

THE EFFECTS OF LUPINOSIS ON WOOL GROWTH OF EWES

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SUMMARY

Wool grown by ewes with chronic lupinosis, induced by injections of phomopsins, was compared to that grown by control ewes and by ewes pair-fed to the feed intake of the phomopsin-treated ewes, during and after the period of phomopsin administration. Wool growth was not different between the groups during treatment. Fibre diameter and length of wool grown by the phomopsin-treated ewes were reduced in the period following treatment, compared to the control and pair-fed ewes ($P < 0.01$), while the pair-fed ewes grew shorter ($P < 0.05$) but not finer wool than the control ewes. These results indicate that the reduction of wool growth of ewes with lupinosis is not solely due to the decreased feed intake which occurs with the disease.

Keywords: wool growth, phomopsins, lupinosis, sheep.

INTRODUCTION

Lupinosis is a mycotoxicosis resulting from the ingestion of phomopsins (Culvenor *et al.* 1977), which are toxins produced on lupin plants by the fungus *Phomopsis Zeptostromiformis* (van Warmelo *et al.* 1970). The toxins are primarily hepatotoxic, causing inappetance, jaundice and depression in affected animals. Phomopsins interact with tubulin and disrupt microtubules within the cells, interfering with those cell processes which require functional microtubules, such as mitosis (Dustin 1984; Tonsing *et al.* 1984).

We have previously reported that intake of phomopsins can reduce both the length and fibre diameter of wool grown during the period of exposure to the toxins (Barnes *et al.* 1990). The effect on wool grown after cessation of phomopsin administration, and the mechanism by which the reduction in wool growth occurred, were not investigated. The aims of this experiment were to separate the direct effects of phomopsins from the nutritional consequences of inappetance by including a pair-fed group, to monitor wool growth after the treatments had ceased, and to examine skin sections histologically for evidence of a direct effect of phomopsins upon the wool follicle.

MATERIALS AND METHODS

Animals

Two hundred and ten 3.5 year old ewes were selected at the Wongan Hills Research Station following the weaning of their lambs and shearing in mid October 1990. They were transported to the Medina Research Station and individually penned in a sheep shed 10 days after shearing (day 0). All ewes were drenched with levamisole hydrochloride and oxfendazole to remove internal parasites at the time of entering the shed.

Treatments and diet

The ewes were randomly allocated to 1 of 3 groups. The Control group received 500 g wheaten chaff and 300 g clean lupin seed per day. The Toxin group were fed the control diet, and given subcutaneous (SC) injections of phomopsins diluted in phosphate buffered saline (PBS) (27 µg phomopsin A/mL) every 2 to 3 days, commencing on day 0, to give a total dose equivalent to 26.1 µg phomopsin A/kg liveweight. Each ewe in the Pair-fed group was paired to a ewe in the Toxin group on the basis of ranked liveweight when they entered the shed, and the feed supplied daily equalled the feed intake of the Toxin ewe on the previous day. Pair feeding started on day 10. Ewes in the Control and Pair-fed groups were given a 1 ml SC injection of PBS on the days of the phomopsin injections. These treatments were continued for 47 days, after which the ewes were returned to Wongan Hills where they were run as a single mob on summer pastures.

Measurements

Feed intakes were monitored daily, with the lupin seed and chaff components measured separately. Liveweights were measured on days 0, 7, 21, 33 and 45 of treatment, and 41 and 125 days after the

treatments ceased. Liver damage was evaluated by the measurement of plasma activities of gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) and plasma concentration of bilirubin on days 7, 21, 28, 33 and 45 of treatment and 41 and 125 days after treatment on 20 ewes from each group selected on the basis of ranked liveweight. Liver function was assessed in 7 ewes per group by the determination of the biological half life for clearance of bromosulphophthalein (BSP clearance) from the plasma (Lanigan and Peterson 1979) on the same days.

Wool growth was measured in all ewes using **dyebands** (Chapman and Wheeler 1963). These were applied when the animals entered the shed (day 0), when they left the shed (day 47) and 41 days later. The **dyebands** were harvested at lamb marking, 125 days after the ewes had left the shed. The length of wool grown in each period was measured with a ruler, with each recorded value being the mean of 4 measurements on each sample. Snippets of wool were taken immediately below each **dyeband**, and at the cut end of the staple, and the fibre diameter measured in a CSIRO Fibre Fineness Distribution Analyser.

A skin biopsy 5mm in diameter was taken from over the ribs from 25 ewes per group at the same time as the **dyebands** were applied. The biopsy was fixed in 10% buffered **formalin** and skin sections were stained in haematoxylin and eosin for examination under the light microscope.

RESULTS

The average feed intake of the Toxin group decreased gradually during the period of toxin administration, until by day 47 they were consuming approximately two-thirds of the control diet. The amount consumed by the Pair-fed group closely followed that of the Toxin group, while the Control ewes consumed over 90% of the feed offered throughout this period. The average liveweights of the ewes are shown in Table 1.

Table 1. The effect of phomopsin administration and pair-feeding on mean (± s.e.m.) liveweight (kg) (n=70)

Means within columns with different letters are significantly different : ab, $P < 0.001$; cd, $P < 0.01$; ad, $P < 0.05$. Statistical analysis : 1 way analysis of variance.

