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Title: INFLUENCE OF DIETARY FACTORS ON TANSY RAGWORT TOXICOSIS IN RATS AND HORSES

Abstract approved:

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Several experiments were conducted to investigate the effect of dietary factors such as amino acids, B vitamins, butylated hydroxyanisole (BHA) and phenobarbital on pyrrolizidine alkaloid toxicosis in rats and horses. The influence of St. John's wort, bracken, comfrey, and alfalfa on tansy ragwort toxicity was also examined.

Effects of dietary tansy ragwort (Senecio jacobaea), comfrey (Symphytum officinale), bracken (Pteridium acquilinum) and alfalfa (Medicago sativa) on hepatic drug-metabolizing enzymes in rats were measured. Tansy ragwort and bracken increased ($P<0.05$) the activity of glutathione transferase and epoxide hydrolase. Comfrey and alfalfa increased ($P<0.05$) the activity of aminopyrine N-demethylase. Feeding bracken or St. John's wort (Hypericum perforatum) in conjunction with tansy ragwort did not influence chronic toxicity of tansy ragwort as assessed by rat survival time. Dietary tansy ragwort resulted in increased ($P<0.05$) hepatic copper levels; the other plants did not affect copper levels. The results do not suggest any major interaction in the toxicity of tansy

ragwort with bracken or St. John's wort.

Dietary supplementation of rats with branched chain amino acids (BCAA: leucine, isoleucine, valine) did not alter their susceptibility to chronic poisoning by tansy ragwort (Senecio jacobaea), which contains hepatotoxic pyrrolizidine alkaloids (PA). Phenobarbital in the diet, which alters liver microsomal enzyme activity, also did not alter susceptibility to PA poisoning. A combination of BHA, cysteine and BCAA did increase ($P<.05$) survival time of rats fed tansy ragwort. Dietary BHA and cysteine increased the survival times of rats injected with the PA monocrotaline, with evidence that addition of vitamin B_{12} and folic acid improved the effectiveness of this treatment. In a chronic feeding trial with tansy ragwort, a combination of BHA and cysteine increased ($P<.05$) the survival times of rats, showing protective activity against PA poisoning. A mixture of B-complex vitamins, or vitamin B_{12}-folic acid, was not effective in improving the response.

Dried tansy ragwort, which contains pyrrolizidine alkaloids, was fed as 10% of a complete diet to horses, with and without a mixture of additives. The additives provided a dietary supplement equivalent to 1% cysteine, 0.75% BHA, 200 ppb vitamin B_{12} and 5 ppm folic acid. The additives did not alter tansy ragwort toxicity, as assessed by survival time, liver histology, bromosulphalein clearance rate and serum gamma glutamyl transpeptidase activity. In horses fed tansy ragwort, bromosulphalein clearance rate was a sensitive indicator of liver damage, and declined to a level of

about 20% of control values. Gamma glutamyl transpeptidase levels showed considerable variability but in general were elevated in animals fed tansy ragwort. Liver iron and copper levels were elevated, and liver zinc declined in tansy ragwort-fed horses.

Influence of Dietary Factors on Tansy Ragwort Toxicosis in Rats and Horses

by

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Influence of Dietary Factors on Tansy Ragwort Toxicosis in Rats and Horses

INTRODUCTION

The livestock industry (Duby, 1975) and the human population (Hill, 1960; Huxtable, 1980) endure illness and death due to pyrollizidine alkaloid (PA) toxicity. The PAs are found in a number of plants including various species of Senecio, Crotalaria, Heliotropium, Echium, and Amsinckia. Amsinckia, Senecio vulgaris (common groundsel) and Senecio jacobaea (tansy ragwort) are the common hepatoxic plants found in the western United States, particularly the Pacific coast states (Kingsbury, 1964; Gibbons, 1972).

Humans sometimes use these PA containing plants as medicines and herbal teas in various parts of the world. In addition, animal health is endangered when either they have inadvertently been fed contaminated hay or the pasture becomes sparce and the animal is forced to eat the hepatotoxic plants. Cattle and horses are the major livestock species affected by tansy ragwort poisoning. Some animals, like sheep, are resistant to PA's. Probably because of the nature of their hepatic metabolism. Shull et al., (1976) related species susceptibility to PAs by the mixed function oxidase (MFO) enzyme PA dehydrogenase level, in his in vitro studies.

The PAs are bioactivated by the MFO enzyme system to pyrroles (dehydropyrrolizidine) causing characteristic changes in liver morphology. These changes include megalocytosis, fibrosis, necrosis,

bile duct proliferation, cirrhosis and vascular lesions (McLean, 1970).

Clinical signs do not appear until weeks to months after onset of PA consumption. Once disease signs are apparent, in most instances, the pathological damages are irreversible and incurable. Control of the hepatotoxic plants is of great importance since no consistant means of treating PA produced liver disease has been discovered.

The objective of this study was to explore various aspects of the influence of dietary factors on tansy ragwort toxicoses. These aspects included:

1. determining the influence of the poisonous plants St. John's wort and bracken on tansy ragwort toxicity. In addition, the effect of tansy ragwort, bracken, and comfrey on the activity of hepatic drug-metaboizing enzymes were studied.
2. evaluating various types of dietary supplements to determine if animals susceptible to PA poisoning could be rendered more resistant. This would initially be carried out using laboratory rats. Information from these preliminary studies would then be used to evaluate the protective effects on susceptible livestock. It is hoped that information gained will contribute to stopping or even reversing the course of the disease. This would ultimately be of great economic importance to the livestock producer.

3. examining the bromsulphalein clearance liver function test as a potential screening device to be used in diagnosis of the disease.

CHAPTER I.

Consumption of Poisonous Plants (*Senecio Jacobaea*, *Symphytum Officinale*, *Pteridium Aquilinum*, *Hypericum Perforatum*) by Rats: Chronic Toxicity, Mineral Metabolism, and Hepatic Drug-Metabolizing Enzymes[1]

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Consumption of Poisonous Plants (*Senecio Jacobaea*, *Symphytum Officinale*, *Pteridium Aquilinum*, *Hypericum Perforatum*) by Rats: Chronic Toxicity, Mineral Metabolism, and Hepatic Drug-Metabolizing Enzymes

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SUMMARY

Effects of dietary tansy ragwort (*Senecio jacobaea*), comfrey (*Symphytum officinale*), bracken (*Pteridium aquilinum*) and alfalfa (*Medicago sativa*) on hepatic drug-metabolizing enzymes in rats were measured. Tansy ragwort and bracken increased ($P<0.05$) the activity of glutathione transferase and epoxide hydrolase. Comfrey and alfalfa increased ($P<0.05$) the activity of aminopyrine N-demethylase. Feeding bracken or St. John's wort (*Hypericum perforatum*) in conjunction with tansy ragwort did not influence chronic toxicity of tansy ragwort as assessed by rat survival time. Dietary tansy ragwort resulted in increased ($P<0.05$) hepatic copper levels; the other plants did not affect copper levels. The results do not suggest any major interaction in the toxicity of tansy ragwort with bracken or St. John's wort.

INTRODUCTION

Tansy ragwort (Senecio jacobaea) contains PAs which cause irreversible liver damage in livestock consuming the plant. It has caused extensive livestock losses in the U.S. Pacific Northwest and in other temperate regions such as Nova Scotia, New Zealand, Australia, South Africa and western Europe. The PAs are bio-activated by hepatic enzymes to pyrroles, which react with hepatic macromolecules, causing liver necrosis (McLean, 1970). In many areas where tansy ragwort grows, such as the Pacific Northwest, bracken (Pteridium aquilinum) and St. John's wort (Hypericum perforatum) are also widespread toxic weeds. Bracken contains at least two toxic factors: a thiaminase and a "cattle bracken poisoning factor," which causes destruction of bone marrow, anemia, and internal hemorrhaging (Evans, 1976). This plant also causes bladder and intestinal tummors (Evans, 1979). St. John's wort contains the toxin hypericin which causes primary photosensitization (Kingsbury, 1964). Because pastures frequently contain all three of these plants, there is a likelihood that livestock could consume them simultaneously. The objective of this study was to determine if the toxicity of tansy ragwort is influenced by the consumption of St. John's wort and bracken. In addition, the effect of tansy ragwort, bracken, and comfrey (Symphytum officinale) on the activity of hepatic drug-metabolizing enzymes was studied. Comfrey contains PAs (Furuya and Araki, 1968; Culvenor et al., 1980; Culvenor et al., 1980) and is carcinogenic (Hirono et al., 1978).

MATERIALS AND METHODS

The plant material was collected in the vicinity of Corvallis, OR. The tansy ragwort was in full bloom; bracken fern was collected at the fiddle-head stage before the fronds were completely open; St. John's wort was in full bloom; and comfrey was harvested in the prebloom vegetative stage. The plants were dried at 45°C, finely ground in a Wiley mill, and stored at room temperature.

