THE EFFECT OF ENZYMES ON \textit{CAMPYLOBACTER} AND \textit{SALMONELLA} IN BROILERS

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\textbf{Summary}

The action of exogenous enzymes resulted in a reduction in zoonotic bacteria, \textit{Campylobacter jejuni} and \textit{Salmonella enteritidis}, in the caeca of broilers fed either wheat- or corn-based diets. The feed ingredient type and the level of starting pathogenic challenge influenced the effect of enzymes on microbial population change. The reductions in \textit{Campylobacter} and \textit{Salmonella} observed in the reported studies indicated that exogenous enzyme supplementation, through its effect on intestinal environment, offers a useful addition to other management practices presently employed to improve food safety of poultry meat.

\section{I. INTRODUCTION}

Most countries with systems for reporting cases of foodborne diseases have documented significant increases over the past few decades in the incidence of diseases caused by micro-organisms in food including \textit{Salmonella spp.}, \textit{Campylobacter jejuni}, \textit{Listeria monocytogenes} or \textit{E. coli} 0157 among others (FOA/WHO, 2002).

\textit{Campylobacter} spp. induced enteritis continues to be a significant public health problem throughout the world. In the USA alone, it is estimated that more than 2.4 million cases have been occurring annually, of which 80\% are considered to be foodborne (Stern, 2002). A study in New Zealand found that campylobacteriosis occurrence was strongly associated with consumption of chicken meat (Eberhart-Phillips \textit{et al}., 1997). In the UK, poultry contamination levels with campylobacter currently average 50\% (Lister, 2002).

Compared to the control of \textit{Salmonella} spp., there is limited information available on methods for decontamination of poultry carcasses or a use of other management practices during poultry growth and processing to reduce \textit{Campylobacter} contamination. In feed, for example, Wagenaar and Jacob-Reitsma, (2002) and Mead (2002) reported that there is no existing competitive exclusion product effective against \textit{Campylobacter}.

Bedford (2000) reported that exogenous enzymes might play a role in microbial population changes in broilers. Chocq \textit{et al}.
(1999) reported that the anti-nutritive effect of soluble non-starch polysaccharides (NSP) present in wheat and barley is related to their ability to increase digesta viscosity along the gut of broilers, which in turn causes changes (types and levels) in gut microflora. Exogenous carbohydrases have been shown to reduce intestinal viscosity and improve nutrient digestibility in broilers fed different diet types. Against this background it seems that exogenous enzymes may indirectly influence the microbial activity, including zoonotic bacteria, in the birds gastrointestinal tract.

\section{II. METHODS}

Fifteen studies were conducted to evaluate the effect of exogenous enzyme supplementation either on \textit{Campylobacter jejuni} or \textit{Salmonella enteritidis} in wheat- and corn-based broiler diets. One-day-old broiler chicks (Ross-1) were randomly assigned to different treatments (wheat +/- enzyme or corn +/- enzyme) and kept in floor pens (12 to 36 broilers per treatment depending on a study). The chicks were challenged orally with variable suspension dilutions of \textit{C. jejuni} or \textit{S. enteritidis}. Diets were given \textit{ad libitum} from day one.

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\textbf{127}
Exogenous enzymes (Avizyme® 1300 in wheat diets, Avizyme® 1500 in corn diets) were used at the standard recommended dose rates of the appropriate product and no antibiotics or coccidiostatics were used (Table 1).

Table 1. Ingredient composition (g/kg) of experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Wheat diet</th>
<th>Corn diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>546.3 - 547.3</td>
<td>-</td>
</tr>
<tr>
<td>Corn</td>
<td>-</td>
<td>541.5 - 542.5</td>
</tr>
<tr>
<td>Soybean meal 48%</td>
<td>348.9</td>
<td>376.7</td>
</tr>
<tr>
<td>Soy oil</td>
<td>42.6</td>
<td>17.5</td>
</tr>
<tr>
<td>Tallow</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Salt</td>
<td>3.8</td>
<td>4.1</td>
</tr>
<tr>
<td>DL Methionine</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Limestone</td>
<td>12.2</td>
<td>12.2</td>
</tr>
<tr>
<td>Dical Phosphate</td>
<td>13.5</td>
<td>15.5</td>
</tr>
<tr>
<td>Vit/Min premix</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Avizyme 1300 (wheat) or 1500 (corn)</td>
<td>+/- 1</td>
<td>+/- 1</td>
</tr>
</tbody>
</table>

