EXAMINATION OF TROPICAL LEGUMES FOR DELETERIOUS EFFECTS ON ANIMAL REPRODUCTION

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Summary

The effects of dietary plant material from 27 pasture legumes on reproduction of mice have been examined. Seed of ***Indigofera spicata*** and the amino acid mimosine, extracted from ***Leucaena leucocephala,*** caused death of embryos when fed to pregnant mice. Leaf material of these species at levels up to 30% had only slight effects on pregnancy. Lactation, as measured by weaning weight of litters, was reduced in mice fed mimosine, but was unaffected by diets containing ***L. leucocephala*** and ***Trifolium repens.*** The response by the mouse to gonadotrophin was reduced following a three-day feeding period on ***L. leucocephala.*** Pregnant ewes, fed ***ad libitum*** on ***L. leucocephala*** from 30 or 90 days post-coitus produced lambs of low birth weight and low viability. These lambs and lambs of ewes fed ***L. leucocephala*** from the time of parturition gained less weight than lambs from ***ewes*** fed ***Medicago sativa*** hay.

I. INTRODUCTION

With the development of pasture plants, especially legumes (Hutton 1962; Bryan 1965; Davies and Eyles 1965), for use in northern Australia, it has become necessary to institute a program of screening for deleterious substances. The C.S.I.R.O. Division of Tropical Pastures conducts biochemical studies of known plant toxic substances (for example, see Hegarty, Schinckel and Court 1964) and arrangements have also been made for chemical screening of new legumes for oestrogens. This paper describes screening studies in which leaf and seed from legumes were incorporated into the diet of mice to investigate possible deleterious effects on reproduction. An experiment was also carried out in sheep to extend information gained in one plant to a domestic animal species.

II. MATERIALS AND METHODS

(a) Animals

Nulliparous albino mice of the Sydney White strain, aged eight weeks and weighing in excess of 22 g, were maintained under uniform conditions of lighting, temperature and humidity. The animals were fed ad ***libitum*** on mouse cubes offered in a wire feeding basket.

The sheep used were four-year-old, parous Merino ewes kept in covered concrete yards. They were fed milled hay in troughs. Water was available at all times.

(b) Plant material

Air-dried plant (leaf and stem) material was ground, mixed with crushed mouse cubes and re-pelleted so as to provide experimental diets containing from 1 to 30% of legume. Seeds were crushed, mixed with ground mouse cubes and offered in that form inside the mouse cage. All feeding was ***ad libitum.***

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The following legumes were screened: ***Trifolium repens*** (leaf), ***Leucaena leucocephala*** (formerly ***L. glauca)*** (leaf), ***Indigofera spicata*** (tetraploid and diploid strains) (leaf and seed), ***Soya maxima*** (seed), *Phaseolus* ***lathyroides*** (seed), *Phaseolus* ***atropurpureus*** (leaf, seed) **,** *Phaseolus* ***bracteaticus*** (leaf), ***Desmodium intortum*** (leaf, seed), ***Desmodium uncinatum*** (leaf, seed), ***Lotononis baineseii*** (leaf, seed), ***Lotononis angolensis*** (leaf), ***Glycine javanica*** (seed), ***Centrosema*** *pubescens* (seed), ***Dolichos*** *lablab* (leaf), ***Dolichos axillaris*** (leaf), ***Crotalaria rhodeseii*** (leaf), ***Vigna vexillata*** (leaf), *Vigna* ***CQ 502*** (leaf), ***Vigna 25380*** (leaf), ***Vigna*** *marina* (leaf), ***Stylosanthes fruticosa*** (leaf), ***Stylosanthes*** 9215 (leaf), *Cassia rotundiflora* (leaf), ***Alysicarpus rugosus*** (leaf), ***Alysicarpus*** CQ5 12 (leaf), *Desmanthus* spp. (leaf), *Teramnus* ***uncinatus*** (leaf). In addition, the amino acid, mimosine, obtained from ***Leucaena leucocephala*** (Hegarty, Schinckel and Court 1964), was studied.