Exp. 1. The effect of several plants in the diet of rats on hepatic drug-metabolizing enzymes was studied. Six Long-Evans male rats of about 140 g initial weight were assigned to each diet. Diets included a corn-soy control, 5, 10 and 30% bracken, 5, 10 and 30% comfrey, 1% tansy ragwort, and 30% alfalfa. Rats fed bracken were given an injection of 2 mg thiamine hydrochloride in 0.2 ml of saline once per week to prevent thiamine deficiency due to the thiaminase in bracken. A control group also received thiamine injection. Alfalfa was included as a non-poisonous plant to ascertain if any effects observed on hepatic enzymes were characteristic of plants in general or were due to the specific poisonous plants. The basal diet was that of Miranda et al., 1980; the dried plants were added in place of corn in the basal diet. The rats were fed the diets ad lib. for 21 days and were then killed, the livers removed, weighed, minced and homogenized. Cytosol and microsomes were prepared as described by Miranda et al., 1980. The microsomal protein and activities of epoxide hydrolase, glutathione S-transferase and aminopyrine demethylase were determined

by the methods of Miranda et al., 1980. Data were analyzed by analysis of variance (Steel and Torrie, 1960) and treatments compared to appropriate controls by lsd.

Exp. 2. Eight male Long-Evans rats of about 90 g initial weight were assigned to each of the 7 treatments: basal, basal + tansy ragwort, basal + bracken, basal + St. John's wort, basal + tansy ragwort + bracken, basal + tansy ragwort + St. John's wort, and basal + tansy ragwort + bracken + St. John's wort. The St. John's wort was fed at 10% of the diet for the first 12 days and thereafter at 5%, because of unpalatability. Bracken was fed at the 10% level, and tansy ragwort was fed at 1% for the first 41 days and 2.5% thereafter. The basal diet was that of Miranda et al., 1980; the plant meals were added in place of complete diet (e.g. 90% basal, 10% bracken). Growth and feed intake data were recorded for 178 days, at which time surviving animals were killed. On day 119, 4 of the rats from the basal + St. John's wort groups were sacrificed and necropsied. The rats fed bracken received 2 mg of thiamine hydrochloride per week as in Exp. 1. Liver copper, iron and zinc were determined. Approx. 1.5 g wet liver samples were dried in acid-washed flasks at 100°C. The liver samples were wet-ashed with 4:1 HNO_3:$HClO_4$. The ash was dissolved in 0.1 N HCl and analyzed on a Perkin-Elmer Model 303 atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Exp. 1. Weight gains were reduced ($P<0.05$) in rats fed tansy ragwort and 30% comfrey (Table 1). Bracken and alfalfa did not affect gains. The activity of the three hepatic enzymes examined reflects the ability of the animals to metabolize and conjugate foreign compounds. Tansy ragwort has been previously reported to stimulate activity of glutathione S-transferase and epoxide hydrolase in rats (Miranda, 1980). Levin et al. reported that epoxide hydrolase activity is elevated by carcinogens and suggested that this enzyme may have a role in the neoplastic process. The PAs are reported to be carcinogenic (McLean, 1970). Also, jacobine in tansy ragwort is an epoxide and could probably serve as a substrate for epoxide hydrolase. Bracken at the 10 and 30% levels in the diet also stimulated the activity of these two enzymes (Table 1). Bracken contains carcinogenic compounds (Kingsbury, 1964); their chemical nature is unknown. Presumably epoxides could be involved. Comfrey and alfalfa stimulated the activity of aminopyrine N-demethylase but did not affect glutathione S-transferase or epoxide hydrolase activities.

The results of this experiment indicate that tansy ragwort and bracken have similar effects on glutathione S-transferase and epoxide hydrolase activities, so could conceivably show an interaction in their toxic effects.

Exp. 2. The survival time of rats fed tansy ragwort was not

TABLE 1. WEIGHT GAINS AND LIVER ENZYME ACTIVITIES OF RATS FED TANSY RAGWORT, COMFREY, BRACKEN AND ALFALFA.

Treatment	Avg. daily gain (g)	Enzyme Activity nmoles product min^{-1} (mg $protein^{-1}$) Aminopyrine N-demethylase	Glutathione S-transferase	Epoxide hydrolase
control	7.1[a]	7.05±0.05	239±16	3.90±0.33
control + thiamine	6.3[a]	7.30±0.37	189±19	3.27±0.39
1% tansy ragwort	5.1[b]	7.53±0.29	296[d]±13	8.97[g]±0.43
5% comfrey	6.9[a]	8.75±0.48	254±18	4.59±0.73
10% comfrey	6.4[a]	9.26[d]±0.74	222±20	3.74±0.47
30% comfrey	3.3[c]	8.64[d]±0.44	262±24	4.31±0.42
5% bracken	5.8[a]	8.04±0.40	236±12	4.10±0.29
10% bracken	6.8[a]	8.60±0.64	252[e]±14	4.71[e]±0.48
30% bracken	5.5[a]	10.28[e]±0.80	335[f]±23	6.84[f]±0.71
30% alfalfa	5.5[a]	9.74[d]±0.42	270±23	5.04±0.40

[a,b,c] are different at $P<.05$

[d] is different ($P<.05$) than control

[e] is different ($P<.05$) than control + thiamine

[f] is different ($P<.01$) than control + thiamine

[g] is different ($P<.001$) than control.

not decreased when either bracken or St. John's wort was fed with it (Table 2), indicating that these plants do not potentiate the toxicity of tansy ragwort. Survival time was longest for those rats receiving all three toxic plants; this may be a reflection of a lower feed intake, causing a longer period required for intake of a lethal dose. Consumption of tansy ragwort resulted in an increase in liver copper concentration, as reported earlier for rats (Swick et al., 1981c) and sheep (Bull et al., 1956). Neither bracken nor St. John's wort directly affected liver copper levels. No major effects on liver zinc or iron levels were observed.

This experiment indicates that bracken and St. John's wort do not interact in a significant manner with tansy ragwort to influence its toxicity.

No significant tissue lesions were found in four rats fed St. John's wort. Feeding trials with rats fed this plant have not previously been reported. Photosensitization was not observed, as the animals were not exposed to ultraviolet light.

TABLE 2. WEIGHT GAINS, SURVIVAL TIMES AND LIVER MINERAL CONTENT FOR RATS FED TANSY RAGWORT, BRACKEN AND ST. JOHN'S WORT

Treatment	Avg. daily gain[a], g	Tansy ragwort intake, g	Bracken intake g	St. John's wort intake g	Survival time, days	Liver minerals, ppm Cu	Fe	Zn
Control	6.9[a]	--	--	--	178[g]	11.12[a]	377.2	53.4[a,b]
Control + tansy ragwort (TR)	4.7[c,d]	42.7[a,b]	--	--	123.4[a]	39.87[b,c]	458.2	59.7[b]
Control + bracken (Br)	6.2[b]	--	428[a]	--	178[g]	11.02[a]	323.6	46.6[a]
Control + St. John's wort (SJW)	5.2[c]	--	--	133[a]	178[g]	12.88[a,b]	349.8	53.7[a,b]
Control + TR + Br.	4.7[d]	51.7[b]	255[b]	--	136.5[a]	44.36[c]	355.4	60.5[b]
Control + TR + SJW	3.7[e]	41.2[a]	--	100[b]	127.6[a]	20.75[a,b,c]	379.6	61.2[b]
Control + TR + SJW + Br.	3.2[f]	51.0[a,b]	219[b]	110[a,b]	164.6[b]	17.10[a,b]	406.5	53.0[a,b]

[a,b,c,d,e,f], Values with different superscripts differ ($P<.05$).

[g], All animals survived until 178 days.

[+] 42 days.

CHAPTER II.

Evaluation of Amino Acids, B-vitamins and Butylated Hydroxyanisole as Protective Agents against Pyrrolizidine Alkaloid Toxicity in Rats[1]

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Evaluation of Amino Acids, B-vitamins and Butylated Hydroxyanisole as Protective Agents against Pyrrolizidine Alkaloid Toxicity in Rats

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SUMMARY

Dietary supplementation of rats with branched chain amino acids (BCAA: leucine, isoleucine, valine) did not alter their susceptibility to chronic poisoning by tansy ragwort (Senecio jacobaea), which contains hepatotoxic pyrrolizidine alkaloids (PA). Phenobarbital in the diet, which alters liver microsomal enzyme activity, also did not alter susceptibility to PA poisoning. A combination of butylated hydroxyanisole (BHA), cysteine and BCAA did increase ($P<.05$) survival time of rats fed tansy ragwort. Dietary BHA and cysteine increased the survival times of rats injected with the PA monocrotaline, with evidence that addition of vitamin B_{12} and folic acid improved the effectiveness of this treatment. In a chronic feeding trial with tansy ragwort, a combination of BHA and cysteine increased ($P<.05$) the survival times of rats, showing protective activity against PA poisoning. A mixture of B-complex vitamins, or vitamin B_{12}-folic acid, was not effective in improving the response.

