242[b]	303[c]	20	.01	.01[e]	.01
LDH, U/liter	175[a]	277[ab]	337[b]	496[c]	627[c]	49	.01	.01[e]	.01
ALK-P, U/liter	145[a]	146[a]	171[ab]	165[ab]	196[b]	14	.20	.01[e]	.65
Tot. Prot., g/dl	6.12[a]	5.50[b]	5.45[b]	5.62[ab]	5.68[ab]	.18	.43	.01[e]	.81
Albumin, g/dl	3.48[a]	3.37[ab]	3.25[b]	3.38[ab]	3.43[a]	.05	.14	.03[e]	.19
Globulin, g/dl	2.63[a]	2.13[b]	2.20[ab]	2.23[ab]	2.25[ab]	.15	.69	.01[e]	.95
Alb/Glob	1.37[a]	1.58[b]	1.48[ab]	1.52[ab]	1.53[ab]	.07	.92	.04[e]	.72

[abc]Means in the same row with different superscripts differ (P<.05).
[d]Standard error of least squares means.
[e]Main effect of levels is significant; linear contrast is significant.
[f]Main effect of levels is significant; quadratic contrast is significant.

TABLE 3. SERUM CONSTITUENTS THAT DIFFERED IN YOUNG ADULT MALE ALBINO RATS FED DRIED KOCHIA HERBAGE, EITHER LOW OR HIGH IN ALKALOIDS, AS 0, 12.5 OR 25 PERCENT THROUGH NINE WEEKS AND THEN FED DIETS SHOWN FROM WEEK TEN THROUGH FIFTEEN

Item	Group mean					SELSM[d]	Probability		
Diet	Control	Low kochia		High kochia					
Level	0%	12.5%	25%	12.5%	25%		Diet	Level	D x L
Diet, weeks 10 to 15:	Control	25% Kochia		Control					
Serum Values, 15th week:									
K, mEq/liter	4.60[a]	5.20[b]	4.60[a]	4.60[a]	4.87[ab]	.14	.34	.13[f]	.02
iP, mg/dl	5.03[ab]	4.83[ab]	5.60[a]	4.47[b]	4.90[ab]	.26	.11	.05[f]	.43
Uric acid, mg/dl	1.43[a]	.87[b]	1.10[ab]	1.20[ab]	1.13[ab]	.17	.40	.09[e]	.58
BUN, mg/dl	21.7[ab]	23.0[a]	20.0[b]	23.0[a]	21.0[ab]	.87	.63	.03[f]	.79
ALK-P, U/liter	137[a]	205[b]	149[a]	138[a]	158[a]	15	.15	.12	.05
Globulin, g/dl	2.47[ab]	2.20[a]	2.27[a]	2.43[ab]	2.70[b]	.10	.02	.23	.14
Alb/Glob	1.33[a]	1.57[b]	1.50[b]	1.33[a]	1.27[a]	.06	.01	.15	.09

[abc]Means in the same row with different superscripts differ (P<.05); comparisons based on initial diets.
[d]Standard error of least squares means, based on three rats per group.
[e]Main effect of levels is significant; linear contrast is significant.
[f]Main effect of levels is significant; quadratic contrast is significant.

Proceedings, Western Section, American Society of Animal Science

Vol. 38, 1987

TOXICOSIS OF RATS FED KOCHIA HERBAGE AND ALLEVIATION BY SUPPLEMENTAL VITAMIN MIXTURE, ZINC, OR PARENTERAL ACETYLCYSTEINE PLUS TRANS-STILBENE OXIDE[1]

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Summary

Three collections of Kochia scoparia (L.) Schrad. herbage grown in east-central New Mexico (Clovis), and differing in content of substances reactive to Dragendorff's reagent (alkaloids), were selected pre-bloom, harvested as whole-plant herbage, dried, ground (2-mm screen) and incorporated into mixtures for rat diets, as 50% of diet dry matter, replacing commercial rat feed. Thus, four diets were prepared [0% kochia, control; 50% low-alkaloid kochia; 50% medium-alkaloid kochia; and 50% high-alkaloid kochia]. All were made isonitrogenous by adding casein as 3 to 3.5% of diet mixtures as needed. Likewise, all diets were made isofibrous in terms of acid detergent fiber by adding alpha-cellulose as 17% of the control diet, replacing commercial rat feed. Immature, male, Sprague-Dawley rats were randomly allocated to these four diets and fed throughout 8 wk. Within each diet, four treatments were imposed in a 4 x 4 factorial design: none, supplemented vitamins (VIT), supplemental zinc (ZN), or parenteral acetylcysteine plus trans-stilbene oxide (CYS + TSO). Vitamin mixture provided 80 mg retinyl palmitate, 80 mg α-tocopherol, 3 g choline chloride, 200 mg myo-inositol and 4 mg folic acid per kg of diet. Zinc sulfate was added to provide 200 mg Zn per kg of diet. N-acetyl-L-cystine (50 mg/kg body wt, in saline) and trans-stilbene oxide (400 mg/kg body wt, in corn oil) were dosed i.p. twice weekly. Each of the diet x treatments combinations was imposed on six rats. Body weights were monitored through 8 wk. Blood was collected at 4 wk and sera were analyzed for 22 components. Body weight gains were depressed 16 to 22% below controls ($P<.05$) by dietary kochia. Serum enzymes (ALK-P, GGTP, AST, ALT), BUN, uric acid, creatinine and globulin were altered ($P<.05$), but not bilirubin ($P>.10$), reflecting early, chronic toxicosis, roughly proportional to alkaloid levels in kochia. Each of the treatments tended to alleviate body wt suppression by kochia diets ($P<.05$) and alleviated most of the blood changes reflecting toxicosis.

Introduction

Kochia scoparia (L.) Schrad. (kochia, fireweed) is a valuable forage plant on arid and semiarid land (Erickson and Moxon, 1947). It has potential as a valuable agronomic crop in dryland agriculture (Finley and Sherrod, 1971; Sherrod, 1971; 1973), but its value is diminished by occasional livestock poisonings (Sprowls, 1981). Kochia toxicosis in livestock has been characterized (Galitzer and Oehme, 1978; Dickie and Berryman, 1979; Dickie and James, 1983), and oxalate has been identified as a primary toxicant in drought-stricken, mature or overgrazed kochia. In recent studies, other toxicants have been implicated, especially in toxicity of lush forage (Kiesling et al., 1984). Smith et al. (1986) implicated substances reactive to Dragendorff's reagent, presumably alkaloids, as related to the toxicity of lush kochia fed to rats. That study also showed rats are susceptible to toxic kochia herbage, and mild toxicosis in rats clinically resembles early stages of toxicosis in cattle and sheep.

Identification of toxicant(s) and a better understanding of toxicosis is necessary for agronomic development of kochia as a forage crop. The present study was conducted to further relate alkaloid content of lush kochia with degree of toxicity and to evaluate prospective treatments that might prevent or alleviate kochia toxicity.

Materials and Methods

Herbage was collected from prebloom kochia grown under controlled conditions at Clovis, NM. Three batches were composited that varied in response to Dragendorff's reagent; high, medium and low. Specimens were frozen as lush, green herbage, dried at 50 C for 48 h in a forced-air oven, and ground to pass a 2-mm screen. Herbage was then mixed with ground (2 mm) commercial rat feed[2] as 0 or 50% of mixture. All diets were made isonitrogenous and isofibrous (ADF) by addition of casein (3 to 3.5%) to diets containing kochia, and α-cellulose (17%) to the control diet.