(c) Screening procedure

(i) Effects ***on oestrous cycles and conception***

Adult female mice, four per cage, were fed ***ad libitum*** on diets containing 10 or 30% of plant material for seven days (not including ***I. spicata*** or ***L. leucocephala).*** Body weight changes were recorded during this period. On the eighth day the normal diet replaced the test diet and males were introduced. There were eight mice per treatment. Daily examination for the vaginal plug revealed the date of mating.

(ii) Effects on pregnancy

Groups of eight pregnant mice were fed diets containing various amounts of legume leaf or seed for six days, commencing on the 12th day post-coitus. The animals were slaughtered on the day of completion of feeding period and the number of normal and degenerating foetuses counted. Pregnant mice fed a normal diet were slaughtered at similar times.

(d) Further ***studies***

Plants and extracts considered to have possible deleterious effects were studied in greater detail, as described in the next section.

III. RESULTS

Non-pregnant female mice generally failed to maintain body weights during a seven-day period on diets containing 30% legume. Apart from a slightly longer interval to mating after introduction of the male, compared with mice on a normal diet or a diet containing 10% legume, there was no adverse effect on mating performance with any of the plants studied.

Tests on pregnant mice were also negative for all plants except ***I. spicata.***

(a) Studies with Indigofera spicata

Leaf and seed of two strains of ***I. spicata*** were fed to groups of mice in late pregnancy and additional tests were carried out with leaf material fed during early pregnancy. The numbers of normal and degenerating foetuses observed at slaughter are shown in Table 1. Results for mice fed normal laboratory diet and a diet containing 10% or 30% ***T. repens*** are included for comparison.

I. spicata seed when included at a level of 5 % had deleterious effects when fed during days 12-18 of pregnancy. Leaf material from the tetraploid strain at

TABLE 1

Summary of effects of dietary Indigofera spp. on pregnancy of the mouse

Diet	Dietary level (%)	No. of Mice	Feeding Period (days*)	Mean No. of Foetuses at Slaughter ± S.E. Normal	Degenerate
Control (no treatment)		20	10-18	7.2±0.3	0.5±0.2
		20	5-12	8.0±0.3	0.3±0.2
Trifolium repens leaf	10	20	12-18	7.1±0.4	0.6±0.3
	30	20	12-18	6.7±0.6	0.3±0.1
	10	8	5-12	7.5±1.1	0.4±0.3
	30	8	5-12	7.5±1.3	1.4±0.6
I. spicata seed (CPI. 30492) tetraploid	5	5	12-18	2.4±1.0	3.8±1.6
	10	5	12-18	5.0±1.9	3.4±1.7
	20	5	12-18	1.0±1.0	5.0±1.9
I. spicata seed (CPI.18557) tetraploid	5	5	12-18	3.7±1.0	3.5±0.7
	10	5	12-18	2.6±1.6	5.6±1.5
	20	5	12-18	0.0	5.0±2.1
I. spicata leaf (CPI.18557) tetraploid	0.8	5	12-18	9.0±0.3	0.8±0.2
	4.0	5	12-18	7.4±0.7	0.4±0.2
	20.0	5	12-18	6.8±0.1	1.4±0.7
	30.0	8	12-18	9.4±0.4	0.1±0.1
I. spicata leaf (CPI.24205) diploid	10	5	12-18	9.2±0.4	0.4±0.2
	30	5	12-18	5.6±2.1	2.2±1.3
	30	8	5-12	0.5±0.5	0.4±0.4
I. spicata hepatotoxin†	0.5	5	6- 8	6.2±0.5	1.4±0.7
	seed	5	6-12	6.2±0.8	1.4±0.7
	equiv.	6	12-14	6.0±0.9	0.5±0.2
	/day	6	12-18	5.2±0.6	1.2±0.3

* Days post-coitus.

† Injected subcutaneously each day.

a level of 30% had no effect at this stage of pregnancy while leaf material from the diploid strain fed at the same level (30%) caused a slight reduction in the number of normal foetuses when fed during days 12-18, and a very marked reduction when fed during days 5-12.

(b) Studies with Leucaena leucocephala *and mimosine*

(i) Effect on body weight changes

Adult female mice maintained body weight on 10% dietary ***L.*** *leucocephala* and 0.2% mimosine but lost weight (0.1 g/head/day) on ***30% L. leucocephala*** and 0.8% mimosine (0.4 g/head/day) during a 10-day feeding period ***(ad libitum*** but unknown intake) . Replacement of normal diet and introduction of males was followed by mating and conception in these mice.