INTRODUCTION

Tansy ragwort (Senecio jacobaea) is a poisonous plant responsible for extensive livestock losses in the Pacific Northwest, and other temperate areas such as Nova Scotia, New Zealand, Australia, South Africa and western Europe. Its toxicity is due to pyrrolizidine alkaloids (PA) that are bioactivated by hepatic enzymes to pyrroles, which react with hepatic macromolecules, causing liver necrosis.

Cattle and horses are the major livestock species affected by tansy ragwort poisoning. Some animals, like sheep, are resistant to PA's, probably because of the nature of their hepatic metabolism (Shull et al., 1976; Swick et al., 1982b). The possibility exists that dietary supplements provided to susceptible animals could modify their hepatic enzyme activity to increase their resistance to PA's. Buckmaster et al. (1976) reported that dietary cysteine increased the resistance of rats to tansy ragwort poisoning. Miranda et al. (1981b) found that butylated hydroxyanisole (BHA) provided protection against PA toxicity. Gulick et al. (1980) observed that in horses poisoned by PA, the plasma amino acid patterns were altered; treatment of a horse suffering from clinically severe Senecio vulgaris poisoning with a paste of branched chain amino acids (BCAA) was effective in restoring the animal to an apparently satisfactory state of health (Rogers et al., 1979).

The objective of this study was to evaluate various types of

dietary supplements to determine if animals susceptible to PA poisoning could be rendered more resistant.

MATERIALS AND METHODS

Tansy ragwort, in full bloom, was collected in the vicinity of Corvallis, Oregon. The plant material was dried at 45°C, finely ground in a Wiley mill and stored at room temperature. Statistical analysis of data involved one way analysis of variance with treatments compared to appropriate controls by least significant difference (Steel and Torrie, 1960). The amino acids used were DL, except for cysteine; L-cysteine was used.

Exp. 1. The objectives of this experiment were to determine if various dietary supplements modified the susceptibility of rats to tansy ragwort poisoning, as assessed by growth and survival time. The BCAA (leucine, isoleucine, valine) were used to test the suggestion of Rogers et al. (1979) that they have protective activity against PA. Protective effects have previously been reported for cysteine (Buckmaster et al., 1976) and BHA (Miranda et al., 1981b); these compounds were tested in conjunction with the other additives. BHA may exert its effects through stimulation of glutathione-S-transferase activity (Miranda et al., 1981b) while cysteine increases glutathione formation, so these compounds might have synergistic action. The aromatic amino acids phenylalanine and tyrosine were used because of the suggestion of Rogers et al. (1979) that the blood plasma balance of BCAA and the aromatic

amino acids may influence the degree of liver damage or encephalopathy. Phenobarbital was used as a hepatic enzyme inducer, to determine if it influenced the susceptibility of rats to tansy ragwort toxicity.

Sixty male Sprague-Dawley rats of about 117 g initial weight were randomly distributed into twelve treatments of five rats each. Dietary treatments were as follows:

1. Control
2. Basal (Control + 5% tansy ragwort)
3. Basal + .15% phenobarbital (PB)
4. Basal + .75% BHA
5. Basal + 1% cysteine
6. Basal + 3% branched-chain amino acids (BCAA--1% leucine, 1% isoleucine, 1% valine)
7. Basal + .15% PB + 3% BCAA
8. Basal + 3% BCAA + 1% cysteine
9. Basal + 3% BCAA + 1% cysteine + .15% PB
10. Basal + 3% BCAA + 1% cysteine + .75% BHA
11. Basal + 3% BCAA + 1% cysteine + .75% BHA + .15% PB
12. Basal + 1% phenylalanine + 1% tyrosine

The basal diet was that of Miranda et al. (1980). All substances were added in place of the complete diet (eg. 92% control, 5% tansy ragwort, 3% BCAA). Rats were caged individually in galvanized cages, and fed and watered ad libitum. The diets with the additives were fed for a one-week pretreatment period before the tansy ragwort was added, to allow hepatic enzyme induction

prior to exposure to PA.

Exp. 2. The results of Experiment 1 suggested protective activity against PA toxicity for some of the additives. The objective of this experiment was to evaluate the effects of B vitamins on the responses to cysteine and BHA. B vitamins are cofactors in enzymatic reactions. Vitamin B_{12} and folic acid were of particular interest, as they are involved in hematopoesis; pronounced anemia occurs in PA poisoning (Swick et al., 1982c). The following dietary treatments were used:

1. Control (saline injected)
2. Control (monocrotaline injected - MI)
3. Control (MI) + 1% cysteine
4. Control (MI) + .75% BHA
5. Control (MI) + vitamin B_{12} + folic acid
6. Control (MI) + B vitamin mixture
7. Control (MI) + .75% BHA + 1% cysteine
8. Control (MI) + .75% BHA + 1% cysteine + B_{12} + folic acid
9. Control (MI) + .75% BHA + 1% cysteine + B vitamin mixture
10. Control (MI) + .75% BHA + B_{12} + folic acid
11. Control (MI) + .75% BHA + B vitamin mixture
12. Control (MI) + 1% cysteine + B vitamin mixture
13. Control (MI) + 1% cysteine + B_{12} + folic acid

The compositions of the B vitamin mixture, and of the vitamin mixture used in the control diet, are shown in Table 3. The vitamin mixtures were used at a dietary level of 1%. Folic acid and vitamin B_{12} were fed at levels of 5 ppm and 200 ppb respectively.

TABLE 3. COMPOSITION OF THE VITAMIN MIXTURES USED IN THE BASAL AND VITAMIN-SUPPLEMENTED DIETS - EXPERIMENT 2

Vitamin (units)[a]	Basal Diet	Vitamin-supplemented diet
A (IU)	2600	2600
D (IU)	1000	1000
E (mg)	21	21
K (as menadione) (µg)	500	500
Folic acid (mg)	.5	5.2
Niacin (mg)	20	200
Ca pantothenate (mg)	.2	20
Riboflavin (mg)	1.81	18.1
Thiamin (mg)	2.84	28.4
Pyridoxine (mg)	3.6	36
Vitamin B_{12} (µg)	50	500

[a]Amount per kg of diet.

The control diet had the following composition: ground corn, 61%; soybean meal, 32%; soybean oil, 3%; and Jones and Foster (1942) mineral mix, 4%. The test compounds, and either the basal or B vitamin mix, were added in place of the complete diet (eg. 98% control, 1% cysteine, 1% basal vitamin mix).

Sixty-five male Sprague-Dawley rats of about 100 g initial weight were randomly distributed into the 13 treatments of five rats each. The animals were fed their respective diets for 7 days, and then were given a series of intraperitoneal doses of the PA monocrotaline, which totalled 230 mg monocrotaline per kg body weight. Multiple injections were given to approximate chronic feeding doses. Dosages of 30 mg/kg were injected on days 7, 9, 11, 13 and 15; 20 mg/kg was injected on day 17, and 15 mg/kg on days 19, 21, 29 and 33. Animals in control group I received the vehicle alone. The experiment was terminated on day 55 when all monocrotaline-injected rats had died.

The monocrotaline solution was prepared by dissolving the alkaloid in .2 N HCl, neutralizing with .2 N NaOH, and diluting to volume with saline.

Exp. 3. The results of Experiment 2 suggested possible synergistic protective activity of cysteine, BHA and folic acid-vitamin B_{12}. The objective of this experiment was to evaluate these compounds as protective agents against PA toxicity when included in diets containing tansy ragwort.

Forty-two male Sprague-Dawley rats of about 95 g weight were

randomly distributed into 7 treatments of 6 rats each. The control diet was that of Miranda et al. (1980); test substances were added in place of the complete diet. Treatments were as follows:

1. Control
2. Control + 5% tansy ragwort (basal)
3. Basal + vitamin B_{12} + folic acid (200 ppb and 5 ppm, respectively)
4. Basal + .75% BHA
5. Basal + .75% BHA + vitamin B_{12} + folic acid
6. Basal + .75% BHA + 1% cysteine
7. Basal + .75% BHA + 1% cysteine + vitamin B_{12} + folic acid

Diets with additives were fed for 7 days before the tansy ragwort was added. After 17 weeks of tansy ragwort exposure the diets were fed for six weeks without tansy ragwort; for the remainder of the experiment, the surviving rats were fed Purina Rat Chow ad libitum. All surviving rats were sacrificed on day 307.

