The effect of organic acids on chickens infected with Eimeria

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Coccidiosis is a parasitic disease in chickens which is caused by the protozoan parasite of the genus Eimeria. Coccidiosis is a worldwide disease and on cost basis the most important infectious disease in the intensive poultry industry. The disease is estimated to cost producers worldwide more than €2.2 billion annually.

Seven pathogenic species are identified which cause lesions in characteristic parts of the gastro-intestinal tract. These species are: Eimeria acervulina, E. brunette, E. maxima, E. necatrix, E. tenella, E. mitis and E. praecox.

Effects of Eimeria infection

Infection with Eimeria results in reduced feed intake and nutrient absorption, less growth and an increased susceptibility to other diseases such as necrotic enteritis caused by Clostridium perfringens. Mortality on the whole, however, is usually low.

Symptoms of the disease can be any or all of the following: ruffled feathers, unthriftiness, hunched appearance and diarrhoea which may have blood in it. At flock level the main symptom will be a costly production loss.

Anticoccidial feed additives have become an essential part of poultry production management. However, there have been reports recently of resistance growing to the regularly used anticoccidial drugs. Furthermore, article 11 of EC regulation 1831/2003 states: 'with a view to a decision on the phasing out of the use of coccidiostats and histo-monostats as feed additives by 31st December 2012, the Commission shall submit to the European Parliament and the Council before 1st January 2008 a report on the use of these substances as feed additives and available alternatives, accompanied, where appropriate, by legislative proposals'.

Although this has not happened as of yet, naturally derived products which control and protect against intestinal infections, for example coccidia, may provide a useful alternative.

Organic acids have long since been used as feed preservatives to inhibit pathogenic bacteria but are gaining interest as gut health regulators and, in particular, butyric acid, a saturated fatty acid with four carbon atoms, is noted for its specific effect on cell proliferation of colon epithelial cells as well as a modulator of the composition of intestinal micro-population and a stimulator of the gut immune system.

Study aims

The aim of this study was to identify plant and/or organic acid derived in-feed additives which would control coccidiosis. Several products were identified and their in feed concentrations decided upon based on literature searches.

A total of eight pens of 19 birds per pen were selected for enrolment into eight treatment groups: T01 (not infected, not treated control), T02 (infected, not treated control), T03 (additive a), T04 (additive b), T05 (additive c), T06 (additive d), T07 (monoglyceride of butyric acid) and T08 (organic acid blend).

The organic acid blend was included as a control to ensure that the acid blend (which was included with all products with the exception of the monoglyceride of butyric acid) did not have an effect. The chicks were allocated into treatment groups as per randomised complete block design with blocking based on body weight on day one.

Seven of the eight treatment groups received a single challenge dose of Eimeria spp. on day 16. Lesion scoring on four birds from each group was done on day 22.

Gut samples were taken from four birds in each of groups T01, T02, T07 and T08 on days 22 and 35. Faecal oocysts counts were carried out on days 16, 20, 22, 24 and 35. The study terminated on day 35.

- **Day 1.** All chicks were weighed and their bodyweight recorded. Just prior to enrolment on day one, a physical examination of the candidate chicks was performed and this was recorded. Chicks that satisfied all of the inclusion criteria and none of the exclusion criteria were selected for enrolment in the study. A total of 152 chicks were selected for enrolment based on body weight.
- **Day 16.** The animals in groups T02-T08 were inoculated with approximately 0.5ml of the Eimeria spp. suspension by individual oral gavage, using a syringe fitted with a blunt ended needle. Group T01 (uninfected and untreated) were administered in the same way as the infected birds with the equivalent volume i.e. 0.5ml of potassium dichromate without oocysts. Oocysts were counted using the McMaster technique and expressed in oocysts per gram of faeces (OPG). This was performed to confirm naivety.