(ii) Effect ***on pregnancy***

Leucaena leucocephala (8% and 15%) and mimosine (0.05% and 0.5 %) were fed to groups of pregnant mice during 6-12 days post-coitus. Fewer

TABLE 2

Summary of *effects* of ***dietary*** Leucaena leucocephala ***and mimosine on pregnancy*** of ***the mouse***

Diet	Dietary Level (%)	No. of Mice	Feeding Period (days*)	Mean No. of Embryos at Slaughter ± S.E. Normal	Degenerate
L. leucocephala (leaf)	8	8	6-12	5.9±0.4	1.1±0.5
	15	8	6-12	8.2±0.4	0.4±0.8
	8	8	12-18	7.8±0.2	0.0
	15	8	12-18	7.5±0.7	0.4±0.1
Mimosine	0.05	4	6-12	3.3±0.3	0.2±0.2
	0.5	4	6-12	2.5±2.5	0.0
	0.05	4	12-18	3.0±1.7	0.2±0.2
	0.5	4	12-18	2.0±1.4	0.5±0.5
Control (no treatment)		8	6-12	8.0±0.5	0.2±0.1
		8	12-18	7.4±0.6	0.0

* Days post-coitus.

embryos were present at slaughter in the mice treated with mimosine than in the other groups. Resorption of dead embryos must have occurred since few were degenerating at the time of slaughter. These results are presented in Table 2.

(iii) Effect on lactation

Lactating mice, each with four offspring, were fed ***ad libitum*** during either days 1-22 or 7-22 post-parturition on ***L. leucocephala*** (1, ***8,*** 15 %) , ***Trifolium repens*** (15%) or mimosine (0.5%) . The body weight changes of all mice were recorded. There were seven litters per treatment group. The results, presented in Table 3, indicate reduced litter growth and greater body weight loss in dams

TABLE 3

Effect of ***dietary legume fed to lactating mice on weight change*** of ***dam and weaning weight*** of ***litter (4 offspring per litter)***

Diet	Dietary Level (%)	No. of Litters	Feeding Period (days*)	Mean Weight Change of Dam (g)	Mean Litter Weaning Weight ±S.E. (g)
L. leucocephala	1	7	1-22	—0.7	49.5±1.4
	1	7	7-22	+1.2	47.2±2.3
	8	7	1-22	—0.5	48.4±2.0
	8	7	7-22	—1.0	48.7±2.7
	15	7	1-22	—0.6	45.6±1.3
	15	7	7-22	+0.3	47.8±1.8
Trifolium repens	15	7	1-22	+1.6	44.2±2.8
	15	7	7-22	+1.0	47.8±1.7
Mimosine	0.5	7	1-22	—5.3	31.6±2.0
	0.5	7	7-22	—4.8	43.0±2.1

* Days post-parturition.

fed a diet containing 0.5% mimosine. The other diets had no adverse effect on these responses.

(iv) Effect on the mouse uterine response to gonadotrophin

In the second experiment, three doses (chosen to include the linear portion of weaned) were fed for periods of five or three days post-weaning ***ad libitum*** on diets containing ***L. leucocephala.*** In the first experiment, the mice were fed for five days post-weaning and were then killed and uterine weights were obtained. In the second experiment, three doses (chosen to include the linear portion of the dose-response line) of pregnant mare serum gonadotrophin (PMS) were injected into groups of four mice at the completion of the feeding period of three days post-weaning and the uterine weights were determined 48 hours later. Mice on the standard laboratory diet or a diet containing ***T. repens*** were treated with PMS also. The results are presented in Table 4. In the second test the results show a reduced uterine response to PMS after a short period of ***L. leucocephala*** when compared with the response after either control or ***T. repens*** diets. In neither test was the observed difference attributable to body weight differences of the test animals.

