EFFICACY OF ALTERNATIVES TO AGPS IN BROILERS CHALLENGED WITH
CLOSTRIDIUM PERFRINGENS

A. KOCHER, N. J. RODGERS and M. CHOCT

Summary

The effects of two alternative feed supplements (Additive A, a prebiotic, and Additive B an organic acid-based product) alone or in combination were investigated on the growth performance of broiler chickens using a NE challenge model. The effects of these supplements were evaluated against a negative control (no additives) and a positive control (with monensin and Zn-Bacitracin). The positive control birds had a numerically higher bodyweight and significantly (P<0.05) higher FCR compared to the negative control birds. The addition of the prebiotic (Additive A) had a marked beneficial effect (P<0.05) on FCR as well as the relative weight of the spleen. However, there were no further improvements when the Additive A was added in combination with the organic-acid based product (Additive B).

I. INTRODUCTION

One of the most common and financially devastating bacterial diseases in modern poultry flocks is necrotic enteritis (NE). The disease is caused by the α-toxin of Clostridium perfringens (CP) types A or C. In its acute form, birds often die without clinical signs. However, in its subclinical form the disease is much more financially damaging for the producer. The commonly observed symptoms of the disease vary with age of the birds (van der Sluis, 2000) and early signs of an NE outbreak such as wet litter, diarrhoea and a small increase in mortality of less than 1% are often overlooked.

Outbreaks of NE can be effectively treated or prevented with antibiotics such as virginiamycin, bacitracin, penicillin, tylosin or flavomycin (Watkins et al., 1997). When included at subtherapeutical dosage these antibiotics can be very effective in controlling and preventing NE by selectively modifying the gut flora, suppressing bacterial catabolism and reducing bacterial fermentation. All these changes lead to increased nutrient availability for the animal and increased growth performance. In 1999, the European Union has placed a partial ban on the use of anti-microbial growth promotants (AGP) and it was recently announced by the EU that a total ban all of AGP will be banned in the future. Therefore alternative methods to control NE will become inevitable.

It has been recognised that modulations of the natural bacterial population in the intestine of broilers through the use of alternate feed supplements such as prebiotic (oligosaccharides), probiotic or organic acids have some effect in controlling the proliferation of CP. This paper reports the result of a study investigating the effect of manno-oligosaccharides (Additive A: Bio-MOS®) and an organic-acid-based product with lactic acid bacteria, organic acids, enzymes and electrolytes (Additive B: Acid-Pak 4-Way™) on the performance of broiler chickens challenged with Clostridium perfringens.

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II. MATERIALS AND METHODS

A total of 150 male and 120 female broiler chickens (Cobb) were purchased from the local hatchery and raised in brooders on commercial starter crumbles. At 14d the birds were weighed in nine single sex groups of 6 birds (n=6) and transferred to 45 metabolism cages located in two adjacent rooms. Five experimental diets based on wheat (70%), SBM (17%) and fishmeal (5%) replicated nine times (5 male and 4 female) were fed from 14 to 42d.

The treatment groups were: 1) negative control (no antibacterial or anticoccidial additives); 2) positive control (with monensin and Zn-Bacitracin); 3) Additive A 1kg/t; 4) Additive B 2kg/t; and 5) Additive A 1kg/t plus Additive B 2kg/t. All supplements were kindly supplied by Alltech, Australia.

The challenge model used in this study consisted of a dual infection with *Eimeria* spp. on 18d followed by the challenge with *Clostridium perfringens* (d20, 21 and 22). From 14 to 18d birds received a mix of experimental diets and fishmeal (1:1) with the full dosage of the respective additives. On d 18 all feed was removed and replaced with 100% of the experimental diets. At this point all birds, including the positive control were challenged with 10,000 each of sporulated oocysts of *E. acervulina* and *E. brunetti* using a commercially available crop needle. On d 20 birds were starved overnight (~20h) and fed a 1:1 of experimental diets and broth culture containing approximately 10^5-10^6 CFU of *C. perfringens* type C. This procedure was repeated twice, however on d 21 and 22 feed was withheld for 3-4 h only. The inoculum was prepared daily using a commercially available broth (Thioglycollate broth, Oxoid, Heidelberg, Australia). Once all birds had eaten the inoculated feed, free access to experimental feed was restored. On d 28 half of the birds per cage were killed by cervical dislocation and the spleen and bursa of Fabricius were collected and weighed.

Feed intake and weight gain were recorded on a weekly basis throughout the experimental period. Mortality was recorded daily and a likely cause of death was given. SAS systems (SAS Institute Inc., 2001) as was used to perform the statistical analyses used in this study. Data were analysed according to the GLM procedure for ANOVA. Duncan’s multiple-range test was used to separate means when significant effects (P<0.05) were detected by analysis of variance.

III. RESULTS AND DISCUSSION

In the current study birds did not die or develop a clinical form of clinical necrotic enteritis. However, the negative control birds (without antibacterial or anticoccidial feed additives) had a numerically lower bodyweight at 42 d of age and a significantly increased FCR in comparison to the positive control birds (Table 1). These findings are consistent with findings from the literature which showed that damage to the small intestine caused by *C. perfringens* will result in a reduction in bodyweight and FCR of approximately 5% (Corpet, 1999; Kaldhusdal and Hofshagen, 1992; Kaldhusdal and Lovland, 2000; van der Sluis, 2000). It was concluded that the challenge model used in the present study was effective in inducing a subclinical form of NE.