RESULTS AND DISCUSSION

Exp. 1. The phenobarbital treatment did not affect survival times ($P>.05$) as compared to similar treatments without it, but in each case that phenobarbital was used, survival time was slightly less than in the treatments without its addition (Table 4). Allen et al. (1972) found that following stimulation of microsomal enzymes with phenobarbital, rats died more rapidly than those that received monocrotaline alone. Phenobarbital also increased

TABLE 4. THE EFFECT OF BHA, PHENOBARBITAL AND AMINO ACID SUPPLEMENTS ON TANSY RAGWORT TOXICITY IN RATS - EXPERIMENT 1

	Average 27 day gain (g)	Survival time (days)	Tansy intake (g)	Tansy intake as % of initial body weight (g)
1. Control	6.5 ± 1.8[c]	--[f]	--	--
2. Control + 5% Tansy Ragwort (Basal)	3.8 ± .5[a]	77 ± 14[ab]	62 ± 9[a]	53 ± 7[ab]
3. Basal + .15% phenobarbital (PB)	4.1 ± .7[ab]	75 ± 12[ab]	60 ± 14[ab]	51 ± 11[a]
4. Basal + .75% BHA	4.4 ± .5[ab]	83 ± 33[abc]	71 ± 24[abcd]	62 ± 21[abc]
5. Basal + 1% cysteine (Cys)	5.0 ± .5[b]	89 ± 18[abc]	78 ± 8[bcd]	68 ± 8[bc]
6. Basal + 3% branched chain amino acids (BCAA)	4.1 ± .5[ab]	73 ± 16[ab]	59 ± 13[a]	51 ± 13[a]
7. Basal + .15% PB + 3% BCAA	4.1 ± .7[ab]	70 ± 18[a]	57 ± 18[a]	49 ± 16[a]
8. Basal + 3% BCAA + 1% Cys	4.4 ± .5[b]	93 ± 12[bcd]	75 ± 13[abc]	62 ± 9[abc]
9. Basal + 1% Cys + 3% BCAA + .15% PB	4.3 ± .5[ab]	81 ± 8[abc]	70 ± 10[abc]	59 ± 11[abc]
10. Basal + 1% Cys + .75% BHA + 3% BCAA	4.0 ± .9[a]	107 ± 16[de]	82 ± 10[cd]	68 ± 9[bc]
11. Basal + 1% Cys + .75% BHA + 3% BCAA + .15% PB	4.5 ± .6[b]	101 ± 10[cde]	90 ± 17[d]	73 ± 15[c]
12. Basal + 2% aromatic amino acids	3.8 ± .7[a]	80 ± 16[abc]	63 ± 15[ab]	52 ± 13[ab]

[a,b,c,d,e]Means with different superscripts are different (P<.05).

[f]All animals survived until termination of the experiment at 118 days.

the susceptibility of guinea pigs to the acute hepatoxic effects of the PA retrorsine (White et al., 1973). The lack of effect of phenobarbital on tansy ragwort toxicity might be a result of induction of enzymes involved in PA conjugation and excretion, as well as pyrrole production. Also, the effects of phenobartital may vary according to the specific PA's involved.

The BCAA had no effect ($P>.05$) on survival time, which does not support the suggestion of Rogers et al. (1979) that they may have protective effects against PA toxicity. Cascino et al. (1978), Gulick et al. (1980) and Rosen et al. (1977) reported that in various species, similar changes in plasma amino acid patterns are seen in animals with hepatic disease. Neurological improvement and increased survival time have been reported in man when the plasma amino acid pattern was restored toward normal by supplementation with the appropriate amino acids (Fischer et al., 1975, 1976). Rogers et al. (1979) reported that treatment of a horse poisoned by PA with a paste of BCAA restored its plasma amino acid pattern to normal and improved its physical condition. The aromatic amino acids, phenylalamine and tyrosine, increase as the BCAA plasma levels decrease during hepatic disease. The aromatic amino acids are neurotransmitter precursors. These observations may relate to the hypothesis by which James et al. (1979) related encephalopathy and hepatic coma to liver damage. When liver function is reduced, the blood ammonia concentration is increased. They suggest that this high ammonia level may stimulate the secretion of glucagon which in turn may cause a

high insulin secretion. Insulin stimulates the uptake and catabolism of branched-chain amino acids (BCAA), leucine, isoleucine, and valine, by muscle. The entry into the brain of the aromatic amino acids may be enhanced because of less competition due to the lowered BCAA level in the plasma. The increased level of these neurotransmitter precursors could alter the balance of neurotransmitter substances in the brain and cause hepatic encephalopathy. Thus it was hypothesized that feeding an elevated level of aromatic amino acids might increase tansy ragwort toxicity, and decrease survival time. This did not occur (Table 4).

Evidence of protective activity was observed in the treatments with cysteine, BHA, and BCAA, in which increased survival time occurred (Table 4). This may be due to an interaction of BHA and cysteine. BHA is an inducer of glutathione S-transferase in mice (Miranda et al., 1981b), while cysteine is a precursor of glutathione, so the combined treatment could allow increased synthesis of the enzyme, which is involved in conjugation of PA metabolites.

Exp. 2. The longest survival times were with the treatments with BHA and cysteine, with or without vitamin B_{12}-folic acid (Table 5). There was evidence that the vitamin B_{12}-folic acid may have had some influence on PA metabolism, because at day 42, all rats on cysteine + vitamin B_{12}-folic acid were still alive, while mortality had occurred in all other treatments (Table 5). It is likely that the dosage of monocrotaline given substantially exceeded the lethal dose, as there can be a large lag time between

TABLE 5. SURVIVAL, GAINS AND FEED INTAKE OF RATS INJECTED WITH MONOCROTALINE - EXPERIMENT 2

Treatment	Avg. daily gain (g) for 0-29 days	Total diet intake (g)	Survival time (days)	% alive at 42 days
1. Control (saline injection)	10.4 ± .9[a]	1134 ± 76[a]	--[f]	100[a]
2. CIM[g] (monocrotaline injection)	7.9 ± 1.1[b]	537 ± 122[cde]	42.2 ± 6[abc]	40[bc]
3. CIM + Cysteine (cys)	8.7 ± .7[b]	546 ± 75[cde]	43.8 ± 4[abc]	40[bc]
4. CIM + BHA	8.6 ± .2[b]	577 ± 56[cde]	44.0 ± 6[abc]	60[abc]
5. CIM + vitamin B_{12} + folic acid (BF)	8.0 ± 2.3[b]	558 ± 179[cde]	40.2 ± 10[cd]	60[abc]
6. CIM + high B vitamins (HV)	8.7 ± 1.3[b]	596 ± 150[cd]	46.4 ± 6[abc]	80[ab]
7. CIM + BHA + cys	8.1 ± .3[b]	752 ± 211[b]	50.0 ± 8[a]	80[ab]
8. CIM + BHA + cys + BF	8.1 ± .3[b]	654 ± 131[bc]	48.6 ± 4[ab]	100[a]
9. CIM + BHA + cys + HV	7.8 ± .3[b]	575 ± 75[cde]	44.6 ± 7[abc]	20[c]
10. CIM + BHA + BF	7.6 ± .8[b]	428 ± 35[e]	37.2 ± 5[d]	20[c]
11. CIM + BHA + BF	8.0 ± .6[b]	538 ± 113[cde]	37.6 ± 9[d]	40[bc]
12. CIM + cys + HV	8.5 ± 1.1[b]	497 ± 125[de]	37.6 ± 7[d]	20[c]
13. CIM + cys + BF	8.4 ± .9[b]	519 ± 69[de]	40.6 ± 6[bcd]	20[c]

[a,b,c,d,e]Means with different superscripts are different ($P<.05$).

[f]All animals survived until termination of the experiment at 55 days.

[g]control diet + monocrotaline injection.

intake of PA and mortality (Swick et al., 1979). A greater effectiveness of the vitamin treatment might have been apparent if a lower dosage of monocrotaline had been used. There was no indication that a high level of other B vitamins had favorable effects.

Exp. 3. The survival time was increased ($P<.05$) in tansy ragwort-fed rats that received BHA and cysteine, with or without vitamin B_{12}-folic acid (Table 6). The BHA without cysteine was not effective, in agreement with Experiments 1 and 2.

General Discussion. This study has demonstrated that a combination of cysteine and BHA has protective activity in rats against PA toxicity. The probable mechanism of action is that BHA stimulates glutathione-S-transferase activity, while cysteine provides a precursor for glutathione synthesis. The favorable results warrant evaluation of the protective agent mixture with cattle and horses. Cysteine is not available commercially at an economical price, so an alternative precursor would be needed. One possibility is methionine, which can be converted to cysteine *in vivo*. For cattle, methionine hydroxy analog, which is less subject to ruminal degradation than methionine, might be useful.