The four diets, 0% kochia (control), 50% high-alkaloid kochia, 50% medium-alkaloid kochia, and 50% low-alkaloid kochia, were fed for 8 wk in conjunction with four treatments: no treatment, N-acetyl-L-cysteine (CYS, 50 mg/kg body wt, dosed i.p. in saline twice weekly) and trans-stilbene oxide (TSO, 400 mg/kg body wt, dosed i.p. in corn oil twice weekly), vitamins (VIT, 80 mg retinyl palmitate, 80 mg α-tocopherol, 3 g choline chloride, 200 mg m-inositol and 4 mg folic acid per kg of diet) and zinc (ZN, 202 mg of Zn provided as 500 mg zinc sulfate per kg of diet). Diets and treatments were arranged factorially (4 x 4, 6 rats/group).

Male albino rats were purchased from Simonsen Labs (Gilroy, CA), caged in plastic, box-type cages on aspen wood litter, and housed in research facilities at the main campus in Las Cruces. Ninety-six rats were caged, two per cage, and assigned randomly by cage to receive diets with 0 or 50% kochia and either no treatment, CYS + TSO, VIT or ZN. Diets were provided ad libitum in stainless steel, well-type feeders. Water was provided ad libitum in plastic bottles fitted with stainless steel, ball-and-tube fountains fitted in Neoprene stoppers. Feed and water consumption were monitored daily. Body weights of rats were recorded weekly.

[1] Scientific Paper 284 of the New Mexico Agr. Exp. Sta., Las Cruces.

[2] Purina Laboratory Rodent Chow 4001, Ralston-Purina Co., St. Louis, MO.

Four rats from each diet x treatment group were bled at 4 wk by venipuncture of infraorbital vasculature, using heparinized glass capillary tubes (2 x 30 mm). Approximately 3 ml of blood were collected into sterile serum separator tubes, held 30 min at room temperature and centrifuged at 2500 x g for 15 min at 4C. Separated serum was stored frozen until analyzed. Serum constituents were determined in an automated serum analyzer operated by personnel of the Southwest Diagnostic Medical Laboratory (Las Cruces, NM). At 8 wk, half of the rats were sacrificed and examined grossly postmortem. The remaining rats were fed commercial rat feed for an additional 6 wk to monitor any residual effects of kochia.

Data were analyzed as a completely randomized design arranged factorially using GLM procedures of the Statistical Analysis System (SAS, 1982). Where F values were significant (P<.05), means were separated using the least significant difference method.

Results and Discussion

Body weights of rats fed high- and low-alkaloid kochia were depressed 21% below controls (P<.05), and those fed medium-alkaloid kochia were depressed 16% (P<.05) at 8 wk (table 1). Treatment with CYS + TSO and VIT increased body wt by 9 and 8%, respectively (P<.05) (table 2). Rats fed kochia diets increased water consumption 1.6-fold over controls, and feed intakes were the same for all diets. Table 3 shows serum constituents affected by varying kochia at 4 wk. There were no treatment x diet interactions (P>.10); therefore, means were pooled across treatments and, in table 4, pooled across diets. Kochia decreased calcium, glucose, cholesterol, creatinine, globulin and total protein, with the largest depression related to high-alkaloid kochia (P<.05). Blood urea nitrogen, BUN/creatinine and uric acid were increased (P<.05). Enzyme activities (alkaline phosphatase, gamma-glutamyl transpeptidase, aspartate aminotransferase, and alanine amino transferase) were elevated (P<.05). Treatments also affected serum constituents (table 4). Treatment with supplemental VIT or ZN increased sodium, potassium and chloride, and lowered lactic dehydrogenase (P<.05). Treatment with CYS + TSO increased sodium and decreased alkaline phosphatase (P<.05). Other serum constituents did not differ from control values (P>.10).

Treatment with CYS + TSO, VIT or ZN showed beneficial effects for alleviating kochia toxicosis. All treatments increased body wt above controls and treatments were also beneficial in maintaining serum constituents at control values. This study further confirms that rats exhibit toxicosis clinically resembling the early stages of toxicosis in livestock, and severity of serum and weight changes parallels the level of substances reactive to Dragendorff's reagent, presumably alkaloids. Further work is needed to specifically identify the toxicant(s), and to develop treatments and protocols that will prevent or alleviate toxicosis.

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TABLE 1. EFFECTS OF VARYING KOCHIA HERBAGE ON BODY WEIGHTS (GRAMS) OF YOUNG, MALE ALBINO RATS FED FOR EIGHT WEEKS TREATED WITH ACETYL-CYSTEINE + TRANS-STILBENE OXIDE OR VITAMINS OR ZINC.[a]

	Diet				
Week	Control	30% Low Alkaloid	30% Medium Alkaloid	30% High Alkaloid	SE[b]
0[c]	186	190	193	191	2.8
4	306[d]	235[e]	245[e]	239[e]	3.5
8	371[d]	294[efg]	311[eg]	289[ef]	7.0

[a]Analysis of variance revealed no treatment x diet interactions (P>.05); therefore, means were pooled across treatments.

[b]Standard error of the mean, n=24.

[c]Row values do not differ (P>.10).

[defg]Row values with different superscripts differ (P<.05).

TABLE 3. EFFECTS OF VARYING KOCHIA HERBAGE ON BLOOD SERUM CONSTITUENTS OF YOUNG, MALE ALBINO RATS FED FOR FOUR WEEKS AND TREATED WITH ACETYL-CYSTEINE + TRANS-STILBENE OXIDE OR VITAMINS OR ZINC.[a]

	Diet				
Constituent	Control	50% Low Alkaloid	50% Medium Alkaloid	50% High Alkaloid	SE[b]
Sodium, mEq/l	157	159	156	154	1.7
Potassium, mEq/l	7.5	7.4	7.4	7.0	.20
Chloride, mEq/l	109	112	111	109	1.3
Bicarb/CO_2, mEq/l	22.5	21.9	22.3	22.6	1.1
Anion gap, mEq/l	18.3	17.2	17.8	18.1	1.1
Calcium, mg/dl	10.5[c]	10.2[ce]	10.1[de]	9.7[d]	.15
Phosphorus (inorganic), mg/dl	9.4	9.5	8.5	8.7	.49
Iron, mcg/dl	207[c]	213[c]	235[d]	214[c]	6.8
Glucose, mg/dl	127[c]	114[de]	120[ce]	108[d]	2.9
Cholesterol, mg/dl	65[c]	63[c]	54[d]	54[d]	1.7
Triglycerides, mg/dl	148	81	67	112	34
Blood urea nitrogen, mg/dl	25[c]	35[d]	36[d]	33[d]	1.0
Creatinine, mg/dl	41[c]	57[d]	60[d]	61[d]	2.4
BUN/Creatinine, mg/dl	63[c]	61[c]	60[c]	55[d]	.02
Uric acid, mg/dl	.31[c]	.52[d]	.44[cd]	.35[c]	.05
Albumin, g/dl	5.1	5.0	5.0	4.8	.09
Globulin, g/dl	1.6[c]	1.5[cd]	1.4[d]	1.4[d]	.05
Albumin/globulin	3.2	3.4	3.7	3.5	.13
Protein (total), g/dl	6.7[c]	6.5[ce]	6.4[de]	6.2[d]	.12
Bilirubin (total), mg/dl	.18	.15	.15	.17	.02
Alkaline phosphatase, U/liter	281[c]	487[d]	465[d]	504[d]	22
Creatine kinase, U/liter	668	548	558	511	99
α-glutamyl transpeptidase, U/liter	0.0[c]	0.7[d]	0.6[d]	1.1[d]	.22
Lactic dehydrogenase, U/liter	456	584	505	495	49
Aspartate aminotransferase, U/liter	103[c]	129[d]	133[d]	119[d]	5.5
Alanine aminotransferase, U/liter	56[c]	128[e]	112[d]	125[e]	4.7
AST/ALT	1.9[c]	1.0[e]	1.2[d]	1.0[e]	.06

[a]Analysis of variance revealed no treatment x diet interactions (P>.10); therefore, means were pooled across treatments.

