Efficacy test of a hydrolysable tannin extract against necrotic enteritis in challenged broiler chickens

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Abstract

A hydrolysable tannin extracted from chestnut (SaviotaN®) was tested for efficacy in controlling the proliferation of Clostridium perfringens in the gut of broiler chickens challenged via oral gavage first with coccidia (Eimeria tenella, Eimeria acervulina, Eimeria maxima) at the age of 10 days, and then with Clostridium perfringens at the age of 15 days. We randomly allocated 150 broiler chickens within 5 poultry isolators (30 birds each). Dietary treatments consisted of a basal diet (C) composed of corn (575 g/kg on dry matter DM) and soybean meal (100 g/kg DM), barley bran (220 g/kg DM), corn gluten feed (30 g/kg DM), soybean oil (25 g/kg DM), vitamin mineral premix (49.5 g/kg DM), and four other diets obtained by adding chestnut tannin extract (1.5, 3, 5, and 12 g/kg during week 1, 10.0 g/kg during week 2, and 8.0 g/kg during the last two weeks, respectively) to C. At the age of 20 days, 15 birds/group were euthanised and individually examined for the level of gut infection by counting Clostridium perfringens and macroscopic gut lesions. Results demonstrated that chestnut tannin gave significant results even at low concentration levels in the feed (1.5 to 3.0 g/kg), but was actually efficient in controlling necrotic enteritis at levels ≥5.0 g/kg. The treatment (12.0 g/kg during the first week and 8.0 g/kg during the last two weeks of age) resulted very efficient in controlling the proliferation of Clostridium perfringens and in reducing the severity of gut damage compared to the untreated infected group.

Introduction

Necrotic enteritis is one of the world’s most prominent and severe diseases in chickens and, in particular, in broiler chickens (McDevitt et al., 2006). The disease is responsible for high mortality rates and for a significant depression of performance parameters. The disease usually occurs in broiler chickens of 2 to 6 weeks of age and is caused by the overgrowth of Clostridium perfringens (type A and, to a lesser extent, type C) in the small intestine and by the production of extracellular toxins damaging the intestine. Although Clostridium perfringens is recognised as the etiologic agent of necrotic enteritis (Elwing and et al., 1992), other co-factors are usually required to precipitate an outbreak, including environment, climate, management of hygiene and diet. In commercial production, coccidiosis is another important predisposing factor for triggering outbreaks of necrotic enteritis. Experimental induction of intestinal damage to cause necrotic enteritis in broilers has been successfully accomplished by co-infection with Eimeria spp. (Persia et al., 2006).

In many countries, necrotic enteritis is controlled by the use of antibiotics in feed or drinking water. The European Union has enforced a ban on the use of in-feed antibiotics and consumer pressure may force similar restrictions on antibiotic use. Therefore, alternative strategies for the control of necrotic enteritis are needed to limit the economic impact of the disease. Possible alternatives include probiotics, prebiotics, organic acids and products extracted form plants. In human medicine, plants are known to be a source of bioactive compounds useful as alternative to drugs, but the applications in veterinary medicine are very limited. Tannins are a complex mixture of polyphenolic compounds characterised by a high variability in molecular structure. In particular, hydrolysable tannins (HT) are characterised by the presence of a core of glucose esterified with gallic and hexahydroxydiphenic acids. Tannins extracted from chestnut wood (Castanea sativa Miller), commonly found in the central Mediterranean area, are an example of HT. In literature, a considerable number of publications demonstrated the anti-nutritional effects of tannins in poultry diet, including a worsening of productive performances as a consequence of a decrease in organic matter digestibility, especially for the protein component (Chang and Fuller, 1964; Ahmed et al., 1991; Garcia et al., 2004; Barroga et al., 1985; Longstaff and McNab, 1991a, 1991b). In contrast, it is evident from several in vitro studies that the microorganisms in the gastrointestinal tract are strongly sensible to the presence of HT and that tannins from chestnut wood are efficient against coccidiosis and necrotic enteritis in poultry (Elizondo et al., 2010). Hence, it seems very interesting to test in vivo the efficacy of this kind of compounds as protective agents to control the intestinal diseases produced by the most common bacteria and parasites of chickens.

The aim of the present trial was the evaluation of the efficacy of an industrial HT, extracted from chestnut wood (commercial name SaviotaN®; Gruppo Mauro Saviola s.r.l., Viadana, Italy), in controlling necrotic enteritis and multiplication of Clostridium perfringens in the gut of broiler chickens. The study was designed using Eimeria spp. infection as experimental model to produce clinical infection of Clostridium perfringens in experimentally challenged broiler chickens.

Materials and methods

Animals

The broiler chickens used in this study were female Ross 308 chicks, purchased from a local hatchery. The chicks were vaccinates against Marek disease in the hatchery, but not against coccidia. One hundred and fifty birds at one day of age were allocated randomly within...
poultry isolators (Allentown®; Allentown, NJ, USA) equipped with air filtration systems both inward and outward. The poultry isolators were 5, with 30 birds each.

Diets

Feed and drinking water were administered ad libitum throughout the whole trial by means of hopper feeders and bell-shaped drinkers.

