A microencapsulated blend of organic acids and natural identical flavours reduces necrotic enteritis-associated damages in broiler chickens

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Six-hundred ROSS 308 broilers were allocated in 24 pens divided into 3 experimental groups (d0): the control diet (CTR), the control diet added with Galliacid®S at 300 ppm (GAL S), a microencapsulated blend of organic acids and natural identical flavours (EU patent 1391155B1; Vetagro srl, Italy), and the control diet added with a different blend of organic acids (WSB) added at 300 ppm. Birds were fed a wheat-based diet without coccidiostat and at d9 were inoculated with live vaccinal Eimeria oocysts; at d15 animals were infected with Clostridium perfringens. Mortality was registered and dead animals were analysed for necrotic enteritis (NE) lesions; growth performance of animals was calculated and analysed with 1-way ANOVA, whereas mortality analysis was run with chi-square test. At d21, following challenge, mortality was significantly reduced in the GAL S group (GAL S: 21.8%; CTR: 37.4%; WSB: 43.6%; P<0.001). Feed conversion during the challenge period (15-21d) was significantly lower for GAL S than the other groups (GAL S: 2.4; CTR: 3.2; WSB: 4.7. P<0.05). Galliacid®S allowed to improve growth performance in a NE challenge model when compared to a negative control diet and to wider spectrum blend of organic acids.

Keywords: necrotic enteritis, organic acids, natural identical flavours, microencapsulation, broilers.

Introduction

Necrotic enteritis is a multi-factorial disease and there are many predisposing factors which can contribute to pathology outcome, among which chemical composition of the diet, cereal grain selection, protein source, concomitant coccidiosis, and, the presence of high number of Clostridium perfringens cells (McDevitt et al., 2006). The use of in-feed antibiotics has until now been the main strategy for controlling Clostridium perfringens-associated necrotic enteritis in poultry, but inclusion of antibiotics in feed is disappearing due to the concrete threat of development of antibiotic resistant microbes (Dahiya et al., 2006). Public concern has forced the poultry industry to consider alternatives. Strategies to control necrotic enteritis in the absence of antibiotic growth promoters have focused, therefore, both on dietary and management practices and alternative feed additives. Among the candidate replacements for antibiotics there are organic acids and plant extracts. No single satisfactory non-antibiotic measure against Clostridium perfringens has been identified yet, even if it has been demonstrated the in vitro antimicrobial effect of organic acids and essential oils against intestinal pathogens (Kamel, 2000; Dorman and Dean, 2001; Skrivanova et al., 2006).

Aim of this study was to evaluate the role of two different microencapsulated blend of organic acids and natural identical flavours in controlling necrotic enteritis in broilers chickens in a challenge study.
Materials and methods

Animals
Six-hundred ROSS 308 day old female vaccinated for Marek’s and infectious bronchitis were used. On the day of the study start the chicks were weighed and distributed at random in 24 pens, 25 per pen. Chickens were divided into 3 experimental groups (d0): the control diet (CTR), the control diet added with Galliacid®S at 300 ppm (GAL S), a microencapsulated blend of fumaric, malic, citric, sorbic acid and natural identical flavours (EU patent 1391155B1; Vetagro srl, Italy), and the control diet added with a different microencapsulated blend of organic acids containing fumaric acid, calcium formiate and calcium propionate (WSB) added at 300 ppm. The total weight of the chickens, for each pen, was recorded. The floor of each pen was covered with a triple layer of paper.

Management
The room temperature was adjusted at 32°C at start, and it was linearly decreased over time (d1=32°C, d7=29°C, d14=27°C, d21-d42 = 25°C). Adequate ventilation was maintained to avoid any accumulation of ammonia and to control moisture. The lighting period was from 0 to 2 days light:dark 23:1 at 100 lux, and from 3 to 35 days light:dark 18:6 from 100 lux to 10 lux over a 4 days period.

Feeding program and diets
Birds were fed a vegetable diet without enzymes, antibiotic, and anticoccidial, containing 35% wheat (Tab. 1). The feeding program was divided into three phases (0-15 days, starters; 16-28, growers; 29-42 finisher) and designed for female birds. The diets did not differ for energy value and protein content.

Experimental challenge
After 9 days (d9) from their arrival birds were weighed per pen and inoculated with live vaccinal Eimeria oocysts by intracrop administration; at d15 third weighing per pen, feed conversion calculation and animals infection with Clostridium perfringens were made. C. perfringens was inoculated into the crop, twice daily, for 6 inoculations (3 days).