[b]Standard error of the mean, n=6.

[cde]Row values with different superscripts differ (P<.05).

TABLE 2. EFFECTS OF TREATMENT WITH ACETYL-CYSTEINE + TRANS-STILBENE OXIDE (CYS + TSO) OR VITAMINS OR ZINC ON BODY WEIGHTS (GRAMS) OF YOUNG, MALE ALBINO RATS FED KOCHIA HERBAGE FOR EIGHT WEEKS.[a]

Week	Treatment				SE[b]
	None	CYS + TSO	Vitamins	Zinc	
0[c]	185	192	192	193	2.8
4	252[d]	251[d]	263[e]	259[de]	3.6
8	302[d]	329[e]	326[ef]	308[df]	7.0

[a]Analysis of variance revealed no treatment x diet interactions (P>.05); therefore, means were pooled across treatments.

[b]Standard error of the mean, n=24.

[c]Row values do not differ (P>.10).

[def]Row values with different superscripts differ (P<.05).

TABLE 4. EFFECTS OF TREATMENT WITH ACETYL-CYSTEINE + TRANS-STILBENE OXIDE OR VITAMINS OR ZINC ON BLOOD SERUM CONSTITUENTS OF YOUNG, MALE ALBINO RATS FED KOCHIA HERBAGE FOR FOUR WEEKS.[a]

Constituent	Treatment				SE[b]
	None	CYS + TSO	Vitamins	Zinc	
Sodium, mEq/l	153[c]	156[ce]	159[de]	159[de]	1.8
Potassium, mEq/l	7.0[c]	6.9[c]	7.3[d]	7.6[d]	.21
Chloride, mEq/l	108[c]	109[cd]	112[de]	112[de]	1.3
Bicarb/CO_2, mEq/l	21.2	23.1	23.3	21.8	1.1
Anion gap, mEq/l	16.7	18.4	19.2	17.1	1.1
Calcium, mg/dl	9.9	10.0	10.3	10.2	.15
Phosphorus (inorganic), mg/dl	8.8	9.3	9.5	8.7	.49
Iron, mcg/dl	230	204	216	219	6.8
Glucose, mg/dl	116	114	116	123	2.9
Cholesterol, mg/dl	56	48	61	60	1.7
Triglycerides, mg/dl	161	57	85	104	34
Blood urea nitrogen, mg/dl	31	33	32	33	1.0
Creatinine, mg/dl	.59	.58	.61	.61	.02
BUN/Creatinine, mg/dl	53	58	53	54	2.4
Uric acid, mg/dl	.44	.36	.43	.39	.05
Albumin, g/dl	4.9	5.0	5.0	5.0	.09
Globulin, g/dl	1.5	1.5	1.5	1.5	.05
Albumin/globulin	3.5	3.4	3.4	3.5	.13
Protein (total), g/dl	6.4	6.5	6.5	6.5	.12
Bilirubin (total), mg/dl	.19[c]	.19[c]	.11[d]	.16[c]	.02
Alkaline phosphatase, U/liter	451[c]	355[d]	462[c]	468[c]	22
Creatine kinase, U/liter	775	489	483	537	99
α-glutamyl transpeptidase, U/liter	4[c]	6[c]	1[d]	3[c]	.22
Lactic dehydrogenase, U/liter	618[c]	490[c]	441[d]	391[d]	49
Aspartate aminotransferase, U/liter	124	126	121	114	5.5
Alanine aminotransferase, U/liter	103	97	107	114	4.7
AST/ALT	1.3[c]	1.4[c]	1.3[c]	1.1[d]	.06

[a]Analysis of variance revealed no treatment x diet interactions (P>.10); therefore, means were pooled across treatments.

[b]Standard error of the mean, n=6.

[cde]Row values with different superscripts differ (P<.05).

NUTRITIONAL AND TOXICOLOGICAL EVALUATIONS OF KOCHIA HERBAGE FED AS HAY TO FINE-WOOL LAMBS[1]

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ABSTRACT

Kochia herbage, which had tested positive to Dragendorff's reagent (presumptive alkaloids) and had elicited chronic toxicosis when fed to rats, was fed to sheep to characterize early stages of kochia toxicosis and evaluate treatments that might improve tolerance. Twelve fine-wool lambs (46 ± 9 kg body wt) were fed chopped kochia hay (35%) mixed with chopped alfalfa hay (65%) for 4 wk. Kochia had 14.3% crude protein (CP) and 39.9% acid detergent fiber (ADF). Dry matter (DM) intake averaged 3.4% of body wt daily and at 4 wk digestibility coefficients were 59% for DM, 72% for CP and 59% for ADF. Body wt did not change during 4 wk and blood serum components were not changed from values at the onset. Thereafter, kochia was increased to 50% of diet for 5 more wk, during which four treatments were imposed randomly (3 lambs/treatment): 1) none; 2) acetylcysteine plus trans-stilbene oxide,

(Key words: Kochia, Toxicity, Sheep)

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50 mg/(kg)$^{.75}$ and 125 mg/(kg)$^{.75}$, respectively, given parenterally twice weekly; 3) retinyl palmitate, 275 mg, plus alpha-tocopherol, 300 mg, per hd given parenterally twice weekly; and 4) zinc sulfate mixed in the feed to provide 500 mg daily. The diet with 50% kochia had 16% CP and 36% ADF. After 5 wk, blood glucose was elevated slightly, total bilirubin was increased about 1.5-fold ($P < .05$), alanine aminotransferase was elevated slightly ($P < .05$), and inorganic phosphorus and urea (BUN) were diminished ($P < .05$); whereas other serum components, including calcium, were unchanged from initial levels ($P > .10$). Treatments had negligible effects for modifying serum signs of chronic toxicosis associated with kochia hay fed as 50% of diet.

Introduction

Kochia scoparia (L.) Schrad. (kochia, fireweed) is a valuable forage plant on arid and semiarid land (Erickson and Moxon, 1967). It was introduced into the United States as an ornamental but is now being considered as a valuable agronomic crop in dryland agriculture (Finley and Sherrod, 1971; Sherrod, 1971, 1973) because it yields digestible energy and protein comparable to alfalfa with about half the water requirement. However, its value is diminished by occasional poisoning of livestock grazing kochia (Sprowls, 1981). Kochia toxicosis in livestock has been characterized (Galitzer and Oehme, 1978; Dickie and Berryman, 1979; Dickie and James, 1983), and oxalate has been identified as a primary toxicant in drought-stricken, mature or overgrazed kochia. Kiesling et al. (1984) implicated other toxicants, especially in toxicity of lush, green forage. Severity of kochia toxicosis in rats was related to content of substances reactive to

Dragendorff's reagent, presumably alkaloids (Smith et al., 1986). In a companion study, rats were fed kochia herbage and toxicosis paralleled alkaloid content. Moreover signs of toxicosis tended to be diminished by certain treatments, namely, N-acetyl-cysteine plus trans-stilbene oxide or supplemental vitamins or supplemental zinc (Rankins et al. 1989, In preparation). Cheeke (1984) also reported beneficial effects with cysteine supplementation in rats with alkaloid toxicosis. The present study attempted to produce kochia toxicosis in lambs and examine the effects of different treatment regimens in alleviating the toxicosis.