(v) Effect on pregnancy of the ewe

Twenty Merino ewes (the number of animals used was limited by the availability of ***L. leucocephala*** leaf material), diagnosed pregnant by exploratory laparotomy at 30 days post-coitus, were fed a daily ration of approximately 1 kg lucerne ***(Medicago sativa)*** hay from this time. Five ewes were transferred to ***ad libitum*** dried leaf of ***L. leucocephala*** from 30 days and five ewes from 90 days post-coitus. A further group of five ewes was treated in this way from the time of parturition, while another group remained on lucerne throughout. All

TABLE 4

Effect of dietary Leucaena leucocephala *and* Trifolium repens *on the uterine weight response by the immature mouse to gonadotrophin*

Diet	Dietary Level (%)	Feeding Period (days*)	No. of Mice	Gonadotrophin Treatment (i.u. PMS)	Mean Body Wt. ±S.E. (g)	Mean Uterine Wt. ±S.E. (mg)
L. leucocephala	1	5	8	nil	17.6±0.5	14.9±1.4
—expt. 1	15	5	8	nil	16.3±0.6	10.4±0.8
L. leucocephala	15	3	4	0.1	13.7±0.7	12.6±1.4
—expt. 2	15	3	4	0.2	14.0±0.9	20.2±0.6
	15	3	4	0.4	13.6±1.2	25.2±1.7
T. repens	15	3	4	0.1	13.2±0.7	12.4±0.9
—expt. 2	15	3	4	0.2	13.7±0.5	23.9±5.9
	15	3	4	0.4	13.0±0.5	37.0±5.2
Control		3	4	0.1	13.7±0.3	12.2±1.5
—expt. 2		3	4	0.2	13.4±0.6	28.0±3.9
		3	4	0.4	13.3±0.2	33.8±2.2

* Days post-weaning.

TABLE 5

Effect of ***dietary*** Leucaena leucocephala ***on reproduction*** **of** ***the Merino ewe. The ewes received*** Medicago sativa ***prior to receiving*** L. leucocephala

Treatment	Pregnant Ewes Treated	Mean Weight Change 90-150 Days p/c ±S.E. (kg)	No. of Lambs Born	No. of Lambs Surviving to 7 Days Age	Mean Birth Weight ±S.E. (kg)	Mean Lamb Weight Change/Day to 25 Days Age (kg)	
Leucaena leucocephala fed *ad libitum* from 30 days post-coitus (p/c)	5	−0.6 ± 0.5	6	2	2.6 ± 0.1	0.15	(2)*
L. leucocephala fed *ad libitum* from 90 days post-coitus	5	+0.9 ± 0.6	4†	3	2.5 ± 0.2	0.11	(3)
L. leucocephala fed *ad libitum* from parturition	5	+4.8 ± 0.5	5	5	3.7 ± 0.2	0.16	(5)
Medicago sativa (approx. 1 kg/head/day)	5	+4.9 ± 0.9	5	4	4.0 ± 0.2	0.23	(2)

* Bracketed figures are the numbers of lambs contributing to the estimate.

† One animal aborted in late pregnancy.

animals were weighed at approximately weekly intervals and birth weights and growth rates of lambs recorded up to one month post-parturition. Daily intake of plant material was estimated on four occasions during pregnancy and twice during lactation.

The results, shown in Table 5 and Figure 1, reveal that ewes fed ***L. leucocephala*** during pregnancy failed to maintain their body weights during the last 60 days of gestation in spite of intake figures of 1.4-1.6 kg/head/day during pregnancy and 1.8-2.0 kg/head/day during lactation. Birth weights were lower and neonatal mortality higher in lambs from ewes fed ***L. leucocephala*** during pregnancy. Lambs from ewes fed lucerne gained weight more rapidly than those from ewes fed ***L. leucocephala*** during lactation in the period to 25 days post-parturition. Three ewes, including one on lucerne, and four lambs, including one from the lucerne treatment, were killed and examined post-mortem. Both ewes fed ***L. leucocephala*** had oesophageal ulcers and enlarged thyroids (>25 g), and one ewe had an enlarged anterior pituitary gland (2.8 g). The lambs born from these ewes had enlarged thyroids and were devoid of thymus tissue.

