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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 ºC. 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 (Paracox 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 ºC. Individual body weights of 10 birds per pen were registered on day 17 before feed shift and on day 27.

**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.

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**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>n-Hexane</td>
<td>170</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Control, non-infected</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>Lesion score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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 (^{a})</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 (^{ab})</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>

\(^{a}\)Means in the same row with different superscripts differ significantly (P<0.05).

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**Figure 1. Weight gain of broilers in the period from day 17 to day 27.**