TABLE 6. EFFECT OF DIETARY SUPPLEMENTS ON SURVIVAL TIME OF RATS FED TANSY RAGWORT - EXPERIMENT 3

Treatment	Avg. daily gain (g) for 0-28 days	Tansy ragwort intake (g)	Tansy ragwort intake as % of initial body weight	Survival time (days)	% alive at 12 weeks	% alive at 21 weeks	% alive at 43 weeks
1. Control	$6.7 \pm .7^{a}$	--	--	307 ± 0^{a}	100^{a}	100^{a}	100^{a}
2. Basal (control + tansy ragwort)	$4.1 \pm .4^{bc}$	69.8 ± 38.3^{a}	71.1 ± 38.5^{a}	100 ± 42^{b}	50^{b}	17^{b}	0^{b}
3. Basal + vitamin B_{12}+ folic acid (BF)	$4.7 \pm .4^{b}$	71.2 ± 14.3^{a}	72.6 ± 14.7^{a}	99 ± 14^{b}	100^{a}	0^{b}	0^{b}
4. Basal + BHA	$3.8 \pm .8^{c}$	72.5 ± 29.3^{a}	73.6 ± 28.5^{a}	103 ± 28^{b}	83^{a}	0^{b}	0^{b}
5. Basal + BHA + BF	$3.8 \pm .7^{c}$	73.0 ± 11.8^{a}	74.3 ± 11.2^{a}	107 ± 15^{b}	100^{a}	0^{b}	0^{b}
6. Basal + BHA + cysteine (cys)	$3.8 \pm .5^{c}$	122.5 ± 13.8^{b}	119.7 ± 24.7^{b}	234 ± 85^{c}	100^{a}	83^{a}	50^{c}
7. Basal + BHA + cys + BF	$4.3 \pm .7^{bc}$	137.0 ± 21.0^{b}	139.0 ± 16.7^{b}	243 ± 75^{c}	100^{a}	83^{a}	50^{c}

^{a,b,c}Means with different superscripts are different ($P<.05$).

CHAPTER III.

Effects of dietary supplementation with butylated hydroxyanisole, cysteine and B vitamins on tansy ragwort (Senecio jacobaea) toxicosis in horses[1]

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SUMMARY

Dried tansy ragwort, which contains pyrrolizidine alkaloids, was fed as 10% of a complete diet to horses, with and without a mixture of additives. The additives provided a dietary supplement equivalent to 1% cysteine, 0.75% butylated hydroxyanisole, 200 ppb vitamin B_{12} and 5 ppm folic acid. The additives did not alter tansy ragwort toxicity, as assessed by survival time, liver histoloty, bromosulphalein clearance rate and serum gamma glutamyl transpeptidase activity. In horses fed tansy ragwort, bromosulphalein clearance rate was a sensitive indicator of liver damage, and declined to a level of about 20% of control values. Gamma glutamyl transpeptidase levels showed considerable variability but in general were elevated in animals fed tansy ragwort. Liver iron and copper levels were elevated, and liver zinc declined in tansy ragwort-fed horses.

INTRODUCTION

Consumption of plants such as tansy ragwort (Senecio jacobaea), common groundsel (Senecio vulgaris) and fiddleneck or tarweed (Amsinckia intermedia) is one of the major causes of liver disease in horses in Oregon, California and Washington. These plants contain pyrrolizidine alkaloids (PA) which cause irreversible liver damage. Various dietary additives including cysteine and butylated hydroxyanisole (BHA), have shown some potential for reducing the toxicity of PA in laboratory animals (Buckmaster et al., 1976; Miranda et al., 1981b). The mode of action of cysteine is to increase liver sulfhydryl activity for conjugation with PA and PA metabolites, while antioxidants such as ethoxyquin and BHA are inducers of glutathione-S-transferase (Miranda et al., 1981a, 1981b) which may catalyze the reaction of PA metabolites with glutathione. Vitamin B_{12} and folic acid were found to enhance the protective effects of a mixture of cysteine and BHA against tansy ragwort toxicosis in rats (Garrett et al., unpublished data). Therefore, the objective of this trial was to assess the effect of a mixture of cysteine, BHA, vitamin B_{12} and folic acid as a dietary additive for horses consuming tansy ragwort.

MATERIALS AND METHODS

Tansy ragwort flowers and leaves were collected in the vicinity of Corvallis, Oregon. The plant material was dried at 45°C, ground, and stored for several months at room temperature. Nine yearling ponies weighing between 73 and 130 kg were distributed

into three groups, consisting of control, control + tansy ragwort (basal) and basal + additives. Levels of the additives added to the diet were: cysteine, 1%; BHA, 0.75%; vitamin B_{12}, 200 ppb; and folic acid, 5 ppm. The vitamins were mixed in a sucrose premix prior to addition to the diet. To encourage initial acceptance of the diets, tansy ragwort was included in the diet at levels of 2.5% for 3 days, 5% for 5 days, and then 10% for the rest of the tansy ragwort feeding periods. Composition of the control diet is shown in Table 7. Tansy ragwort and additives were added in place of grass hay in the control diet. The horses were housed in individual stalls, and fed their respective diets ad libitum. The tansy ragwort-containing diets were fed at intervals in multiple subchronic doses with control diet fed during intervening periods. Initially the ponies were given about 5% of their body weight in tansy ragwort, and later exposures approximated 1% of their body weight. Because the diet acceptance varied among animals, the number of days on the tansy ragwort diets varied considerably among horses. This regime was followed to mimic the field situation in which consumption of the plant is not usually continuous. Also, by continuous administration of tansy ragwort it is possible to have animals consume more than a lethal dose before toxicity signs are observable, and it was considered desirable in this experiment to administer the minimum lethal dose if possible. A total of 4 feeding periods with tansy ragwort were given, with about 40 days on the control diet between each period. Bromosulphalein (BSP) clearance rates were determined

TABLE 7. COMPOSITION AND ANALYSIS OF CONTROL DIET

Ingredient	%
Alfalfa hay	33.3
Grass hay	31.4
Barley	13.3
Corn	6.7
Cottonseed meal	5
Molasses	1.7
Oats	1.7
Soybean meal	3.3
Dried whey	0.7
Limestone	0.3
Dicalcium phosphate	0.3
Salt	0.3
Bentonite	2
Chemical Analysis (dry matter basis)	
Crude protein	11.9
Ether extract	2.6
Cell Wall Constitutents	41.3
Acid Detergent Fiber	29.0

at the beginning and end of each tansy ragwort feeding period. Twenty ml of a 5% s/v solution of BSP sodium salt in isotonic saline was injected into a jugular vein, and blood samples withdrawn at 2.5 minute intervals from the other jugular, following the procedure of Cornelius and Wheat (1957). The serum BSP concentrations were measured using the method of Seligson et al. (1957). The fractional clearance rates (K) were obtained from the slope which was calculated by least squares linear regression. Only K_1 values, or the rate for the first phase of the biphasic clearance curve, were determined.

Blood for hematocrit, hemoglobin[a], and gamma-glutamyl transpeptidase[b] (GGT) analysis was collected in heparinized tubes via jugular puncture. Hemoglobin (HGB) and GGT were determined weekly until 10 weeks, and thereafter every two weeks. Additional determinations were made when clinical signs warranted more frequent analysis. The mean cor-uscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated by standard methods (Schalm et al., 1975).

Liver biopsies were taken periodically, by a biopsy needle and aspiration, to follow the progression of liver damage. Animals were euthanatized when they became moribund. At necropsy the kidney, liver and spleen were weighed. Microscopic examination of tissues were performed. Liver copper, iron and zinc were determined on control pony biopsy specimens and on tansy ragwort

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[a]Sigma method no. 525, Sigma Chemical Co., St. Louis, MO

[b]Sigma method no. 415, Sigma Chemical Co., St. Louis, MO

fed pony necropsy specimens using an atomic absorption technique (Garrett et al., 1982).

Data were analyzed by one way analysis of variance (Steel and Torrie, 1960) and treatments compared to controls by lsd.

RESULTS

All horses fed tansy ragwort became moribund and were euthanatized. Signs of toxicosis included lethargy, anorexia, ataxia, dullness, rough coat, general unthrifty appearance, and a comatose state. Consumption of tansy ragwort is shown in Table 8.

All the liver biopsies taken after the horses had consumed 5% of their body weight of tansy ragwort had mild to severe histopathologic characteristics of PA poisoning. Hepatocellular degeneration evidenced by nuclear (vesiculation or) vacuolization and mild swelling (Fig. 1) occurred as one of the earliest signs of hepatotoxicity. This was usually accompanied by slight portal fibrosis and biliary hyperplasia which became more pronounced as the disease progressed. In advanced stages there was periportal hepatocytic necrosis and fibrosis, marked biliary hyperplasia, moderate numbers of megalohepatocytes, evidence of bile stasis, and accumulation of lymphocytes and siderophages in portal areas (Fig. 2). Two ponies had multiple hyperplastic hepatocyte nodules in liver specimens taken at necropsy (Fig. 3). At necropsy, all animals fed tansy ragwort had severe chronic hepatic damage. The livers were dark, small, firm in texture.