IV. DISCUSSION

These observations indicate that 26 of the 28 species examined had no deleterious effects on reproduction of the mouse when included at levels up to

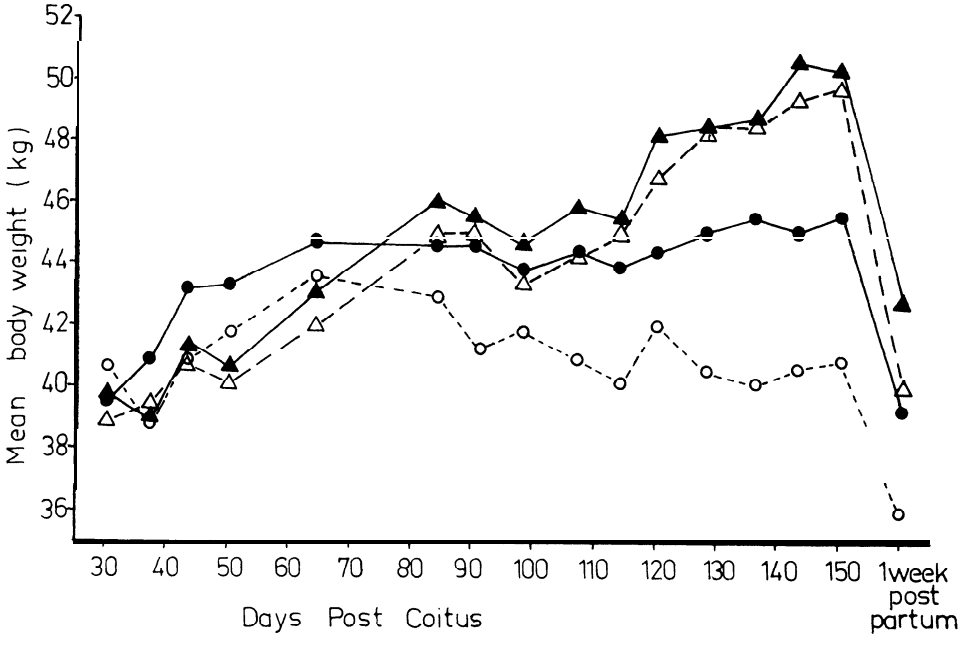

Fig. 1.—Bodyweights of groups of ***five ewes*** fed ***M. sativa*** or ***L. leucocephala*** during pregnancy or early lactation.

0 - - - 0 *L. **leucocephala*** fed ***ad libitum*** from 30 days post-coitus.

● —— ● ***L. leucocephala*** fed ***ad libitum*** from 90 days post-coitus.

A - - - △ *L. **leucocephala*** fed ***ad libitium*** after parturition.

A - - - ▲ *M. **sativa*** fed throughout.

20% (seed) or 30% (leaf) of the normal diet. Body weight loss at high dietary levels was common but without observed effects on reproduction.

Studies with *Indigofera spicata* revealed deleterious effects of seed material on late pregnancy, even at 5% dietary levels. Leaf material was relatively less harmful when fed in late pregnancy but caused failure of pregnancy when fed (30%) from the fifth to the twelfth day of pregnancy.

The incorporation of mimosine into mouse diets during pregnancy caused embryonic losses. Lactation was not impaired in mice on diets of up to 15% *L. leucocephala* and 15% *T. repens* but was reduced, as evidenced by lower weaning weights, in mice receiving 0.5% mimosine. The latter effect may be attributed to reduced intake of the diet, as evidenced by greater weight loss of dams.

Reduced uterine weights after injection of gonadotrophin to immature mice fed *L. leucocephala* for short periods were not due to undernutrition so far as could be judged by body weights.

The effects of dietary *L. leucocephala* on sheep reproduction are difficult to interpret in view of body weight loss of treated groups during the last 60 days of pregnancy. Nutritional restriction alone during this period (2.5 to 6 kg weight loss) has been shown to result in small lambs, increased neonatal mortality, and reduced lamb growth rate (Thomson and Aitken 1959). Ewes in the present study produced lambs with poor birth weights, survival and growth but weight losses were not severe (average of 0.6 kg). It would be of considerable interest to determine whether the results observed were due to reduced availability of nutrients to the animal or a toxic substance (such as mimosine) or a combination of both. It is known that mimosine has depilatory effects (Owen 1958; Hegarty, Schinckel and Court 1964).

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