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The use of *Artemisia annua* in the prevention of necrotic enteritis in a broiler disease model

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Introduction

The plant *Artemisia annua* contains considerable amounts of essential oils, i.e. camphor, 1,8 cineole and artemisia ketone with antimicrobial effect on *Clostridium perfringens* Type A, causing necrotic enteritis in broilers.

Aim

The aim of the study was to investigate the effect of a dietary supplementation of either dried *A. annua* leaves or an extract from dry leaves on the course of necrotic enteritis in broilers applying a disease model.

Material and Methods

Antimicrobial effect of *A. annua* extracts against *C. perfringens*

In order to find the most potent *A. annua* extract to be used as feed additive in the consecutive broiler experiment, the minimal inhibitory concentrations (MIC values) of extracts extracted with either methanol or dichlormethane or *n*-hexane were determined in 96 well microplates. The plant extracts were initially dissolved in dimethylsulfoxide and serial two-fold dilutions were made in Anaerobe Basal Broth (Oxoid). A volume of 20 μl of an overnight *C. perfringens* culture (strain 48) was added to 250 μl medium in the wells of the microplates. The plates were incubated under anaerobic conditions at 38 °C. After 24 h microbial growth was recorded visually and by measuring the absorbance at 650 nm.

The *n*-hexane extract had the lowest MIC value indicating the strongest antimicrobial effect on *C. perfringens* (Table 1) and was therefore used as feed additive in the broiler experiment.

Broiler experiment

A broiler experiment was carried out over 27 days with 320 male broilers divided into 4 experimental groups (4 replicate floor pens/group).

- Group 1 control, non-infected
- Group 2 control, infected
- Group 3 dried plant 10 g/kg, infected
- Group 4 *n*-hexane extract 250 mg/kg, infected

The infection model was based on a sudden shift to a feed providing 30% fish meal at the expense of soya meal on days 17, 18, 19 and 20, a 10 fold overdose of an attenuated live coccidiosis vaccine (Paraxol 5) on day 18, and inoculation of the feed and the individual birds with *C. perfringens* strain 48 isolated from a diseased broiler flock. On each of days 22, 24 and 27, 5 birds per pen were killed. Small intestinal lesions were scored on a scale from 0 (no pathological changes) to 6 (severe diffuse necrosis). In caecal contents, *C. perfringens* numbers were counted on Tryptose Sulphite Cycloserine (TSC) agar incubated anaerobically for 24 hours at 36 °C. Individual body weights of 10 birds per pen were registered on day 17 before feed shift and on day 27.

Table 1. Minimal inhibitory concentration of extracts against *Clostridium perfringens*

<table>
<thead>
<tr>
<th>Solvent extract</th>
<th>MIC (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>&gt; 600</td>
</tr>
<tr>
<td>Dichlormethane</td>
<td>270</td>
</tr>
<tr>
<td><em>n</em>-Hexane</td>
<td>170</td>
</tr>
</tbody>
</table>

Results and Discussion

None of the feed additives could prevent the development of necrotic enteritis in the experimental birds. The most severe small intestinal lesions and the highest *C. perfringens* numbers in caecal content were found at day 24 (Table 2). Birds supplemented with the *n*-hexane extract had lower lesion scores (P<0.05) and lower caecal *C. perfringens* counts (P<0.05) on days 22 and 27 as compared to the other infected groups (Table 1). This indicates a positive influence on the course of the disease in terms of a later disease onset and a faster recovery of the birds. The infection caused a severe growth depression (Figure 1). In the period from 17-27 days, no difference regarding body weight gain was found between the infected control group and the group receiving dried plant material (P>0.05). Birds supplemented with *n*-hexane extract had a higher weight gain (P<0.05) than the other infected groups (Figure 1).

Conclusion

In a necrotic enteritis disease model, the dietary supplementation of a *n*-hexane extract from dried leaves of *A. annua* modulates the course of the infection in a positive way and prevents to a certain extent severe growth depression related to the disease.

Table 2. Small intestine lesion score and numbers of *Clostridium perfringens* in the contents of caeca (log CFU/g)

<table>
<thead>
<tr>
<th>Lesion score</th>
<th>Control, non-infected (log CFU/g)</th>
<th>Control, infected</th>
<th>Dried plant (10 g/kg), infected</th>
<th>n-Hexane extract (250 mg/kg), Infected</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 days</td>
<td>0 ±a</td>
<td>1.75 ±a</td>
<td>1.30 ±a</td>
<td>0.60 ±b</td>
<td>***</td>
</tr>
<tr>
<td>24 days</td>
<td>0 ±a</td>
<td>3.65 ±a</td>
<td>2.75 ±a</td>
<td>2.65 ±a</td>
<td>***</td>
</tr>
<tr>
<td>27 days</td>
<td>0 ±c</td>
<td>1.75 ±a</td>
<td>1.30 ±a</td>
<td>0.60 ±b</td>
<td>***</td>
</tr>
<tr>
<td><em>C. perfringens</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 days</td>
<td>3.27 ±c</td>
<td>8.31 ±a</td>
<td>7.92 ±b</td>
<td>7.10 ±b</td>
<td>***</td>
</tr>
<tr>
<td>24 days</td>
<td>5.45 ±b</td>
<td>8.53 ±a</td>
<td>8.30 ±a</td>
<td>7.90 ±a</td>
<td>**</td>
</tr>
<tr>
<td>27 days</td>
<td>2.31 ±c</td>
<td>6.83 ±a</td>
<td>7.23 ±a</td>
<td>6.09 ±b</td>
<td>***</td>
</tr>
</tbody>
</table>

*Means in the same row with different superscripts differ significantly (P<0.05).