There were no significant differences among treatments for body weight (Table 1). However, significant differences (P<0.05) were found among treatments for FCR. Birds fed manno-oligosaccharides (Additive A) had FCR similar to the positive control and significantly different to the negative control. It is known that manno-oligosaccharides can reduce unwanted enteric pathogens such as *Salmonella* or *Campylobacter* by blocking the type-1 fimbriae which enables the pathogens to attach to the intestinal lining (Dawson and Pirvulescu, 1999). Although Clostridia do not express type 1 fimbriae it was previously reported that the addition of manno-oligosaccharides to diets fed to birds challenged with *CP*
had some effects in reducing mortality as well as reducing secondary effects of NE on feed conversion ratio (Hofacre, 2001, Hofacre et al., 2003).

Table 1. Effects of manno-oligosaccharides and combination probiotic of on feed conversion ratio and relative weight of spleen and bursa of Fabricius

<table>
<thead>
<tr>
<th></th>
<th>Bodyweight g/bird (42d)</th>
<th>FCR 13-d28</th>
<th>FCR 28-d42</th>
<th>Spleen %BW</th>
<th>Bursa %BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control (NC)</td>
<td>2176.0</td>
<td>1.780ᵇ</td>
<td>1.980ᵃ</td>
<td>2.150ᵃ</td>
<td>0.138ᵇᶜ</td>
</tr>
<tr>
<td>Positive Control (PC)</td>
<td>2271.5</td>
<td>1.611ᵃ</td>
<td>1.792ᵇ</td>
<td>1.960ᵇ</td>
<td>0.123ᶜ</td>
</tr>
<tr>
<td>NC + Additive A¹</td>
<td>2243.4</td>
<td>1.728ᵇ</td>
<td>1.862ᵇ</td>
<td>1.999ᵇᶜ</td>
<td>0.156ᵃ</td>
</tr>
<tr>
<td>NC + Additive B²</td>
<td>2208.8</td>
<td>1.769ᵇ</td>
<td>1.908ᵇᵇ</td>
<td>2.038ᵇᵇ</td>
<td>0.148ᵇᵇ</td>
</tr>
<tr>
<td>NC + Additives A+B</td>
<td>2192.6</td>
<td>1.757ᵇ</td>
<td>1.850ᵇ</td>
<td>1.951ᵇᶜ</td>
<td>0.141ᵇᵇ</td>
</tr>
<tr>
<td>Female</td>
<td>2064.3</td>
<td>1.749ᵃ</td>
<td>1.967ᵃ</td>
<td>2.157ᵃ</td>
<td>0.142</td>
</tr>
<tr>
<td>Male</td>
<td>2341.7</td>
<td>1.713</td>
<td>1.808ᵇ</td>
<td>1.910ᵇ</td>
<td>0.141</td>
</tr>
<tr>
<td>SEM</td>
<td>28.47</td>
<td>0.016</td>
<td>0.020</td>
<td>0.030</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Source of variance

<table>
<thead>
<tr>
<th>Diet</th>
<th>Probability of greater F value in analysis of variance³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>***</td>
</tr>
<tr>
<td>Diet*Sex</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a,b,c Values with unlike superscripts differ significantly (P<0.05)
¹ Additive A: Bio-MOS®, Alltech, Australia; ² Additive B: Acid-Pak 4-Way, Alltech Australia
³ * P<0.05, ** P<0.01, *** P<0.001

The addition of Additive B had little effect on bodyweight or FCR compared to the negative control. This is somewhat in contrast with reports by (Hofacre et al., 2003) who found that the addition of a lactic acid producing bacterial culture alone or combined with manno-oligosaccharides was effective in reducing *C. perfringens* related effects on the feed conversion ratio. It appears that Additive B, a combination product with lactic acid bacteria, exogenous enzymes, organic acid and electrolytes was less effective in modifying the intestinal flora in comparison to a product with a purer culture thus allowing *CP* to proliferate in the small intestine.

The severity of necrotic enteritis and the reduction in growth performance is among other factors, influenced by the bird's immune capability (D'Assonville, 2001). It has been demonstrated that in physiologically challenged chickens the relative weight of immunological organs such as the spleen or the bursa of Fabricius decreases (Puvadolpirod and Thaxton, 2000). There was a significant difference (P<0.05) among treatment groups in the relative weight of the spleen. The addition of monensin and Zn-Bacitracin in the positive control group may have reduced the numbers of *CP* in the intestine, and hence reduced the need for the bird's own immune system to act, resulting in a reduction in the size of the immunological organs. On the other hand, the addition of Additive A alone resulted in a significant increase in the relative weight of the spleen, which can be associated with an increase in the cell-mediated immune response.

Based on the results of this study it appears that the reduction in broiler growth performance as a result of a sub-clinical *Clostridium perfringens* infection could be partially
overcome when manno-oligosaccharides (Additive A) are added to the diet. There appear to be no further benefits when the same product is added in combination with an organic-acid based product. However, NE is a complex multifactorial disease with many unknown factors and future research has to focus on the understanding of the disease itself and the development of reliable and repeatable disease models to investigate nutritional manipulation in detail.

REFERENCES

Hofacre, C.L. (2001). In: Biotechnology in the Feed Industry; Alltech’s 17th Annual Symposium, pp. 79-86. T.P. Lyons and K.A. Jacques, Nottingham Press, Nottingham,