Lupinosis in sheep. 1. Laboratory studies

M R. Gardiner
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LUPINOSIS IN SHEEP

LABORATORY STUDIES


In earlier articles by Bennetts (1957, 1960), the clinical and pathological features of lupinosis, as seen in field outbreaks, were described. The suggestion was made that the roughage portion of the lupin plants was responsible for the disease. More recent observations suggest a relationship between parasitism and lupinosis.

Experiments were designed to study various aspects of the problem based on these observations, and also to determine the possible role of fungi in the disease. Therefore, different parts of the lupin plant were fed to small groups of sheep. One half the sheep in each group were given a suspension of mixed parasites, considerably augmenting the natural infestations carried. In another experiment, 2 sheep groups were fed lupins which had been sterilised, sterilised and fungus inoculated, and non-sterilised and fungus-inoculated.

LUPIN-PARASITE EXPERIMENT

Fourteen sheep were used in this experiment and care was taken to procure young animals never exposed previously to lupins and which were lightly parasitised.

The lupin material fed was gathered from a property at Moolinabeenie. These lupins had caused an acute outbreak of the disease on this property and the material was, therefore, considered to be "toxic" material. It was separated into stalk, leaf, husk and seed fractions for the feeding trial. Each component, as fed to the group consuming it, was essentially uncontaminated, although there were small amounts of stalks in the leaf and husk fractions. The stalks were hammer-milled but the other fractions were not processed in any way.

The lupin sheep were group fed and brought into their diets of 500 grams lupins and 100 grams chaff per head per day, while the sheep acting as controls were fed at a calculated rate of 500 grams chaff and 100 grams oats per head per day.

The suspensions of mixed parasites were administered at the beginning of the experiment and again on the 7th and 14th days, and the effect of these drenches on parasitic burdens was assessed throughout the experiment by worm egg counts, and at termination, by a complete count of each parasitic species.

The course of this experiment was followed by regular weighings, feed consumption measurements, clinical examinations and blood studies. The sheep were killed when severely affected with lupinosis and up to about 5 weeks, if they survived this long without clinical signs. At post-mortem, a complete pathological and parasitological study was made on each animal and this was followed by a very detailed histological study of all body organs and tissues.

The consumption of all rations was poor and consequently marked losses in body weight occurred. The mean feed consumption and mean daily weight loss per sheep are shown in the following table:

<table>
<thead>
<tr>
<th>Total feed/day g</th>
<th>Stalks</th>
<th>Leaves</th>
<th>Husks</th>
<th>Seeds</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupins/day g</td>
<td>112</td>
<td>368</td>
<td>336</td>
<td>110</td>
<td>319</td>
</tr>
<tr>
<td>Protein/day g</td>
<td>15-0</td>
<td>55-7</td>
<td>19-2</td>
<td>40-8</td>
<td>15-9</td>
</tr>
<tr>
<td>Loss Bodyweight/lb</td>
<td>0-63</td>
<td>0-49</td>
<td>0-56</td>
<td>0-51</td>
<td>0-25</td>
</tr>
<tr>
<td>Period on lupins (days)</td>
<td>17-18</td>
<td>21-33</td>
<td>15-31</td>
<td>32-33</td>
<td>...</td>
</tr>
</tbody>
</table>
In the majority of sheep the worm egg counts increased slightly in those not receiving, and strikingly in those receiving, larval suspension drenches.

There were few clinical manifestations of lupinosis during this experiment in the sheep fed the leaves and husks, except for loss of weight. The sheep fed lupin seeds and the control sheep all showed a decline in appetite towards the end which was thought to be due to cobalt or Vitamin B12 deficiency in the rations offered. It was found that both the maintenance laboratory ration and the lupin seeds were markedly deficient in cobalt.

In contrast to the sheep in the above groups, those fed lupin stalks developed listlessness and apathy, followed quickly by severe jaundice deepening rapidly from the 14th to 17 days. Both clinically and pathologically these sheep closely resembled sheep undergoing the haemolytic crisis of copper poisoning.