	Day 0	Day 45	Day 172
Control	43.0 ± 0.66	44.7 ± 0.58a	51.5 ± 0.70c
Toxin	42.5 ± 0.63	40.4 ± 0.59b	48.0 ± 0.74d
Pair-fed	43.3 ± 0.63	43.2 ± 0.59a	50.3 ± 0.74a

The activities of GGT and GLDH in the plasma, concentration of bilirubin and BSP clearance time were significantly elevated ($P < 0.001$) in the Toxin group compared to both the Control and the Pair-fed ewes for the period within the shed e.g. on day 28 BSP clearance times were 2310 ± 525.6, 193 ± 14.9 and 171 ± 10.7 s, and GGT activities were 208 ± 15.8, 45 ± 1.6 and 42 ± 1.3 U/L for the Toxin, Control and Pair-fed groups respectively. The GGT activity, bilirubin concentraton and BSP clearance times for the Toxin group remained significantly elevated ($P < 0.01$) compared to the Control and Pair-fed ewes on day 88, although all values were within the normal ranges. The BSP clearance times were

Table 2. The effect of phomopsin administration and pair-feeding on mean (± s.e.m.) length of wool grown (mm) (n=70)

Means within columns with different letters are significantly different : ab, $P < 0.001$; cd, $P < 0.01$; ad, $P < 0.05$. Statistical analysis : 1 way analysis of variance.

	Day 47 (Period within shed)	Day 88 (1st period post toxin)	Day 172 (2nd period post toxin)
Control	11.5 ± 0.16	9.5 ± 0.14a	20.5 ± 0.40
Toxin	11.7 ± 0.14	8.6 ± 0.14bc	20.6 ± 0.41
Pair-fed	11.2 ± 0.14	9.1 ± 0.14d	20.4 ± 0.37

263 $\pm$ 8.3, 207 $\pm$ 14.8 and 191 $\pm$ 9.1 seconds, and the GGT activities were 52.1 $\pm$ 2.6, 38.3 $\pm$ 1.8 and 41.2 $\pm$ 2.2 U/L for the Toxin, Control and Pair-fed groups respectively on day 88.

Results of the wool measurements are shown in Tables 2 and 3. There was no significant difference between the groups in length of wool grown whilst shedded. The length of wool grown in the first period after treatment was reduced in all groups compared to that grown during the shedded period, with the wool grown by the Toxin group significantly shorter than that of the Control ($P < 0.001$) and the Pair-fed ewes ($P < 0.01$). The wool grown by the Pair-fed ewes during that period was significantly shorter than that of the Control ewes ($P < 0.05$). There was no difference between the groups in the following period. The fibre diameter of the wool grown by both the Toxin and Pair-fed groups decreased significantly compared to that of the Control ewes ($P < 0.01$) so that the wool from the Toxin group was finer ($P < 0.01$) than that from the Control ewes by day 47. There was little change in fibre diameter during the next 41 days, but by the time the wool was harvested at lamb marking the fibre diameter of the wool from each group had increased, and there was no difference between the groups. Histological examination of the skin biopsies revealed no morphological differences between the samples.

Table 3. The effect of phomopsin administration and pair-feeding on mean ($\pm$ s.e.m.) diameter of wool grown (μ m) ($n=70$)

Means within columns with different letters are significantly different : ab, $P < 0.001$; cd, $P < 0.01$; ad, $P < 0.05$. Statistical analysis : l-way analysis of variance

	Day 0 (entered shed)	Day 47 (left shed)	Day 88 (41 days post treat.)	Day 172 (84 days post treat.)
Control	21.1 $\pm$ 0.25	20.1 $\pm$ 0.20c	20.1 $\pm$ 0.19c	21.0 $\pm$ 0.35
Toxin	20.8 $\pm$ 0.22	19.3 $\pm$ 0.19d	19.2 $\pm$ 0.21d	21.2 $\pm$ 0.28
Pair-fed	21.3 $\pm$ 0.22	19.6 $\pm$ 0.18	20.0 $\pm$ 0.18c	21.0 $\pm$ 0.32

DISCUSSION

This work confirms that experimentally induced lupinosis can affect both the length and fibre diameter of wool grown, and these effects can persist for 6 weeks after cessation of exposure to phomopsins. Chronic lupinosis, with liver damage and impairment of liver function, was induced in the ewes injected with phomopsins, as assessed by clinical pathology. The return of these values to within normal reference ranges by 6 weeks post toxin indicates cessation of the liver damage, and return to normal function.

The observation that the length of wool grown during administration of phomopsins did not differ from the Control group contrasts with the findings of Barnes *et al.* (1990), and may result from the different route of toxin administration (SC versus oral), although Allen (1989) has found that similar disease and pathology result from the different methods of phomopsin dosing. The reduction in wool growth in all groups in the first post toxin period may have been due to the poorer quality of summer pastures compared to the feed given in the shed. The ewes in the Toxin and Pair-fed groups were lighter and would have less body reserves of both energy and protein and may therefore be more affected by poor nutrition than the controls. Liver damage and other effects of phomopsins in the Toxin group may have further contributed to the group differences. In the second post toxin period, the length of wool grown was not different between the groups, even though the liveweight of the Toxin ewes was still significantly less than the Control ewes. However, the liver damage in the Toxin ewes had resolved by that time, which may indicate the contribution of liver damage to reduced wool growth.

The differences between the Toxin and Pair-fed groups in length and fibre diameter of wool grown indicates that the effects are not attributable solely to a depression of feed intake, and while there were no morphological differences seen in the skin sections, further investigation of mitotic activity in the wool follicles is required to explain the effect of lupinosis on wool growth.

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