TABLE 8. CONSUMPTION OF TANSY RAGWORT BY HORSES

Treatment	Horse No.	Initial body weight (kg)	Amount of Tansy Ragwort consumed (kg)	No. of days fed tansy ragwort	Tansy Ragwort intake as percent of initial body weight	Survival time (days)
Basal	4	80	8.4	159	10.5	297
	5	114	7.2	68	6.3	151
	6	73	3.8	101	5.1	113
Mean		89	6.5	109	7.3	187
Basal + Additives						
	7	130	6.7	43	5.1	64
	8	111	10.1	124	9.1	297
	9	127	11.9	110	9.4	297
Mean		123	9.6	92	7.9	219

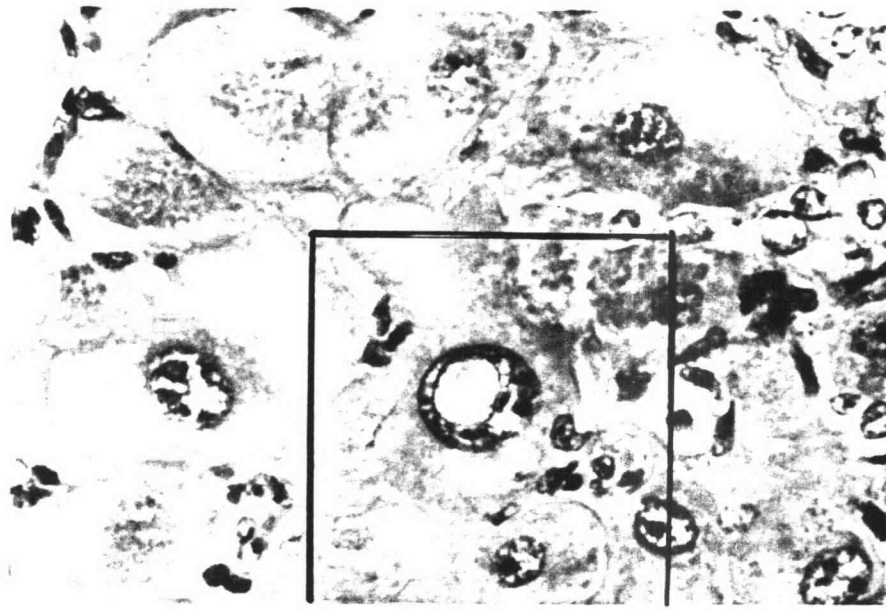

Figure 1. Photomicrograph showing enlarged hepatocyte with enlarged vacuolated nucleus in liver of horse fed basal diet plus Senecio jacobaea. Hematoxylin and eosin, x788.

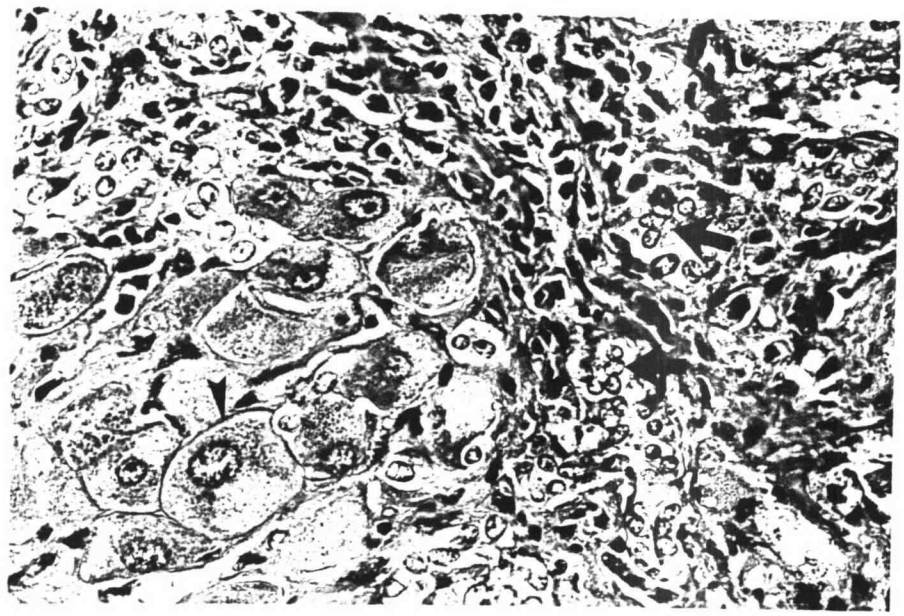

Figure 2. Photomicrograph of portal and periportal area in liver of horse fed basal diet plus Senecio jacobaea. Note portal and periportal fibrosis, biliary epithelial hyperplasia (arrows), and megalohepatocytes (arrow head). Hematoxylin and eosin, x500.

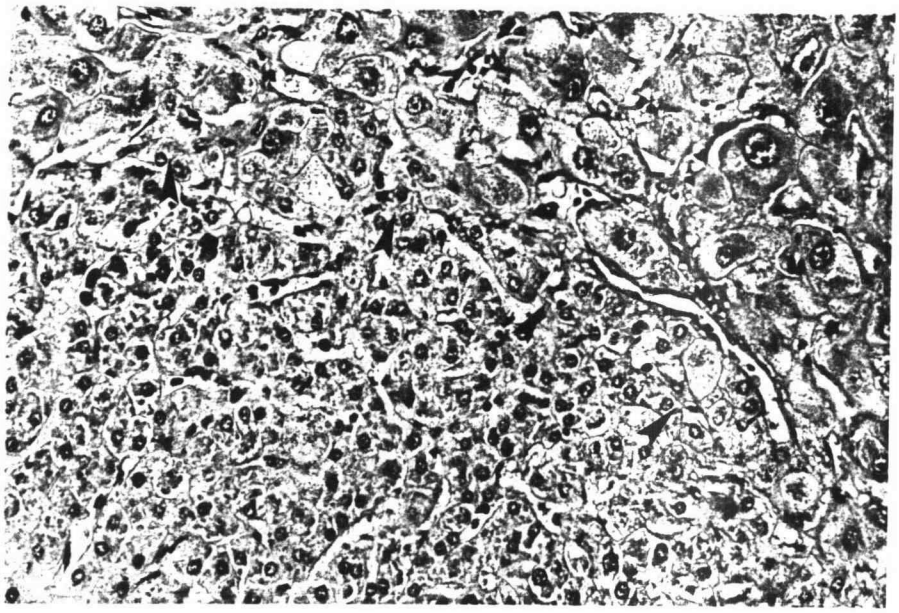

Figure 3. Photomicrograph of liver of horse fed basal diet plus Senecio jacobaea showing portion of hyperplastic hepatocytic nodule. Note dense population of hepatocytes of approximate normal size in lower left portion, abnormally enlarged hepatocytes in upper right portion, and indistinct line of demarcation between the two (arrowheads). Hematoxylin and eosin, x312.

Icterus was evident and three horses had ascites. Several had multiple tiny necrocalcific foci in the collecting ducts of the kidney suggesting diminished urine flow.

There were no pronounced differences in hematological values (Table 9). A marked drop in BSP clearance rate occurred (Table 10) in horses fed tansy ragwort, with a decline of about 80% during the course of the trial. A pronounced elevation in GGT occurred. These changes are shown for individual animals in figures 3 and 4.

Since the control horses were not euthanatized, organ weights were not obtained for this group. Compared to normal values obtained from 2 clinically normal ponies necropsied at the Oregon State University School of Veterinary Medicine, the livers and lungs of tansy ragwort-fed horses appeared smaller than normal (Table 11).

The liver copper and iron levels tended ($P<.05$) to be increased in the tansy ragwort fed horses, while the liver zinc values were lower ($P<.05$) than for the controls (Table 12).

DISCUSSION

Horses fed tansy ragwort developed typical signs of PA toxicosis. The additive mixture of cysteine, BHA and B vitamins did not substantially prevent PA toxicosis, in contrast to previous results obtained with rats (Garrett et al., submitted; Buckmaster et al., 1976) and mice (Miranda et al., 1981a; Miranda et al., 1981b). Johnson (1982) recently reported negative results with

TABLE 9. HEMATOLOTICAL CHARACTERISTICS OF HORSES FED TANSY RAGWORT. RANGES OF VALUES OF EACH PARAMETER DURING THE EXPERIMENTAL PERIOD.

Treatment	Horse number	Hemoglobin (g/dl)	Hematocrit (%)	Mean corpuscular hemoglobin concentration (%)	Red blood cells (no. x 10^6)	Mean corpuscular volume (μ)
Control						
	1	9-15	21-38	31-45	7.7-10.7	29-39
	2	11-16	26-41	36-47	9.8-11.1	24-36
	3	9-17	22-39	25-51	7.0-11.1	33-43
Basal						
	4	9-16	29-35	20-65	6.6-9.9	36-45
	5	9-15	20-36	25-64	4.8-7.9	42-56
	6	10-19	22-47	37-53	7.4-10.4	29-39
Basal + additives						
	7	9-18	21-49	25-54	9.4-14.8	14-36
	8	10-20	22-45	29-45	7.3-10.9	26-40
	9	10-21	20-48	28-49	6.4-10.7	27-51
Base line*		9-17	21-41	25-51	4.8-11.1	25-46

*Values obtained on all animals before feeding of tansy ragwort began.