A battery of biochemical tests was used in the trial, as measurements of liver and kidney functions and as indicators of the progress of the disease.

It has been stated above that the sheep fed the lupin stalks were the only ones developing clinical lupinosis, and that by the 17th day they were so severely affected that they were killed. Nevertheless, the sheep fed lupin leaves developed definite pathological, as distinct from clinical, signs of lupinosis in 21 to 33 days. Only by biochemical and histological criteria could leaf-induced disease be recognised, although in a couple of sheep, an experienced observer might have detected early gross liver changes.

There was little evidence of lupinosis in the sheep fed the husk material although our pathological studies indicated that extremely early changes of the disease were occurring in these livers also.

There was a definite tendency for the development of high liver copper in the sheep fed the lupin leaves and stalks, and for a decline in liver cobalt or liver Vitamin B12 in the experimentally parasitised sheep.

It is interesting that no other organ systems appeared to be affected primarily by the lupins. In particular, the kidney was entirely normal in all sheep. However, there was some evidence of a secondary, probably nutritional, effect on the bone marrow, manifested by a cellular inactivity (hypoplasia), and on the red blood cell. It is not intended to go into the detailed biochemical, haematological or pathological aspects of the experimental disease in this article.

There were clear indications that the onset of parasitism, especially infestations with *Haemonchus contortus* (Barber's pole worm), increased the severity of the liver damage, and accelerated the disease process. Further evidence supporting this contention is given in the article *Field Studies on Lupinosis*.

A study now under way at the Animal Health Laboratory is testing the influence of various deficiencies on the development of Lupinosis. Sufficient work has been done on the Lupinosis problem to indicate the tremendous complexity of the disease and the bewildering variety of factors involved. It would appear that the interplay of these factors including, perhaps some inherent toxic principle in lupin plants, determines the onset and eventual form of the disease in field outbreaks.

**LUPIN-FUNGUS EXPERIMENT**

From time to time the suggestion has been made that fungi growing on the lupin plant are responsible for lupinosis. An experiment designed to answer this question has been conducted at the Animal Health Laboratory, and has succeeded in giving at least a partial answer.

Small groups of sheep were fed sterilised, sterilised and fungus-inoculated, and non-sterilised and fungus-inoculated lupins for 41 days. The lupin material consisted of a mixture of husks (75%), stalks (7%), leaves (7%) and seeds (11%), and was gathered in the metropolitan area. It had not been associated with any clinical disease in sheep. The fungi employed in this study were isolated from "toxic" lupins from a lupinosis property, cultured in the laboratory and sprayed on to the lupin material.

The lupins were well supplemented with chaff or oats or both. The sheep used in this experiment were young, healthy animals, not previously grazed on lupins, and only slightly parasitised.

Detailed pathological studies were carried out in this series in the manner described above in the lupin-parasite experiment. Although a few early changes
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were detected in the livers of most of these sheep, sufficient to indicate the presence of a "lupinosis factor" in the material fed, none of the typical lesions of the disease developed in any animal regardless of the treatment used in preparing the lupins. As in the previous study, liver coppers were high in all the sheep, and in several extended into the "toxic" range, i.e., above 1,000 p.p.m. but, in contrast to the lupin—parasite experimental results, liver cobalts remained in the high normal range. This may have been due to the fact that the rations fed to the sheep were supplemented with cobalt.

Although this experiment did not rule out the possibility of a fungus being involved in the development of the disease, it did show that the fungal species used were not active in inducing lupinosis under the conditions of the trial.

ACKNOWLEDGMENTS

It is with much pleasure that the contributions to these studies of the following persons are acknowledged:

Dr. I. W. Parnell and Mr. Victor Bamford for parasitological work.

