INFLUENCE OF TIAMULIN AND ZINC BACITRACIN ON AVIAN INTESTINAL SPIROCHAETE INFECTION

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Summary

Infection with the intestinal spirochaete Brachyspira (Serpulina) intermedia has been shown to cause increased faecal water content and reduced production in layers. This study aimed to examine the efficacy of the antibiotic tiamulin in controlling infection. The possible role of the antibiotic growth promotant, zinc bacitracin, was also examined. While tiamulin was initially effective in reducing infection, reinfection occurred relatively quickly following the cessation of medication. Evidence was obtained that zinc bacitracin may suppress the growth of intestinal spirochaetes when fed a ration containing this antibiotic.

I. INTRODUCTION

Intestinal spirochaetes of the genus Brachyspira (Serpulina) are anaerobic, spiral-shaped bacteria that colonise the large intestine and can cause enteric disease in a range of animal species (Hampson and Stanton, 1997). Colonisation with intestinal spirochaetes has been recognised as a reason for previously unexplained production losses and/or diarrhoea in layers and meat breeders in Europe, the United States and Western Australia (Davelaar et al., 1986; Griffiths et al., 1987; Dwars et al., 1989; Swayne et al., 1992, 1995; Trampel et al., 1994; McLaren et al., 1996).

In a recent study in Western Australia (WA), intestinal spirochaetes were isolated from birds in 64% of flocks with signs of intestinal disease, compared with 28% of clinically normal flocks (McLaren et al., 1996). Of the WA isolates identified, 41% were identified as B. intermedia, 56.4% as belonging to a previously undescribed and unnamed group of uncertain pathogenicity ( provisionally designated as “Brachyspira pulli” ), and the rest as Brachyspira innocens, a non-pathogenic species known to colonise pigs (McLaren et al., 1997). No isolates of B. pilosicoli or B. alvinipulli were recovered in WA. An isolate of B. intermedia from a WA layer was subsequently used to experimentally infect layer hens, causing increased faecal water content and reduced egg production and thus confirming its pathogenic potential (Hampson and McLaren, 1999).

In 1998 the present authors conducted a survey in which faecal samples from 69 meat breeder, layer or meat chicken flocks in the eastern states of Australia were cultured for intestinal spirochaetes. Overall, birds in 42.9% of meat breeder and 68.2% of layer flocks were colonised with spirochaetes but no birds in meat chicken flocks were infected. There was a significant association between colonisation with spirochaetes and the occurrence of wet litter and/or reduced production (Stephens and Hampson, 1999). A subset of 57 spirochaete isolates from birds in 16 flocks were identified to the species level. Isolates from nine (56%) of these flocks were spirochaetes that are known to be pathogens of poultry: Brachyspira pilosicoli was isolated from birds from five flocks, birds from two flocks were infected with B. intermedia and in two other flocks both species were identified. Isolates

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from the other seven flocks belonged to other *Brachyspira* species of unknown pathogenicity.

A recent trial sought to evaluate the efficacy of antimicrobial control of intestinal spirochaete infection. This paper outlines some preliminary results of this trial.

II. METHODS

Fifty 18-week old layer hens were obtained from a commercial producer. The birds were placed in an airconditioned facility in individual cages with mesh floors. They were fed a commercial, vegetable-based layer diet *ad libitum* and had constant access to water. Individual faecal samples from each bird were cultured once a week for spirochaetes throughout the trial.

Thirty birds were orally inoculated with two mL of an actively growing culture of *Brachyspira (Serpulina) intermedia*, strain HB60, for 3 d per week for a period of three weeks. The broth contained approximately $10^8$ bacterial cells per mL. The remaining 20 birds were placed in another room as control birds and were not inoculated. Ten weeks later, ten infected birds were orally dosed by crop tube with tiamulin in water at a rate of 25 mg per kg bodyweight per d for 5 d.

Initially the diet fed to the birds contained zinc bacitracin at 100 ppm. This was removed after nine weeks, all birds then being given the same diet free of bacitracin. After four weeks, bacitracin was re-introduced into the diet of half (10) of the control birds and half (10) of the infected but untreated birds.

Individual faecal samples were collected from each bird weekly, cultured for spirochaetes and the percentage faecal moisture determined. Egg production and egg weight of each bird were recorded daily and accumulated to provide weekly egg mass output.

III. RESULTS

(a) Faecal moisture and culture

There was no statistically significant difference in the faecal moisture content of the birds in each of the groups. All faecal cultures carried out prior to inoculation of the birds were negative and all faecal cultures of the twenty control birds were negative throughout the trial. In the first six weeks following inoculation, up to five of the 30 inoculated birds were positive on culture. In the week following removal of the zinc bacitracin, as shown in Figure 1 below, this figure rose to 18 positive birds.

![Figure 1. Level of infection before and after removal of zinc bacitracin from the diet.](image)
One week after treatment with tiamulin all ten birds were negative when cultured for spirochaetes. This figure rose slowly over the next month, until seven out of the ten treated birds were positive. At postmortem one week later, that is, six weeks following treatment, nine out of ten birds were positive. In contrast, as shown in Figure 2, in the infected but untreated group between five and seven of the birds remained positive throughout.

![Figure 2](image_url)

**Figure 2.** Influence of tiamulin and zinc bacitracin on infection level.

Of the ten infected birds put back onto the diet containing zinc bacitracin, four birds were positive the following week, with this figure dropping slightly to three birds for the following two weeks. Four weeks following reintroduction of the zinc bacitracin, no birds were culture positive in this group, although three birds were found to be positive on postmortem.

(b) **Egg production**

Egg mass output was calculated by multiplying the number of eggs produced by the mean weight of the eggs in g. This was calculated daily for each bird and accumulated to provide weekly figures for each group.

Egg output from the control birds was significantly better than that from the infected birds. For example, in week 15, total output by the control group was 423.2 g whereas that of the infected group was 340.3 g (P < 0.0182). Treatment with tiamulin significantly improved egg output in infected birds. In week 17, egg output in the treated group was 466.5 g, compared with 400.2 g in the control group and 364.7 g in the infected group.

IV. CONCLUSIONS

This trial has only just been completed and the results are yet to be fully analysed. However, the results confirm the potential that infection with the intestinal spirochaete *B. intermedia* has to depress egg output. Treatment with the antimicrobial tiamulin appeared to be effective in removing the bacteria for a limited time but reinfection occurred. This is not altogether surprising given that the birds were housed in the same room as other infected, but untreated, birds. It would be useful in future studies to determine whether treatment of all birds in a room, together with appropriate disinfection of the environment, would completely eradicate the infection. The fact that the birds were reinfected suggests that infection does not
stimulate a strong protective immunity. Infection levels post treatment were actually higher than those prior to treatment, possibly due to the removal of competing intestinal bacteria.

The results of the trial showed that zinc bacitracin at an inclusion rate in feed of 100 mg/kg suppressed the growth of B. intermedia over an extended period following experimental infection. Subsequent removal of the zinc bacitracin allowed proliferation of the spirochaetes whilst, when reinstated in the diet, it once again reduced growth of the organisms. These preliminary results suggest that removal of zinc bacitracin as a growth promotant may result in increased problems associated with intestinal spirochaete infections in the chicken industries.

V. ACKNOWLEDGEMENTS

The financial support of the Chicken Meat and Egg Industry programs of the RIRDC is gratefully acknowledged.

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