TABLE 10. BROMOSULPHALEIN (BSP) CLEARANCE RATES AND PLASMA GAMMA-GLUTAMYL TRANSPEPTIDASE (GGT) LEVELS IN HORSES FED TANSY RAGWORT

Treatment	Horse Number	BSP clearance rate (K1 value)			GGT (units/ml)		
		Initial value	Final value	% decline in K1 value	Initial value	Final value	Range during trial
Control	1	.23	.22	-4	29	9	0-29
	2	.23	.27	+17	11	9	0-30
	3	.29	.28	-3	22	4	0-22
Basal	4	.28	.05	-82	13	57	0-121
	5	.25	.12	-52	18	204	8-230
	6	.28	.09	-68	7	151	2-151
Basal + additive	7	.29	.03	-89	36	226	4-226
	8	.30	.05	-83	7	43	6-91
	9	.30	.03	-90	29	29	3-110

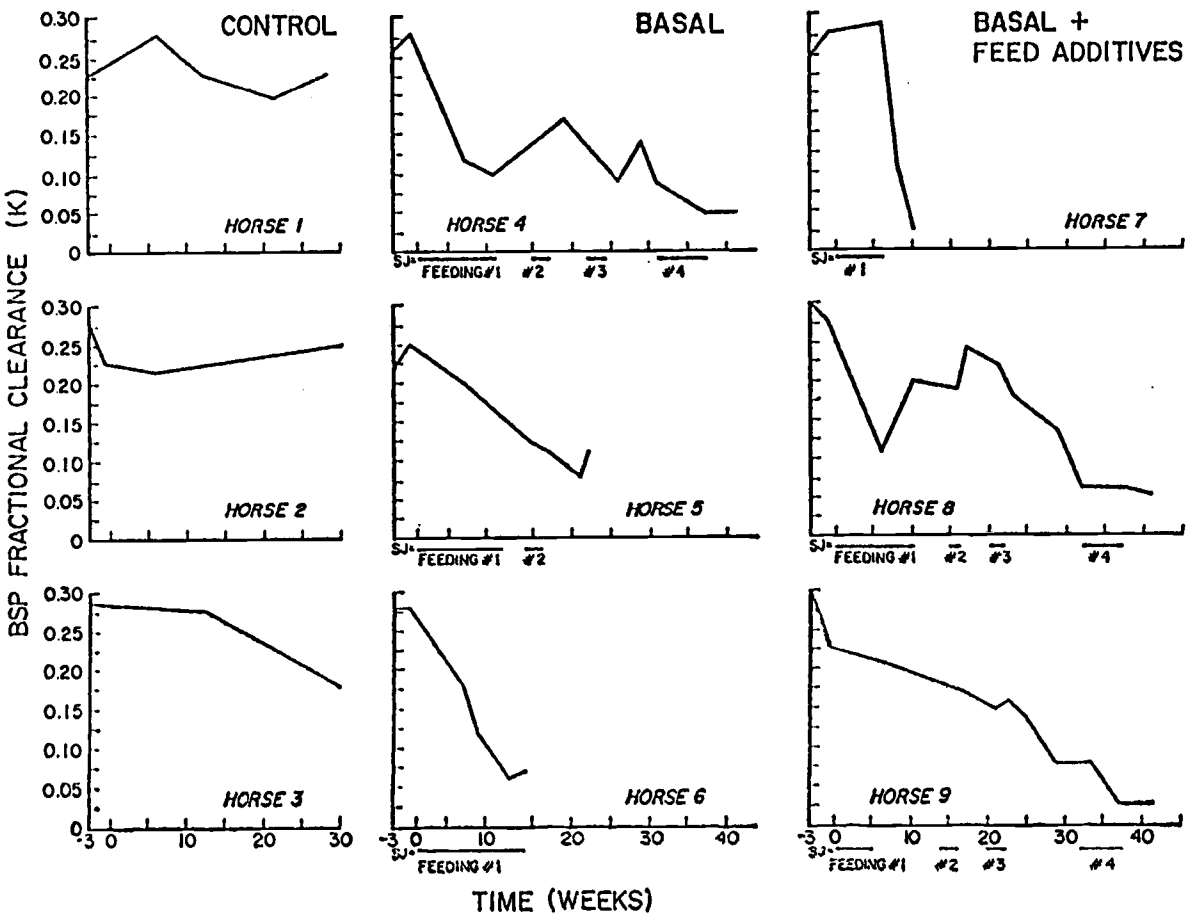

Figure 4. Bromosulphophtalein fractional clearance rates (K_1) in ponies fed control diet and intermittent Senecio jacobaea (SJ) diets. Ponies consumed approximately 5% their body weight in tansy ragwort during first feeding period and variable amounts at subsequent feeding periods.

TABLE 11. ORGAN WEIGHTS FOR HORSES FED TANSY RAGWORT

Treatment	Organ weight as % of body weight				
	Liver	kidney	Spleen	Heart	Lung
Basal	1.28	0.17	0.17	0.58	0.87
Basal + additives	0.94	0.26	0.24	0.59	1.00
Normal values*	1.49	0.17	-	0.64	1.78

*Obtained from 2 clinically normal animals at the OSU School of Veterinary Medicine.

TABLE 12. DIET AND LIVER MINERAL LEVELS OF HORSES FED TANSY RAGWORT

Treatment	Mineral		
	Copper	Zinc	Iron
Diet*			
Control	12.0	40.0	414
Basal	8.9	22.7	169
Basal + additives	17.8	36.7	352
Liver*			
Control +	27	151[a]	694[a]
Basal ‡	155	80[b]	1042[a,b]
Basal + additives ‡	153	98[b]	1505[b]

* Expressed as ppm on dry weight basis.

+ Analysis made on liver biopsies

‡ Necropsy specimen

dietary additives in prevention of tansy ragwort toxicosis in cattle. There are marked species differences in metabolism of PA (Shull et al., 1976) and the susceptibility of animals to PA toxicosis. These differences probably explain why additives such as BHA and cysteine have shown promising activity in laboratory animals but appear to be ineffective in horses.

The BSP clearance rate appears to be a sensitive indicator of hepatic damage due to PA consumption. The values for the control animals (Fig. 4) were similar to the normal values (.255±.05) reported by Cornelius and Wheat (1957). Serum GGT values showed a high degree of variability (Fig. 5), and in horses fed tansy ragwort GGT values fluctuated between elevated and normal values in some cases. Thus the use of GGT as an indicator of exposure to PA's, as proposed by Craig (1979), may have some limitations, depending on time of sampling.

The tendency for increased iron and liver copper and decreased liver zinc in horses fed tansy ragwort is in agreement with previous observations with sheep (Bull et al., 1956; Swick et al., in press), rats (Swick et al., 1982b) and rabbits (Swick et al., 1982c). Liver hemosiderin deposits probably accounted for increased liver iron. The cause(s) of the elevated liver copper seen in PA toxicosis is not conclusively known, but appears to be associated with an impairment of normal subcellular copper excretory mechanisms (Swick et al., 1982d).