Experimental Procedure

Kochia scoparia (L.) Schrad. was harvested pre-bloom (bud stage) as hay from irrigated plots at the Agricultural Science Center in Clovis, NM, in 1986. The kochia hay was chopped to pass a 1.27-cm screen, as was alfalfa hay. The two hays were mixed as 35% kochia and 65% alfalfa (as fed basis) and fed to 12 fine-wool lambs for 4 wk. The 12 lambs (eight wethers and four ruminally cannulated ewes were individually penned and housed inside on concrete floors with wood shavings. All animals were given free access to trace mineralized salt blocks and water and fed ad libitum. Feed intakes were monitored and body wt recorded at wk 0, 2 and 4, and blood samples were taken via jugular venipuncture at onset and at wk 4. Approximately 10 ml of blood was collected into sterile serum separator tubes, held 35 min at room temperature, and centrifuged at 2,300 x g for 15 min at 4°C. Separated serum was frozen until analyzed.

After wk 4, the lambs were fed 50% kochia hay and 50% alfalfa hay

for another 5 wk. Four treatments were imposed randomly on the lambs (one ruminally cannulated ewe was included in each treatment group). Treatments were: 1) None; 2) N-acetyl-cysteine plus trans-stilbene oxide [CYS + TSO: 50 mg acetyl cysteine/(kg body wt)$^{.75}$, dosed i.p. in saline twice weekly and 125 mg TSO/(kg body wt)$^{.75}$, dosed i.p. in corn oil twice weekly]; 3) supplemented vitamin A and E (A + E:300 mg alpha-tocopherol, dosed i.p. twice weekly and 165 mg retinyl palmitate, dosed i.m. twice weekly); and 4) zinc (200 mg zinc/d as 500 mg zinc sulfate in the diet). Body wt were recorded and blood samples collected at wk 4, 6 and 8. All serum analyses were contracted to Southwest Medical Laboratories[1], where serum constituents were determined in an automated serum analyzer. At wk 9, whole blood samples were collected into sterile, heparinized tubes and immediately analyzed for hemoglobin, hematocrit and red and white blood cell counts.

At wk 5 all four rumen-cannulated lambs were dosed with cobalt-ethylene diaminetetracetic acid (EDTA) to determine fluid rate of passage. Lambs were dosed with 169.2 mg of CoEDTA into the rumen and samples were collected 0, 4, 8, 20 and 24 h after dosing. Ruminal samples were strained through four layers of cheesecloth, acidified with 1 ml 7.2 N H_2SO_4/100 ml of ruminal fluid and stored frozen. Ruminal samples were thawed at room temperature and centrifuged at 10,000 x g for 10 min. Supernatant fluid was analyzed for cobalt by atomic absorption spectroscopy (air:acetylene flame).

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At wk 5, six lambs were randomly allotted to a digestibility study for 5 d. Lambs were fitted with collection bags and fed 50% kochia + 50% alfalfa ad libitum. Total dry matter intake and dry matter fecal output were recorded. Feed and fecal samples were composited across days and analyzed for dry matter and nitrogen (AOAC, 1985) and acid detergent fiber (Goering and Van Soest, 1970).

Calculations

Fluid dilution rates were calculated by regressing the natural logarithm of cobalt concentrations on time. Ruminal fluid volume was calculated by dividing marker dose by marker concentration in the rumen extrapolated to 0 h. Fluid flow rate was calculated as ruminal volume multiplied by fluid dilution rate and turnover time was calculated as ruminal volume divided by fluid flow rate.

Statistical Analysis

Analyses of variance were executed using GLM procedures of the Statistical Analysis System (SAS, 1982). Effects of 35% kochia on serum constituents, body wt and feed intakes were analyzed as a completely randomized design. Effects of treatment on serum constituents were analyzed as a split-plot design with treatment as main plot and time as sub-plot. Because of treatment x time interactions, some variables are presented within sampling time. Effects of treatment on body wt and whole blood analysis were analyzed as a completely randomized design. Means were separated by least significant difference (Steel and Torrie, 1980).

Results and Discussion

Fine-wool lambs fed 35% kochia hay and 65% alfalfa hay for 4 wk showed few indications of toxicosis. Body wt and dry matter intake remained constant throughout the 4 wk (Table 1). Table 2 shows serum constituents in lambs fed 35% kochia hay throughout 4 wk. Kochia decreased sodium levels, bicarbonate/CO_2, anion gap and calculated osmolality ($P < .01$). These depressions were statistically significant; however, the biological significance seems slight. Triglyceride levels were decreased 28% and glucose was depressed while BUN was elevated by 14% ($P < .05$). Uric acid was unusually high at wk 0 but this was caused by one lamb having a uric acid level of 5.3 mg/dl. Serum enzymes, calcium and bilirubin were unchanged ($P > .10$).

At wk 4, treatments were imposed on the 12 lambs and the diet was changed to 50% kochia hay and 50% alfalfa hay. Treatments were selected as possible ways to help characterize as well as alleviate alkaloid toxicosis. Acetyl-cysteine was intended as a stimulator of glutathione synthesis and injected i.p., in order to maximize delivery through blood to the liver. Trans-stilbene oxide was provided in conjunction with acetyl-cysteine because it is a potent inducer of phase II enzymes, including glutathione-S-transferase. This regimen could stimulate glutathione synthesis as well as conjugation with toxicant(s) and thereby increase detoxification by elimination. Supplemental vitamins A and E were intended to protect against possible oxidative damage. Supplemental zinc was intended to stimulate heme metabolism and to enhance protein synthesis (on assumptions that the toxicants impairs heme and(or) protein metabolism in the animal).

Table 4 shows effects of 50% kochia hay on serum constituents.

Means were pooled across treatments because split-plot analysis of variance revealed no treatment x time interactions. Serum electrolytes remained unchanged except for a slight increase (8%) in potassium. Calcium was increased slightly at wk 2 and decreased to original values by wk 4. No decrease was observed in calcium concentration from original values of 10.0 mg/dl. This eliminates oxalate from consideration as a major toxicant in this specimen of kochia hay. Both phosphorus and BUN were slightly decreased by kochia, and uric acid was increased ($P < .05$). Total bilirubin concentration was elevated ($P < .05$) approximately 1.5-fold, accompanied by an increase in ALT ($P < .05$), reflecting mild hepatic involvement.