REFERENCES


FIELD STUDIES

By M. R. GARDINER, B.S., V.M.D., Chief Veterinary Pathologist, and A. W. WILLIAMS, B.V.Sc., Veterinary Officer, Geraldton

To complement the laboratory studies reported in a companion paper, a field study was set up on a property where severe outbreaks of lupinosis had occurred for several years. Mr. Eric Smart, of "Erregulla Springs," Mingenew, placed the necessary sheep at our disposal and provided the paddocks and labour essential for the conduct of the trial. We wish to extend to Mr. Smart our thanks for his co-operation and aid.

This trial had two main objectives:

(1) To study the development and progress of a typical field outbreak of lupinosis, and evaluate the factors associated with the toxigenesis.
(2) To assess the effect of alternative feeding on the course of lupinosis.

A mob of 100 healthy weaners, not previously exposed to lupins, was divided into 2 groups of 50 sheep each. One of these groups grazed for 1 week on the "toxic" lupin paddock followed by one week on a non-lupin paddock. The second group was grazed on the "toxic" lupins for a fortnight and then spelled for 3 days on the non-lupin grazing.

Both groups were carried through an entire summer grazing period, from early January to late May, on their respective feeding cycles.

Each fortnight one sheep from each group was shipped to the Animal Health
Laboratory for a complete pathological and parasitological examination, exactly comparable to that discussed in the paper on "Laboratory studies on lupinosis." Ecological data such as rainfall, temperature, feed availability and so forth were collected throughout the trial. The alternative feed in the non-lupin paddock consisted of (approximately) Flatweed 26 per cent., sub-clover 24 per cent., Wimmera rye 28 per cent., Brome grass 10 per cent. other species 12 per cent.

Lupin material and alternative feed were subjected to standard feed analyses at the Government Chemical Laboratories. The chief points of interest in these feed analyses were the high crude protein content of the lupin seeds; low phosphorous values for the lupin leaves and stalks; low copper values for all the feeds available, both lupin and non-lupin; and very low cobalt values for the lupin seeds and lupin roughage.

RESULTS

The following facts have emerged from this study:

1. There was no significant difference in the development and progress of lupinosis with respect to time on alternative feeding. That is, the sheep on 50 per cent. lupin-50 per cent. alternative feeding were as quickly and as severely affected as those on 82 per cent. lupin feeding.

2. The early changes of lupinosis, designated as a mild subacute lupinosis, were apparent by pathological criteria from the earliest days of the trial; that is, the two sheep examined after two weeks on their respective grazing regimens, were in no essential way different from those at the eighth week. It was quite clear, therefore that from the beginning the lupinosis "factor" was present and causing subclinical damage. There were few changes visible in the livers of these early sheep on gross inspection, and it was necessary to judge the lupin effect on the basis of pathological and biochemical observations.

3. Towards the end of March there was a marked exacerbation of lupin damage in the sheep from both groups, and from this time onwards liver damage was obvious on gross inspection, as well as by laboratory criteria.

4. The relevant events concurrent with this rapid progression of the disease in the second half of March were an increase in rainfall and a definite increase in parasitism. Especially noticeable was the appearance of infestation by the Barbers' pole worm (Haemonchus contortus). This worm was entirely absent in the parasitological spectrum during the first two months, thereafter quickly gaining the dominant position among the various parasite species found in the abomasum and intestines.

5. The liver changes were followed closely by a general deterioration in the condition of the sheep on both feeding regimens, characterised by steady loss of weight and ill-thriftiness.

6. There was an increasing disturbance in both copper and cobalt metabolism, with an elevation of liver copper and a fall of liver cobalt or Vitamin B12 in the sheep examined.

7. In May there were definite indications of a reversal of the pathological picture of which the most prominent sign was active regeneration of liver cells. This was due probably to the availability of new green feed at this time.

8. Although many sheep had reached the point of no return as far as liver damage was concerned, it is probable that those animals showing a minimum of fibrotic and cirrhotic changes would have made satisfactory recoveries provided adequate nutrition were available.

9. Lupinosis would appear to be a toxic nutritional cirrhosis.
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