Growth performance parameters
The chickens were weighed per pen 6 times: at d0, d9, d15, d21 and d42. Feed intake and feed conversion were calculated between each period for each pen. Mortality was recorded daily and dead animals were analysed for necrotic enteritis (NE) lesions.

Statistical analysis
Daily feed consumption and feed conversion were adjusted for dead chickens body weight. Growth performance were analysed with 1-way ANOVA and when significant differences were detected between treatments a multiple comparison test (Fisher’s LSD) was done using the NCSS 2001 software (NCSS, Kaysville, Utah 84037, USA). Mortality was analysed with chi-square test.

Tab.1: Nutrients composition of the diets (% as fed basis)

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Starters (0-15d)</th>
<th>Growers (16-28d)</th>
<th>Finisher (28-42d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>23.3</td>
<td>21.7</td>
<td>19.2</td>
</tr>
<tr>
<td>Ether extract</td>
<td>3.1</td>
<td>3.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.26</td>
<td>1.08</td>
<td>0.79</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>0.96</td>
<td>0.74</td>
<td>0.61</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Water</td>
<td>11.4</td>
<td>11.4</td>
<td>12.1</td>
</tr>
</tbody>
</table>

Results and discussions
Throughout the study the group GAL S had a lower mortality rate than the other groups (GAL S: 31.3%; CTR: 38.5%; WSB: 47%; P<0.01). Especially at d21, following challenge, mortality was significantly reduced in the GAL S group (GAL S:21.8%; CTR: 37.4%; WSB: 43.6%; P<0.001). Feed conversion during the challenge period (15-21d) was significantly lower for GAL S than for the other groups (GAL S: 2.4; CTR: 3.2; WSB: 4.7. P<0.05).

Tab.2: Mortality and growth performance at between d15-21, during 6 days after inoculation with C. perfringens. Values with different superscript within the same column are significantly different (P<0.05).
Necrotic enteritis (NE) is a disease frequently observed in commercial poultry. The occurrence of necrotic lesions in the intestinal tract is associated with the proliferation of the anaerobic bacterium *Clostridium perfringens*, and, it has been commonly accepted that this pathogen is the causative factor of NE. This economically relevant disease is spread in most areas where poultry are produced and is characterized by decreased appetite, depression of the growth, decreased feed conversion, diarrhea and an high mortality rate in acute outbreaks (Mitsch et al., 2004).

Antibiotics and coccidiostats have been used for years to control this disease, but, since the European Union laws have banned the use of all antibiotic growth promoters and ionophores, as of January 1st 2006, alternatives to prevent the impact of NE are needed.

In this experiment two different microencapsulated blends of natural identical flavours and organic acids have been tested to counteract the wasting effects of NE in an experimental challenge model. Our findings, such as the lower mortality rate in GAL S fed group are aligned with the ones of previous works. Mitsch et al. (2004) found that feeding two different blends of essential oils to Ross broilers in a commercial flock significantly reduced *Clostridium perfringens* intestinal and fecal counts throughout the study. Since it is well known that it is *C. perfringens* the main causative agent of NE those results demonstrate that essential oils, and flavours can prevent *C. perfringens* proliferation in the intestine, as it is suggested by *in vitro* studies (Dormans and Deans, 2000).

Although in our study we did not perform *C. perfringens* counts, we can assume that the lower incidence of mortality in GAL S group can be associated to a reduction of *C. perfringens* cell in small intestine and cecum of the birds. A further effect of essential oils and flavours is the stimulation of digestive enzymes, thereby digestibility of nutrients can be improved (Platel and Srinivasan, 2000; William and Losa, 2001). This is well observed in the present study, where the feed conversion ratio during the week immediately after challenge, and throughout the first 21 d of the study, was significantly lowered by the inclusion of the GAL S in the feed.

All those factors contribute to a better stabilization of the gut microflora. There is evidence that an healthy microflora inhibits the growth of pathogenic bacteria, such as *C. perfringens*, and that a well developed digestive enzymatic pool can inactivate bacterial toxins, such as α-toxins of *C. perfringens*. Thus, the combined antibacterial effect of flavours and organic acids, slowly released along the intestine, added to the digestive enzymes stimulation of flavours, can reduce the *C. perfringens* colonization of the gut. Moreover, since the other blend (WSB) was not effective in reducing mortality or improving performance of broilers, it appears that different compositions of the blend can significantly affect the final results, suggesting that the selection of the right ingredients and combinations is a tool in controlling certain pathogens growth or pathologies damages.
In conclusion Galliacid®S allowed to improve growth performance in a NE challenge model when compared to a negative control diet and to wider spectrum blend of organic acids. Further studies are needed to find a correspondence between a lower mortality and a lower colonisation of gut by *C. perfringens* cells.

References