Because toxicosis was neglible in lambs fed 35% kochia hay for 4 wk and during the first 2 wk on 50% kochia, treatment effects were minimal. Body wt remained constant throughout the 5-wk treatment period while lambs were consuming 50% kochia hay ($P > .10$; Table 3). Table 5 shows means of serum constituents pooled across weeks because a split-plot analysis of variance revealed no treatment x time interactions. The electrolytes were unchanged as a result of treatment; however, urea nitrogen levels were slightly depressed by the acetyl-cysteine plus TSO treatment ($P < .10$) and this treatment also increased uric acid by 25% ($P < .05$). Treatment with ZN decreased gamma-glutamyl transpeptidase levels but increased alanine amino-transferase, as did supplemental vitamins ($P < .10$). Split-plot analysis of variance revealed a treatment x time interaction for sodium, alkaline phosphatase, lactic dehydrogenase and aspartate aminotransferase. Therefore, effect of treatment was examined within week for these constituents. Sodium was increased by vitamins and zinc

at wk 2 but only by vitamins at wk 4 ($P < .05$; Table 6). Table 7 shows treatment with cysteine plus TSO decreased alkaline phosphatase. Supplemental vitamin treatment also decreased alkaline phosphatase, but only at wk 4 ($P < .05$). Lactic dehydrogenase was elevated by vitamin treatment at wk 4 (but this mean was elevated by one lamb having a value of 638 U/L). All treatments tended to increase aspartate aminotransferase levels. None of the treatments were consistent in altering serum profiles; however, effects of kochia were not detrimental enough to notice treatment differences.

Analysis of whole blood collected from the lambs at wk 5 showed no differences due to treatment ($P > .10$; Table 8). However, both red blood cell (RBC) count and the packed cell volume are rather low for ovine species. Since none of the serum constituents were lowered in concentration, hemodilution can be excluded as the major cause. The decreased RBC concentration is likely due to a shortened half-life of the cells or an impairment in RBC synthesis.

Table 9 shows the nutritive value and fluid passage rate measurements of the hay mixture (50% kochia-50% alfalfa). The protein requirement designated by NRC (1975) for 50 kg replacement lambs and yearlings is 8.9% of dietary dry matter. Dietary protein levels should have been adequate for the lambs used in this trial. Dry matter intake at 1.5 kg/d was also adequate. In comparison with a diet containing 50% prairie hay and 50% alfalfa hay, the fluid dilution rate (13.4%/h vs 11.4%/h), ruminal fluid volume (6.0 l vs 5.2 l), turnover time (7.5 h vs 8.9 h) and flow rate (.82 l/h vs .60 l/h) were all quite similar (Estell et al., 1985).

Overall, the dietary quality of the 50% kochia-50% alfalfa hay

mixture was quite high and more than adequate in digestible protein and dry matter. But results support the view that kochia herbage harvested pre-bloom as hay was mildly toxic when fed to fine-wool lambs as 50% of the diet. The treatments evaluated had little if any effects toward alleviation of the mild kochia toxicosis that was elicited.

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TABLE 1. BODY WEIGHTS (KG) AND FEED INTAKE (KG OF DM/D) OF FINE-WOOL LAMBS FED 35% KOCHIA HAY AND 65% ALFALFA HAY FOR FOUR WEEKS

Week	Body weight[a]	SE[b]	DM intake[a]	SE[b]
0	45.9	2.27	1.34	.071
4	43.8	2.27	1.49	.071

[a]Column values do not differ (P > .10).

[b]Standard error of the mean, N = 12.

TABLE 2. BLOOD SERUM CONSTITUENTS IN FINE-WOOL LAMBS FED 35% KOCHIA HAY AND 65% ALFALFA HAY FOR FOUR WEEKS

Constituent	Week 0	Week 4	SE[a]
Sodium, mEq/L	150	147**	0.5
Potassium, mEq/L	4.9	4.8	.10
Chloride, mEq/L	108	108	0.5
Bicarbonate/CO_2, mEq/L	29.9	24.4**	1.0
Anion gap, mEq/L	25.7	20.5**	1.0
Calcium, mg/dl	10.0	9.9	.10
Phosphorus (inorg), mg/dl	5.5	5.6	.18
Calculated osmolality, mOSM/L	299	295**	1.0
Iron, ug/dl	165	164	1.3
Glucose, mg/dl	71	66*	1.3
Total cholesterol, mg/dl	37	36	2.2
Triglycerides, mg/dl	25.1	18.0*	2.4
Urea nitrogen, mg/dl	19.7	23.0*	0.7
Creatinine, mg/dl	.83	.91	.03
BUN/creatinine	23.8	25.3	1.1
Uric acid, mg/dl	.57	.04	.30
Albumin, g/dl	3.8	3.7	.17
Globulin, g/dl	2.2	2.2	.09
Total protein, g/dl	6.0	6.0	.11
Total bilirubin, mg/dl	.15	.15	.03
Alkaline phosphatase, U/L	190	156	17
Creatine kinase, U/L	134	316	129
Gamma glutamyl transpeptidase, U/L	54	55	2.6
Lactic dehydrogenase, U/L	383	379	13
Aspartate aminotransferase, U/L	66	65	4.4
Alanine aminotransferase, U/L	9.8	11.2	1.0

[a]Standard error of the mean, N = 12.

*P < .05.

**P < .01.

TABLE 3. EFFECT OF TREATMENT WITH N-ACETYL-CYSTEINE + TRANS-STILBENE OXIDE (CYS + TSO) OR VITAMINS OR ZINC ON BODY WEIGHTS OF FINE-WOOL LAMBS FED 50% KOCHIA HAY AND 50% ALFALFA HAY FOR FIVE WEEKS

	Treatment[a]				
Week	None	CYS + TSO	Vitamins	Zinc	SE[b]
0	41.9	45.1	44.3	44.0	4.19
5	41.4	43.5	43.1	43.7	4.19

[a]Values do not differ (P > .10).

[b]Standard error of the mean, N = 3.

TABLE 4. BLOOD SERUM CONSTITUENTS IN FINE-WOOL LAMBS 0, 2 and 4 WEEKS POST-TREATMENT WITH N-ACETYL-CYSTEINE + TRANS-STILBENE OXIDE OR VITAMINS OR ZINC AND FED 50% KOCHIA HAY AND 50% ALFALFA HAY FOR FOUR WEEKS[a]

Constituent	Week 0	Week 2	Week 4	SE[b]
Potassium, mEq/L	4.8[c]	5.2[d]	5.2[d]	.10
Chloride, mEq/L	108	110	108	.50
Bicarbonate/CO_2, mEq/L	24.4	23.6	23.3	.49
Anion gap, mEq/L	20.5	19.4	19.1	.48
Calcium, mg/dl	9.9[cd]	10.2[c]	9.6[d]	.12
Phosphorus (inorg), mg/dl	5.6[c]	4.8[d]	5.0[cd]	.19
Glucose, mg/dl	66[c]	73[d]	73[d]	1.5
Cholesterol, mg/dl	36	37	37	0.9
Triglycerides, mg/dl	18.0	18.7	16.0	1.0
Urea nitrogen, mg/dl	23.0[c]	20.8[d]	20.2[d]	.65
Creatinine, mg/dl	.9	.9	.8	.03
BUN/creatinine	25.2	23.3	24.5	.70
Uric acid, mg/dl	.04[c]	.35[d]	.23[e]	.02
Albumin, g/dl	3.8	3.9	3.8	.05
Globulin, g/dl	2.2	2.3	2.2	.04
Total protein, g/dl	6.0	6.2	6.0	.07
Total bilirubin, mg/dl	.15[c]	.18[c]	.23[d]	.01
Creatine kinase, U/L	316	182	389	124
Gamma glutamyl transpeptidase, U/L	55	57	58	1.5
Alanine aminotransferase, U/L	11.2[c]	11.3[c]	13.5[d]	.40

[a]Split-plot analysis of variance revealed no treatment x week interactions ($P > .10$). Therefore, week means are pooled across treatments.