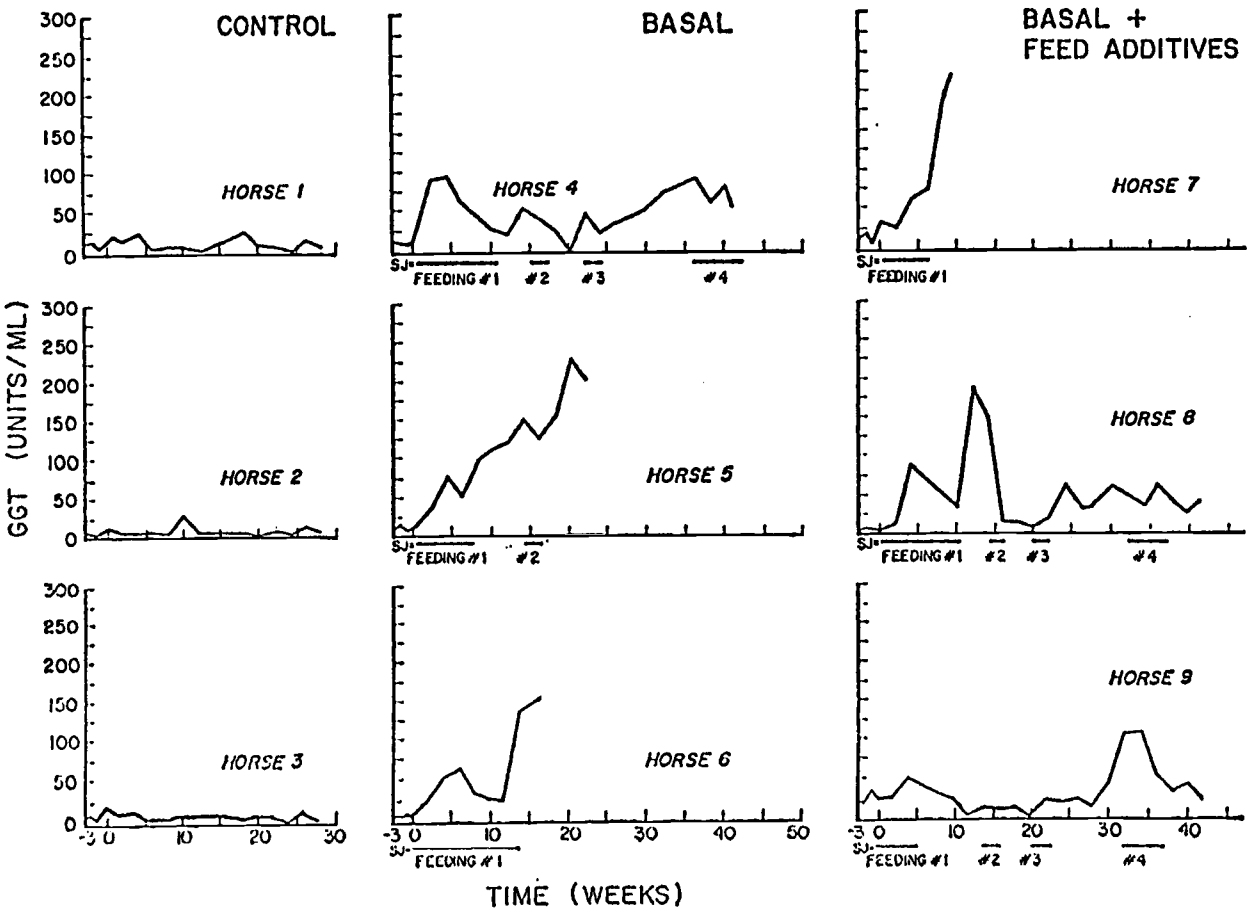

Figure 5. Serum γ-Glutamyl-Transpeptidase levels in horses fed control diet and intermittent Senecio Jacobaea (SJ) diets. Ponies consumed approximately 5% their body weight in tansy ragwort during first feeding period and variable amounts at subsequent feeding periods.

CONCLUSION AND SUGGESTIONS FOR FUTURE RESEARCH

Tansy ragwort toxicity as influenced by dietary factors was studied in rats and horses. These factors included poisonous plants, amino acids, B vitamins, butylated hydroxyanisol (BHA), and phenobarbital.

The parameters examined included survival time, feed intake plus histological and biochemical analyses with prognostic value. It is hoped that information gained from these experiments will ultimately reduce livestock losses due to tansy ragwort poisoning.

During the amino acid, B-vitamin and BHA evaluation for protective activity against tansy ragwort toxicity in rats, greater protection might have been noted if a lower level of tansy ragwort and monocrotaline were used over a shorter period of time. A lower level would have less of a possibility of overwhelming all the mechanisms associated with some of the dietary supplements.

The time for termination of the pyrrolizidine alkaloid exposure could be ascertained by liver histopathology on a predetermined number of rats. Dietary supplementation of feed should continue for the remaining rats. At the time of experimental termination, indices of toxicity could include weight loss, severity and frequency of liver lesions, organ weight as percent of body weight, and liver microsomal enzyme activity.

The above parameters could also be followed using large animals. Liver biopsies, BSP clearance rate, plus clinical signs could serve as indices of when to terminate tansy ragwort feeding. A smaller percent of dietary tansy ragwort then was used in the pony experiment

would improve palatibility and decrease chances of overwhelming protective mechanisms. If at a specific time interval all the large animals were euthanitized it would be of interest to determine liver microsomal enzymes, especially epoxide hydrolase. Epoxide hydrolase activity is elevated by carcinogens and is suggested to play a role in the neoplastic process. This enzyme also increased in rats when fed the poisonous plants, tansy ragwort and bracken. These plants contain known carcinogens. Hyperplastic hepatocyte nodules, as seen in the pony experiment, are considered to be precursors to hepatocellualar carcinomas. Therefore it would be of interest to find out if a correlation between these lesions and the enzyme exists.

The BSP liver clearance test appears to be a sensitive indicator of hepatic damage due to tansy ragwort consumption in horses. It would be of value to determine it's usefullness in other large animal species such as goats, cattle and sheep. Lanigan (1979) has shown that this test has prognostic value in sheep who had consumed Heliotropium europaeum another pyrrolizidine alkaloid containing plant.

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APPENDIX

APPENDIX 1. EVALUATION OF DIETARY SUPPLEMENTATION WITH ZINC, AMINO ACIDS AND BHA AS PROTECTIVE AGENTS AGAINST TANSY RAGWORT (SENECIO JACOBAEA) TOXICOSIS IN RATS

Orally administered zinc salts provide protection against the liver damage induced in poisoning by copper in pigs (Suttle & Mills, 1966), and sheep (Brenner et al., 1976), carbon tetrachloride in rats (Chrapil et al., 1973; Cagen and Klaassen, 1980), sporidesmin in rats (Towers, 1977), cattle (Towers and Smith, 1978) and sheep (Smith et al., 1977), and lupinosis in sheep (Allen and Masters, 1980).

Tansy ragwort (Senecio jacobaea) contains pyrrolizidine alkaloids (PA's) which cause irreversible liver damage in livestock consuming the plant. It has caused extensive livestock losses in the U.S. Pacific Northwest, and in other temperate regions such as Nova Scotia, New Zealand, Australia, South Africa and western Europe. The PA's are bioactivated by hepatic enzymes to pyrroles, which react with hepatic macromolecules, causing liver necrosis (McLean, 1970).

The objective of this experiment was to determine if zinc (Zn) would protect against the chronic toxicity of tansy ragwort. Cysteine has been shown to be protective against tansy ragwort toxicity in rats (Cheeke and Garman, 1974) and when fed in combination with Butylated Hydroxyanisol (BHA), increased the LD_{50} in mice for monocrotaline, a PA from the plant Crotalaria (Miranda, 1980). Branch chained amino acids, valine, leucine and isoleucine (BCAA), are also thought to have protective qualities against PA poisoning (Gulick et al., 1980). A combination

of these compounds fed in a diet might show a synergistic resistance in rats to PA toxicity. This possibility was also examined.

MATERIALS AND METHODS

The plant material was collected in the vicinity of Corvallis, Oregon. The tansy ragwort was in full bloom. The plants were dried at 45°C, finely ground in a Wiley mill, and stored at room temperature.

Five male long-evans rats of about 110 grams initial weight were assigned to each of 6 treatments: control, control + tansy ragwort (basal), basal + Zn, basal + Zn + cysteine, basal + cysteine, basal + Zn + cysteine + BCAA + BHA. Diets were fed for one week before adding the tansy. The tansy was fed at a level of 5%, Zn was fed at 2000 ppm as acetate in a sugar premix, cysteine was fed at a level of 1%, BCAA's were fed at a level of 1% each, and BHA was fed at a level of .75% of the diet. The basal diet was that of Miranda et al. (1980). The protective compounds were added in place of the complete diet (eg. 90% basal, 5% tansy, 5% Zn premix). Rats were housed individually in galvanized cages until death. The rats on the basal diet were sacrificed on day 123. Growth and feed intake data were recorded. Data were analyzed by analysis of variance (Steel and Torrie, 1960), and treatments compared to appropriate controls by lsd.

RESULTS AND DISCUSSION

Compared to the basal diet, none of the treatments improved

gain or increased survival time. The rats fed the basal + Zn + cysteine diet did consume a greater amount of tansy ($P<.05$) and did not have a decreased survival time. This indicates that there was some protective activity from this treatment.

A biological consequence of zinc exposure, regardless if it is injected or included in the diet, is to increase hepatic concentrations of metallothionein (Bremmer and Davies, 1975; Richards and Cousins, 1975; Chen et al., 1974). Metallothionein is a low-molecular weight protein (10,000), which characteristically has a high (30%) cysteine content occuring in the absence of disulfide linkages. It is thought that metals such as cadmium and mercury avidly bind to metallothionein via sulfhydryl groups and this reduces toxic action of metals at other sites (Piotrowski et al., 1973; Winge et al., 1972). The same detoxification mechanism is thought to occur with CCl_4 (Cagen and Klaassen, 1979 & 1980). Therefore the protective activity of the basal + Zn + cysteine might be due to zinc inducing the synthesis of metallothionein, with excess cysteine available for this synthesis, and the sulfhydryl groups on metallothionein being available to sequester the reactive metabolites of tansy ragwort.

The BCAA and BHA additives were slightly antagonistic to the protecive effects of Zn and cysteine.

APPENDIX
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APPENDIX
TABLE 1. WEIGHT GAINS, AND SURVIVAL TIMES FOR RATS FED TANSY RAGWORT AND FEED ADDITIVES

Treatment	Wk 4 ADG	Survival Time(days)	TA intake grams	Tansy as % of Initial Body Wt.
Control	7.2±.5[a]	123[g]		
Control + 5% Tansy ragwort (basal)	4.3±.4[b]	72±26[b]	63.2±26[a]	57.7±24[ab]
Basal + Zinc (Zn)	3.3±.9[c]	95±24[b]	66.2±18[a]	60.6±16[ab]
Basal + Zn + Cysteine (Cys)	4.4±.2[bc]	89±16[b]	76.4±17[b]	69.3±13[b]
Basal + Cys	4.0±.6[bc]	81±11[b]	60 ± 8[a]	54.5± 6[ab]
Basal + Zn + Cys + BHA + Branched chain amino acids	3.3±.9[c]	83±20[b]	54 ± 6[a]	49.2± 6[a]

[a,b]Means±SD values with different superscripts differ (P<.05)

[g]All animals survived until 123 days