In the trials investigating Campylobacter, broiler chicks were challenged orally with the bacteria at four or five days of age, and population numbers were measured between 12 and 33 days of age. In the trials investigating Salmonella the broiler chicks were challenged orally at one day of age and measurements were recorded between 14 and 17 days of age. The contents of caeca were sampled aseptically and inoculated onto campylobacter- or salmonella-selective media. The trials were part of a joint research project between the Dep. of Clinical Vet. Science at the University of Bristol, UK and Danisco Animal Nutrition.

III. RESULTS

In the eight wheat-based trials, there was, on average, a two thirds reduction in the number of Campylobacter found in birds fed the enzyme supplemented diet, and in the four corn-based trials there was a reduction of over a third in birds fed the enzyme treated diet, compared with the control (Figure 1).

![Figure 1](image)

Figure 1. Proportion (%) of Campylobacter jejuni numbers in the caecum of broilers given diets containing enzymes compared to those given control diets without enzymes
In the three corn-based trials, there was, on average, a reduction of almost 60% in the number of *Salmonella* found in birds fed the enzyme treated corn-based diet (Figure 2).

![Graph showing proportions of *Salmonella enteritidis* numbers in the caecum of broilers given diets containing enzymes compared to those given control diets without enzymes.](image)

**Figure 2.** Proportion (%) of *Salmonella enteritidis* numbers in the caecum of broilers given diets containing enzymes compared to those given control diets without enzymes.

Additionally, it was found that significantly fewer birds fed the enzyme treated corn-based diets tested positive to *Salmonella*, when compared with the control (Figure 3).

![Graph showing proportion of *Salmonella enteritidis*-positive birds given diets containing enzymes compared to those given control diets without enzymes.](image)

**Figure 3.** Proportion (%) of *Salmonella enteritidis*-positive birds given diets containing enzymes compared to those given control diets without enzymes.

There was also a tendency for birds inoculated with less *Salmonella* to show higher responses to the enzyme addition.

**IV. DISCUSSION**

A dietary enzyme supplementation initiates fermentation changes in the gastrointestinal tract of broilers (Chocht *et al.*, 1999). These effects are likely related to a reduce undigested substrate reaching terminal ileum and lower gastrointestinal tract due to a viscosity reduction and/or improved nutrient digestibility (Annison and Chocht, 1991, Zannella *et al.*, 1999 Burrows *et al.*, 2002) and production of short chain sugars (from fibre degradation) (Apajalahti and Bedford, 1999).

As a result of such improvements in diet digestibility, there is a significant change in the substrate quality and quantity available to the intestinal microflora in both the upper and
lower gut. Sohail et al. (in press) reported that a multienzyme mixture (protease, amylase, xylanase) addition into a corn/soy-based layer diet changed the microbial profile in the caeca by measuring the percentage of guanine and cytosine of a total microbial DNA profile in digesta. Cowieson et al. (2000) found that the abundance of bacteria with a GC% (guanine and cytosine %) between 20 and 40 was decreased while the abundance of bacteria with a GC% between 40 and 60 was increased in birds fed the control diet supplemented with enzymes.

More specifically, Francis et al. (1999) reported a significant reduction in colony forming units of salmonella and clostridia species measured in caeca with dietary supplementation of the multienzyme mixture product. They also reported an increase in acetic acid concentration in birds fed the enzyme supplemented diets supporting the effect of exogenous enzymes on volatile fatty acid production potentially through the exogenous enzyme effect on production of short chain sugars preferred by some beneficial microflora.

The current results suggested that exogenous enzymes may promote an environment in the intestine that is unfavourable for zoonotic microflora such as Campylobacter and Salmonella. It is possible that a number of different modes of action were responsible for the results observed. Reduced viscosity, improved nutrient digestibility, increased digesta passage rate and increased production of short chain sugars were likely the most important factors contributing to the overall microflora reduction/changes.

The reductions in Campylobacter and Salmonella observed in the reported studies indicated that exogenous enzyme supplementation offers a useful addition to other management practices presently employed to improve food safety of poultry meat.

REFERENCES