[b]Standard error of the mean, N = 12.

[cd]Row values with different superscripts differ ($P < .05$).

TABLE 5. BLOOD SERUM CONSTITUENTS IN FINE-WOOL LAMBS 0, 2 AND 4 WEEKS POST-TREATMENT WITH N-ACETYL-CYSTEINE + TRANS-STILBENE OXIDE (CYS + TSO) OR VITAMINS OR ZINC AND FED 50% KOCHIA HAY AND 50% ALFALFA HAY FOR FOUR WEEKS[a]

Constituent	Control	CYS + TSO	Vitamins	Zinc	SE[b]
Potassium, mEq/L	5.0	5.2	5.0	5.1	.22
Chloride, mEq/L	108	108	110	108	.64
Bicarbonate/CO_2, mEq/L	24.4	23.6	24.5	22.6	.70
Anion gap, mEq/L	20.2	19.5	20.4	18.5	.68
Calcium, mg/dl	9.9	9.6	10.0	10.1	.19
Phosphorus (inorg), mg/dl	5.1[cd]	5.5[c]	5.0[cd]	4.8[d]	.16
Glucose, mg/dl	70	71	74	68	2.0
Cholesterol, mg/dl	40	40	36	39	2.8
Triglycerides, mg/dl	17.2	17.3	17.1	18.6	1.2
Urea nitrogen, mg/dl	20.4[cd]	19.1[c]	22.3[cd]	23.3[d]	1.0
Creatinine, mg/dl	.8	.8	.9	1.0	.05
BUN/creatinine	24.2	24.7	24.3	24.2	2.2
Uric acid, mg/dl	.18[c]	.24[d]	.19[cd]	.22[cd]	.02
Albumin, g/dl	3.7	3.8	3.9	3.9	.12
Globulin, g/dl	2.3	2.2	2.1	2.3	.19
Total protein, g/dl	6.0	6.0	6.0	6.2	.17
Total bilirubin, mg/dl	.19	.17	.21	.19	.02
Creatine kinase, U/L	131	270	395	390	177
Gamma glutamyl transpeptidase, U/L	61[c]	64[c]	55[cd]	46[d]	3.5
Alanine aminotransferase, U/L	8.1[c]	11.8[cd]	13.8[d]	14.3[d]	1.6

[a]Split-plot analysis of variance revealed no treatment x week interactions ($P > .10$). Therefore, treatment means are pooled across weeks.

[b]Standard error of the mean, N = 3.

[cd]Row values with different superscripts differ ($P < .10$).

TABLE 6. SERUM SODIUM CONCENTRATION (mEq/L) 0, 2 AND 4 WEEKS POST-TREATMENT WITH N-ACETYL-CYSTEINE + TRANS-STILBENE OXIDE OR VITAMINS OR ZINC IN FINE-WOOL LAMBS FED 50% KOCHIA HAY and 50% ALFALFA HAY[a]

Week	Treatment[a]				SE[b]
	Control	CYS + TSO	Vitamins	Zinc	
0	148[c]	148[c]	147[c]	146[c]	.91
2	150[c]	152[cd]	153[d]	153[d]	.91
4	148[c]	150[c]	153[d]	149[c]	.91

[a]Split-plot analysis of variance revealed a treatment x week interaction ($P < .05$). Effect of treatment examined within week.

[b]Standard error of the mean, N = 3.

[cd]Row values with different superscripts differ ($P < .05$).

TABLE 7. SERUM ENZYME CONCENTRATIONS (U/L) 0, 2 AND 4 WEEKS POST-TREATMENT WITH N-ACETYL-CYSTEINE + TRANS-STILBENE OXIDE (CYS + TSO) OR VITAMINS OR ZINC IN FINE-WOOL LAMBS FED 50% KOCHIA HAY AND 50% ALFALFA HAY[a]

Week	Treatment[a] Control	CYS + TSO	Vitamins	Zinc	SE[b]
Alkaline phosphatase					
0	160[c]	165[c]	157[c]	142[c]	9.0
2	136[c]	106[d]	149[c]	121[cd]	9.0
4	151[c]	117[d]	115[d]	136[cd]	9.0
Lactic dehydrogenase					
0	346[c]	358[c]	390[c]	421[c]	24.9
2	292[c]	300[c]	339[c]	315[c]	24.9
4	309[c]	367[c]	495[d]	337[c]	24.9
Aspartate amino-transferase[e]					
0	58	66	70	66	9.9
2	63	69	73	75	9.9
4	64	71	95	76	9.9

[a]Split-plot analysis of variance revealed a treatment x week interaction ($P < .05$). Effect of treatment examined within week.

[b]Standard error of the mean, N = 3.

[cd]Row values with different superscripts differ ($P < .05$).

[e]Row values do not differ ($P > .05$).

TABLE 8. EFFECT OF TREATMENT WITH N-ACETYL-CYSTEINE + TRANS-STILBENE OXIDE (CYS + TSO) OR VITAMINS OR ZINC ON WHOLE BLOOD ANALYSIS IN FINE-WOOL LAMBS FED 50% KOCHIA HAY AND 50% ALFALFA HAY FOR FIVE WEEKS

Constituent	Treatment[a]				SE[b]
	Control	CYS + TSO	Vitamins	Zinc	
WBC[c]	8.0	5.5	5.2	6.8	1.3
RBC[d]	4.3	4.2	4.9	4.8	0.6
Hemoglobin (g/dl)	11.0	10.7	11.9	11.0	0.8
Hematocrit (%)	16.8	16.6	19.4	19.3	2.6
MCV[e]	38.7	38.5	39.0	39.0	0.5
MCH[f]	26.0	25.5	24.7	23.7	2.7
MCHC[g]	32.0	28.0	30.3	38.3	3.0

[a]Row values do not differ (P > .10).

[b]Standard error of the mean, N = 3.

[c]White blood cell count x 1000.

[d]Red blood cell count x 10^6.

[e]Mean corpuscular volume (μm^3).

[f]Mean corpuscular hemoglobin (pg).

[g]Mean corpuscular hemoglobin concentration (%).

TABLE 9. CHEMICAL COMPOSITION, APPARENT DIGESTIBILITIES OF CONSTITUENTS AND FLUID PASSAGE RATE MEASUREMENTS OF HAY MIXTURE (50% KOCHIA HAY AND 50% ALFALFA HAY) FED TO SHEEP

Item	Value
Diet composition, %	
Dry matter	92.3
Crude protein	16.0
Acid detergent fiber	36.1
Diet digestibility, %	
DM apparent digestibility	59.1
CP apparent digestibility	71.6
ADF apparent digestibility	59.3
Passage rate parameters	
Fluid dilution rate, %/h	13.4
Fluid volume, L	6.0
Turnover time, h	7.5
Fluid flow rate, L/h	.82

Proceedings, Western Section, American Society of Animal Science

Vol. 39, 1988

DERANGED METABOLIC HORMONES, IMPAIRED NITROGEN RETENTION AND HYPERBILIRUBINEMIA IN EARLY KOCHIA TOXICOSIS[1]