Dietary treatments, formulated according to animal requirement (National Research Council, 1994), consisted of a basal diet [control (C)] composed by corn meal [575 g/kg on dry matter (DM)], soybean meal (100 g/kg DM), barley bran (220 g/kg DM), corn gluten feed (30 g/kg DM), soybean oil (25 g/kg DM) vitamin mineral premix (49.5 g/kg DM with 10 g/kg of Lysine and 3 g/kg of Methionine), and of four other diets obtained by adding to C the chestnut tannin extract (CE) SaviotaN® (equivalent to 570 g of gallic acid/kg DM) in different doses: CE1.5: with 1.5 g/kg SaviotaN®, throughout the whole trial; CE3: with 3.0 g/kg SaviotaN®, throughout the whole trial; CE5: with 5.0 g/kg SaviotaN®, throughout the whole trial; CE12: with 12.0 g/kg SaviotaN® during the first week, 10.0 g/kg during the second week, and 8.0 g/kg further on for the last two weeks.

The chemical characterisation of CE (SaviotaN®) is reported by Romani et al. (2012).

Proximate analysis of feed samples

Samples of C were oven dried at 60°C for 24 h. The dry samples were analysed for crude protein (CP), ash, ether extract (EE) and crude fibre (CF), according to the 954.05, 920.39 and 962.09 procedures of AOAC (1990), respectively. The chemical composition of the control diet was: DM, 895; CP, 205; EE, 55; CF, 920.39 and 962.09 procedures of AOAC (1990), respectively. The chemical composition of the control diet was: DM, 895; CP, 205; EE, 55; CF, 920.39 and 962.09 procedures of AOAC (1990), respectively.
Lupini et al. (2009) demonstrated antiviral activities of CE against avian reovirus and avian metapneumovirus replication because in samples containing CE a reduction of the viral cytopathic effect (CPE) was induced about 80%. The evaluation of lesion score, found in this trial, put in evidence that birds classified with score 0 and 1 were absent in the groups treated with less than 5 g/kg of tannin, but with score 0 and 1 were absent in the groups treated with less than 5 g/kg of tannin, but were 10 out of 15 in the last group at 25 days (Table 2). The lightly affected ones, scored 2 and 3, were greatly represented in the medium tannin groups, while the highly affected chickens, scored 4 to 6, were absent in the treated groups 5 g/kg and upward. Thus, the concentration of 5 g/kg appears the border level to be adopted to protect broiler chickens from necrotic enteritis.

Table 2. Lesion score response to SaviotaN® treatment (number of birds).

|                | Negative (score 0-1) | Positive (score 2-3) | Positive (score 4-6) |
|----------------|----------------------|----------------------|----------------------|
| C, 20 days     | 0                    | 9                    | 6                    |
| C, 25 days     | 0                    | 8                    | 7                    |
| CE1.5, 20 days | 0                    | 13                   | 2                    |
| CE1.5, 25 days | 0                    | 6                    | 9                    |
| CES, 20 days   | 0                    | 13                   | 2                    |
| CES, 25 days   | 0                    | 10                   | 5                    |
| CES, 20 days   | 4                    | 11                   | 0                    |
| CES, 25 days   | 2                    | 13                   | 0                    |
| CE12, 20 days  | 8                    | 7                    | 0                    |
| CE12, 25 days  | 10                   | 5                    | 0                    |

C, control; CE1.5, basal diet with 1.5 g/kg SaviotaN®; CE3, basal diet with 3.0 g/kg SaviotaN®; CE5, basal diet with 5.0 g/kg SaviotaN®; CE12, basal diet with 12.0 g/kg SaviotaN® during the first week, 10.0 g/kg during the second week, and 8.0 g/kg for the last two weeks.

Table 3. Mean values of lesion scores treated with Student’s t-test.

|             | C       | CE1.5   | CE3     | CE5     | CE12    | SEM     |
|-------------|---------|---------|---------|---------|---------|---------|
| 20 days     | 3.13**  | 2.87**  | 2.73a   | 2.00b   | 1.40b   | 0.30    |
| 25 days     | 3.93Aa* | 3.53A** | 3.13Ab  | 2.13B   | 1.33C   | 0.36    |

C, control; CE1.5, basal diet with 1.5 g/kg SaviotaN®; CE3, basal diet with 3.0 g/kg SaviotaN®; CE5, basal diet with 5.0 g/kg SaviotaN®; CE12, basal diet with 12.0 g/kg SaviotaN® during the first week, 10.0 g/kg during the second week, and 8.0 g/kg for the last two weeks.

Conclusions

During the last decades, public awareness of environmental pollution associated with the use of antibiotics has been publicised with the aim to find alternatives to drugs in animal husbandry, increasing the use of bioactive plant extracts to prevent several animal diseases not only in organic but also in conventional livestock. The results of the present trial are to be considered very interesting from the point of view of prevention and, possibly, therapy of necrotic enteritis in broilers in replacement for the chemicals recently banned in Europe as preventing factor. Chestnut tannin extract may help to control pathogen colonisation of the chicken gut without the development of bacteria resistance that commonly occurs when synthetic antimicrobial growth promoters are used. Due to the complexity of poultry digestive tract, further investigations are required to discover the mode of action against bacterial proliferation.
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