- **Day 20, 21, 24 and 35.** Five pooled fresh droppings per pen were collected in labelled plastic pots. Oocysts were counted using the McMaster technique and expressed in oocysts per gram of faeces (OPG).
- **Day 21.** Intestinal lesion scores were evaluated on four birds from each pen/group on day 21 using the system of Johnson and Reid. The birds were selected by randomly sorting the birds within each group and then selecting the first four listed birds from that group. The lesions were scored per area of gut i.e. small intestine, caecum and large intestine accordingly:

0 = gross lesions absent.
1 = a few scattered lesions.
2 = a greater number of discrete lesions involving more of the affected zone of the intestine and marked bleeding with E. tenella.
3 = lesions extensively developed with coalescence and some thickening of the intestinal or caecal walls.
4 = extensive coalescence of lesions with thickening of the wall, bloody intestinal contents with E. maxima and large caecal cores with E. tenella.

Samples from Groups T01, T02, T07 and T08 of ileal and caecal contents were removed after lesion scoring. Samples were transported to a laboratory for qPCR analysis of bacteriology and SCFA analysis.

- **Day 35.** All birds were weighed and their bodyweight recorded. Gut sampling was

Continued on page 19

Continued from page 17
performed as on day 21 for four randomly selected birds from Groups T01, T02, T07 and T08.

● **Day 1-35.** From day 1 until the end of the study at day 35, general health observations were made and recorded by an experienced stock person at least twice daily. If a chick was found dead/euthanased before day 16 a post mortem examination was not performed. Any dead or euthanised chicks after day 16 were examined post mortem, with particular attention being paid to coccidiosis. Parameters measured were: mortality, lesion scoring day 21, bodyweight day 35 and oocyst counts. Furthermore, at day 21 and day 35 samples were taken from the ileum and caeca of four birds each in the groups T01, T02, T07 and T08 for SCFA analysis, bacterial composition and qPCR for Eimeria. This was undertaken to study the effect organic acids may have on these parameters in the presence of an Eimeria infection compared to control.

Results

Mortality was observed in the infected control, T05 group, T06 group and the T08 group. Body weight data was variable between groups and therefore inconclusive as to any treatment effect. Oocyst counts were also inconclusive with the T03 treated group having the highest peak. The infected control had the lowest peak which shows the variation in oocyst output. The uninfected control showed no positive oocyst counts therefore we can assume there was no environmental contamination.

Lesion scoring is a useful aid in identifying key areas affected by particular species. In this study, the main pathogenic Eimeria species used were E. acervulina and E. tenella. The latter gives clinical signs of soft faeces often containing blood, the birds are dull and listless and often results in death if untreated. It affects the caecal region of the intestine and this can be clearly identified on post mortem examination where a lesion score can be given. E. acervulina is characterised by poor weight gains but little mortality. The lesions are very clear in the duodenum and can also be scored accordingly. The most notable lesion scoring results are those of the duodenum and the caeca where, for the caeca the infected control, the T03 group, the T05 group, the T06 group and T08 group showed the highest mean lesion scores. The T07 group showed the lowest mean lesion score in the caeca of 0.5, where the highest score was 3.

The T04 group also showed a lower lesion score. Both of these scores were significantly lower compared to the scores of the other infected groups ($p = 0.0077$ and 0.0026 respectively). The duodenum highest mean scores were between 1.5 and 2.25 in all infected groups. The T07 group, however, had the lowest mean of 1.5.

The results of the qPCR and analysis of the SCFA of the ileal and caecal samples were:

● The Eimeria challenge appeared to affect more significantly caecal than ileal microbiota and fermentation by reducing bacterial biomass and fermentation.

● Both monoglyceride of butyric acid and organic acid blend significantly inhibited Eimeria numbers in caecal samples. The monoglycerid of butyric acid and the organic acid blend shifted the caecal microbiota and fermentation towards that observed in the uninfected control. This result is most likely associated to the reduction of Eimeria infection severity. However at 35 day sampling the microbiota of infected treatments appeared to be normalised.

Conclusion

From these results, the T07 and T04 group are an obvious choice for taking forward for further testing. They showed significant reduced lesion scores compared to other infected groups and there was no mortality observed due to coccidiosis. Although a subjective parameter, it is generally noted that the T07 group (monoglycerid of butyric acid) showed clinical signs less often than the other groups and generally appeared more alert throughout most of the critical period. It is recommended that further work involving dose titration is performed using these particular feed additives. ■