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Summary

Kochia scoparia (L.) Schrad toxicosis in livestock progresses from hyperbilirubinemia to photosensitization and polyuria. To identify earliest metabolic dysfunctions and evaluate treatments, 12 wether lambs (34 ± 3 kg) were fed kochia hay (15% crude protein) and treated with aq. $ZnSO_4$ drench (ZN: 30 mg Zn/kg body wt) daily or i.p. injection twice weekly with N-acetyl-L-cysteine (CYS: 21 mg/kg, in saline) plus trans-stilbene oxide (TSO: 27 mg/kg, in corn oil). Treatments (ZN or CYS + TSO) were imposed factorially (2 x 2), three lambs per group. Kochia intake averaged .57 kg/hd daily (1.7% of body wt) for 80 d, and digestibility of dry matter and crude protein was 44% and 59% at wk 4, but body wt loss was severe (6 to 11 kg/hd) and biological value of N averaged 16%. At 14 d, serum insulin and prolactin were depressed severely ($P<.05$), from .48 to .11 and 102 to 28 ng/ml, respectively. Growth hormone (GH) increased ($P<.05$) from 4.5 to 6.8 ng/ml at wk 4. Serum bilirubin (unconjugated) increased threefold ($P<.01$) at wk 3. Early changes in blood enzymes reflected hepatotoxicosis, but histopathology (at 80 d) showed mainly renal damage. Treatment with ZN exacerbated kochia toxicosis ($P<.05$) and CYS + TSO had no effect ($P>.10$) except that CYS + TSO prevented elevation of blood GH ($P<.05$) by kochia. Early kochia toxicosis involved deranged blood metabolic hormone levels, impaired nitrogen retention and impaired bilirubin conjugation, related to hepatocyte damage. Treatment to stimulate hepatic glutathione (CYS) and conjugase activity (TSO) provided little benefit (if any), but $ZnSO_4$ drench exacerbated kochia toxicosis.

Introduction

Kochia scoparia (L.) Schrad. (kochia, fireweed) is widespread on arid and semiarid land. It was introduced into the United States as an ornamental, but is now considered a valuable agronomic crop in dryland agriculture (Sherrod, 1973) because it yields digestible energy and protein comparable to alfalfa with about half the water requirement. However, its value is diminished by occasional livestock poisonings (Sprowls, 1981). Kochia toxicosis in livestock has been characterized (Dickie and James, 1983), and oxalate has been identified as a primary toxicant in drought-stricken, mature or overgrazed kochia. In recent studies, other toxicants have been implicated, especially i toxicity of lush forage (Kiesling et al., 1984) or o hay from lush forage (Rankins, 1987). Smith et al (1986) implicated substances reactive t Dragendorff's reagent, presumably alkaloids, i toxicity of lush kochia fed to rats. Rankins an Smith (1987) also implicated alkaloids in kochi toxicosis and showed some beneficial effects fo alleviating kochia toxicosis by treatment wit N-acetyl-L-cysteine + trans-stilbene oxide, vitamin and zinc sulfate. In recent work implicatin alkaloids as the major toxicant, the earlies clinical changes involved hyperbilirubinemia an hepatocyte dysfunction.

This study was conducted to identify earl metabolic and hormonal changes associated with kochi toxicosis, to measure digestibility and nitroge metabolism in lambs fed kochia hay, and to evaluat prospective treatments for kochia toxicosis.

Materials and Methods

Herbage was collected as hay from pre-bloo kochia grown under irrigation at Clovis, NM. Kochi hay was chopped to pass a 1.27-cm screen and fed a libitum to 12 fine-wool, wether lambs (34 ± 3 kg for 80 d. All lambs were individually penned in 1. x 6 m pens with access to trace mineralized salt an water. Feed intakes and body wt were monitored. Tw treatments were randomly imposed in a 2 x 2 factoria arrangement. Three lambs received no treatment three received N-acetyl-L-cysteine plus trans-stilbene oxide [CYS + TSO: 50 mg acetyl cysteine/(kg body wt)$^{.75}$, dosed i.p. in saline twice weekly and 6 mg TSO/(kg body wt)$^{.75}$ dosed i.p. in corn oil twice weekly]; three received aqueous zinc sulfate (ZN: 13 mg zinc sulfate to provide 30 mg zinc/kg body wt, orally drenched every day beginning 3 d before kochi feeding) and three lambs received CYS + TSO and ZN.

Weekly blood samples were collected into sterile serum separator tubes, and allowed to clot at roo temperature for 35 min. Serum was obtained by centrifugation at 2,300 x g for 15 min at 4 C and stored at -20 C until analyzed for serum constituents.[3]

At days 0, 14 and 28, lambs were subjected to intensive sampling of blood. Feed was removed at 1800 h on days before sampling. At 0600 and 0700 on day of sampling, lambs were bled, treatments were administered and then feed was offered until 0800, when feed was removed for 4 h. Blood was collected hourly from 0800 to 1200. Serum insulin (Sanson and Hallford, 1984), growth hormone (Hoefler and

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[1]Scientific paper 296 of the New Mexico Agr. Exp. Sta., Las Cruces. Supported in part by a grant from the New Mexico Water Resources Research Institute. Appreciation is expressed to NIDDK and NHP for assay materials.

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Hallford, 1987) and prolactin (Spoon et al., 1988) were quantified by double antibody radioimmunoassays.

Ten d after initiating kochia feeding, all lambs were placed in metabolism stalls to determine digestibility and nitrogen balance. After a 7 d adjustment period, feces and urine were collected for 10 d. Lambs were then returned to individual pens for the remainder of the 80-d trial. During the collection period, lambs were fed 95% of ad libitum intake. All orts, feces and urine were collected, measured and sampled (10%) daily. Feed and fecal samples were oven-dried (50 C) for 48 h, ground to pass a 2-mm screen in a Wiley mill and analyzed for dry matter, nitrogen (AOAC, 1985), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and acid detergent insoluble nitrogen (ADIN) (Goering and Van Soest, 1970). Urine samples were composited daily with .5 ml toluene/150 ml urine and stored at -20 C until analyzed. Urine was analyzed for nitrogen (AOAC, 1985).

At d 80, all lambs were euthanized and examined grossly postmortem. Samples of livers and kidneys were collected into neutral buffered formalin, fixed, processed and examined microscopically by a veterinary pathologist[4].

Analyses of variance were executed using GLM procedures of the Statistical Analysis System (SAS, 1982). Levels of insulin, growth hormone and prolactin were subjected to split split-plot analysis of variance for repeated measurements over time (Lentner and Bishop, 1986). Serum constituents were evaluated by split-plot analysis of variance for repeated measurements on animals (Gill and Hafs, 1971). Means across time were separated by Duncan's new multiple range test (Bender et al., 1982). Effects of treatment on digestibilities and nitrogen balance were analyzed as a completely randomized design with a 2 x 2 factorial arrangement (Steel and Torrie, 1980).

Results and Discussion

Kochia hay contained 69% NDF, 46% ADF, 15% crude protein (CP), 8.5% ADL and .39% ADIN. About 13% (of DM) was available CP [i.e., (N-ADIN) x 6.25]. The protein was highly digestible (table 1). Energy intake of lambs was also adequate as indicated by NDF and ADF contents and respective digestibilities (table 1). However, biological value of nitrogen (N) was extremely low (table 1). Because ADIN was only .39% of dry matter, most of the N was likely proteinaceous and the extremely low biological values probably resulted from derangement of N metabolism in lambs fed kochia. Table 2 shows dry matter intake ranging from .44 to .70 kg/d, which is about 1.7% of body wt. In spite of adequate intakes of digestible nutrients, all lambs lost wt throughout the 80-d trial (table 2), and had negative N balance after only 2 wk. Treatment with CYS + TSO showed no benefit. Zinc sulfate (ZN) treatment exacerbated kochia toxicosis (tables 1 and 2).

Serum insulin (INS) was depressed within 2 wk of kochia ingestion ($P<.01$; table 3). Because INS response in ruminants is associated with certain amino acids, in addition to glucose, this four-fold reduction in INS levels is probably a reflection of inadequate amino acids as a result of impaired N metabolism, possibly over-active deamination. Prolactin (PRL) levels are depressed in livestock grazing tall fescue (Hurley et al., 1980; Elsasser and Bolt, 1987), presumably caused by alkaloids. Thus it is important to examine PRL levels in lambs exhibiting kochia toxicosis, presumably a result of alkaloids (Smith et al., 1986; Rankins and Smith, 1987). Prolactin levels (table 3) were depressed ($P<.01$) within 2 wk, but that depended on each lamb serving as its own control. (It is possible that PRL levels of 102 ng/ml at day 0 may reflect excitement, and levels of 28 to 36 ng/ml may represent more normal values). Treatments had no effect ($P>.10$) on INS or PRL. Serum growth hormone (GH) was elevated from about 4.5 to 7.8 ng/ml by week 4 ($P<.05$) as a result of dietary kochia, and treatment with CYS + TSO lowered the elevation to 5.5 ng/ml ($P<.08$). Elevation of GH is consistent with wt loss and impaired metabolism observed in these lambs.

Table 4 shows effects of kochia on blood bilirubin and enzymes throughout 10 wk. Hyperbilirubinemia was evident within 3 wk ($P<.05$) and caused by decreased conjugation [i.e., increase of indirect (free) bilirubin]. Hepatocyte dysfunction is also indicated by increased lactic dehydrogenase aspartate aminotransferase and alanine aminotransferase ($P<.05$). Treatment with CYS + TSO did not alleviate hepatocyte dysfunction. (Analysis of variance revealed a ZN x week interaction for each of these constituents, and further analysis within week indicated a detrimental effect from ZN).

Histopathology revealed diffuse hepatocyte swelling and dilatation of small bile ducts in all lambs. Nephrosis was evident in all ZN-treated animals. Crystals resembling oxalate were evident in kidneys of two ZN-treated lambs.

Very early changes in kochia toxicosis were associated with liver dysfunction and impairment of hepatocellular conjugation of bilirubin. Severe derangement of metabolic hormones and N metabolism occurred within 2 wk. Kochia scoparia hay was adequate in content of digestible protein and energy; however, kochia toxicosis blocked effective use of nutrients. Subsequently, nephro-toxicosis occurred. Use of CYS + TSO, aimed at enhancement of hepatic detoxification, offered little benefit and drenching with $ZnSO_4$ exacerbated kochia toxicosis.

[4]Dr. John Thilstead, Veterinary Diagnostics Services, N.M. Dept. Agr., Albuquerque.

TABLE 1. APPARENT DIGESTIBILITY AND NITROGEN BALANCE IN LAMBS FED KOCHIA FOR 3 WEEKS AND TREATED THROUGHOUT WITH N-ACETYL-CYSTEINE (CYS) + TRANS-STILBENE OXIDE (TSO) OR ZINC SULFATE (ZN)

Parameter	CYS + TSO[a]			ZN[a]		
	No	Yes	SE[b]	No	Yes	SE
DM digestibility, %	45	42	3.9	52[c]	35[d]	4.3
NDF digestibility, %	48	47	3.5	50	44	3.7
ADF digestibility, %	31	31	3.8	34	28	4.0
CP (apparent) digestibility, %	57	61	3.5	67[c]	50[d]	3.8
ADIN in - ADIN out, g/d	-2.0	-14.4	4.6	-9.7	-6.6	5.0
Nitrogen balance, g/d	-2.9	-1.7	.8	-.5[c]	-4.1[d]	.9
Biological value[e], %	11.7	20.2	4.4	26.5[c]	5.4[d]	4.8

[a]Analysis of variance revealed no CYS + TSO x Zn interactions ($P>.25$). Therefore, main effect means were reported. Doses were 21 mg CYS, 27 mg TSO and 30 mg ZN$\cdot kg^{-1} \cdot d^{-1}$.

[b]Standard error of the mean, n = 5.

[c,d]Row values with different superscripts differ ($P<.05$).

[e]Assumes that metabolic fecal n = .5g/100g DM intake and that endogenous urinary n = .033g/kg body wt.

TABLE 2. FEED INTAKE (KG DM/D) AND BODY WEIGHT CHANGES OF FINE-WOOL WETHERS FED KOCHIA SCOPARIA HAY AND TREATED WITH N-ACETYL-CYSTEINE (CYS) + TRANS-STILBENE OXIDE (TSO) OR ZINC SULFATE (ZN) FOR 80 DAYS

Parameter	CYS + TSO[a]			ZN[a]		
	No	Yes	SE[b]	No	Yes	SE
Feed intake, kg dm/d	.58	.56	.068	.70[b]	.44[c]	.075
Body weight change, kg	-8.4	-8.2	.96	-5.6[b]	-11.0[c]	1.05

[a]Analysis of variance revealed no CYS + TSO x ZN interactions ($P>.10$). Therefore, means were pooled across treatments. Doses were 21 mg CYS, 27 mg TSO and 30 mg ZN$\cdot kg^{-1} \cdot d^{-1}$.

[b,c]Within treatments, row values with different superscripts differ ($P<.05$).

TABLE 3. EFFECTS OF KOCHIA SCOPARIA ON SERUM INSULIN (NG/ML) AND PROLACTIN (NG/ML) IN LAMBS TREATED WITH N-ACETYL-CYSTEINE + TRANS-STILBENE OXIDE OR ZINC SULFATE FOR 4 WEEKS[a]

Hormone	Week			SE (n = 70)
	0	2	4	
Insulin	.48[b]	.11[c]	.08[c]	.045
Prolactin	102[b]	28[c]	36[c]	17

[a]Split split-plot analysis of variance revealed no treatment x week interactions ($P>.50$). Therefore, means were pooled across treatments. Animals were bled at hourly intervals for 7 h.

[b,c]Row values with different superscripts differ ($P<.01$).

TABLE 4. EFFECTS OF KOCHIA HAY ON BLOOD BILIRUBIN AND ENZYMES IN LAMBS TREATED WITH N-ACETYL-CYSTEINE (CYS) + TRANS-STILBENE OXIDE (TSO) OR ZINC SULFATE (ZN) FOR 10 WEEKS[a]

Constituent	Week 0	3	6	8	10	SE (N = 10)
Total bilirubin, mg/dl	.18[b]	.50[d]	.39[cd]	.27[bc]	.36[c]	.033
Direct bilirubin, mg/dl	.01	.03	.01	.01	.01	.004
Indirect bilirubin, mg/dl	.16[b]	.46[d]	.36[cd]	.26[bc]	.35[c]	.031
Lactic dehydrogenase, U/liter	524[b]	527[b]	1071[c]	709[bc]	840[bc]	134
Aspartate aminotransferase, U/liter	117[b]	152[bc]	256[cd]	246[cd]	341[d]	31.7
Alanine aminotransferase, U/liter	16[b]	11[b]	31[bc]	43[cd]	60[d]	5.6

[a]Split-plot analysis of variance revealed no CYS + TSO x week interactions (P>.10). Therefore, means were pooled across treatments.

[bcd]Means in the same row that do not have a common letter in their superscripts differ (P<.05).

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