Is a hydrolysable tannin extracted from chestnut wood efficacious against necrotic enteritis?

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Necrotic enteritis is one of the most severe problems in the poultry industry. The disease, responsible for the poor performance along with high mortality rates, is caused by an overgrowth of *Clostridium perfringens* in the small intestine, releasing extra-cellular toxins. Other co-factors such as poor management conditions and coccidiosis contribute to the onset of the disease. One hundred and fifty female Ross 308 broiler chicks were randomly allocated to 5 isolators equipped with air filtration systems (30 birds per isolator). Feed mixtures were formulated according to the NRC requirements on a base of maize and soybean meal. The hydrolysable tannin to be tested (commercial name SaviotaN®) was equivalent to 570 g gallic acid per kg DM. The treatments were: isolator 1, no tannin (control); isolators 2, 3, 4 and 5, increasing levels of tannin, from 1.5 g/kg feed up to 12 g/kg feed. At 10 days the birds were orally challenged with 3,000 oocysts per bird of *Eimeria tenella*, *E. acervulina* and *E. maxima* and after 5 days with *Clostridium perfringens* (10^7 cfu/bird). At 20 days 15 birds per isolator were euthanized and examined for the level of gut infection and macroscopic gut lesions. The same on the other 15 birds, after another 5 days. The first level of tannin which significantly depressed the count of *Clostridium perfringens* in the gut resulted 3.0 g/kg at 20 days and 1.5 g/kg at 25 days ($p<0.01$), while the severe lesions of the gut mucosa were actually absent with level 5.0 g/kg only. In conclusion, the tannin tested in the present trial resulted efficient in preventing and/or controlling a severe necrotic enteritis in challenged birds starting from the level of 3.0 g/kg feed.

Key words: broilers; necrotic enteritis; chestnut tannin.

Introduction

Antibiotics as growth promoters in feeds, due to their controlling action on the gut micro-population, have been banned in Europe since 2006, because of the potential risk of inducing resistance in pathogenic microbes. As a consequence, efforts to find valid alternatives were made in several directions. Our research unit at the University of Florence has been dealing with a monoglyceride of butyric acid, a synthesis product (Leeson et al., 2005; Antongiovanni et al., 2006; 2007a; 2010) and with a tannin from chestnut wood, a natural product (Buccioni et al., 2011) with satisfyingly good results. The tannin tested in our trials, along with the anti-coccidia and anti-clostridium effect described in the present paper, confirmed its nitrogen saving action in ruminants, swine and broilers (Antongiovanni et al., 2007b)

One of the most prominent and severe diseases which may affect the poultry industry and, in particular, the broiler chickens industry, is represented by necrotic enteritis, responsible for a heavy and significant depression of performance and of unbearably high mortality rates (McDevitt et al., 2006). *Clostridium perfringens* is recognized as the etiologic agent of necrotic enteritis (Elwinger et al., 1992), greatly facilitated by the co-infection with *Eimeria* spp (Persia et al., 2006), along with poor management conditions.

Aim of the present work was to confirm the controlling effect of a chestnut hydrolysable tannin upon very severe co-infection conditions in orally challenged broiler chickens with *Eimeria tenella, E. acervulina* and *E. maxima* and, as the second step, with *Clostridium perfringens.*

Materials and methods

One hundred and fifty female Ross 308 one-day-old broiler chicks were randomly allocated to 5 poultry isolators (Allentown®), 30 birds per isolator, equipped with air filtration systems. The animals were vaccinated against Marek disease at the hatchery, but not against coccidiosis.

Feed and drinking water were *ad libitum* throughout the whole trial which lasted 25 days. The feed ingredient composition and the protocol of treatments are presented in *tables 1* and *2*.

Table 1 Composition of feed, common to all the 5 isolators.

Ingredient	%
Maize meal	57.5
Barley bran	22.0
Soybean meal	10.0
Maize gluten feed	3.0
Soybean oil	2.5
Vitamin mineral premix	3.7
Lysine	1.0
Methionine	0.3

Table 2 Protocol of treatments, levels of SaviotaN® tannin added (g/kg feed)

	Isolator 1	Isolator 2	Isolator 3	Isolator 4	Isolator 5
First week	0	1.5	3.0	5.0	12.0
Second week	0	1.5	3.0	5.0	10.0
Third week	0	1.5	3.0	5.0	8.0
Fourth week	0	1.5	3.0	5.0	8.0

The birds in isolator 1 were the control animals, with no tannin added to their feed. The birds in isolators 2, 3 and 4 received the same constant amounts of tannin throughout the trial, while the animals of isolator 5 received 12 g/kg tannin the first week, 10 g/kg tannin the second week and down to 8 g/kg tannin the final two weeks.

At day 10 of age, all the birds were challenged orally with a mixture of *Eimeria tenella, Eimeria acervulina* and *Eimeria maxima*, (3,000 oocysts per bird in a phosphate buffer solution). The oocysts were collected from the gut content of naturally affected unvaccinated birds and allowed to sporulate before challenging. At day 15 all the chickens were challenged again, with a toxigenic strain of *Clostridium perfringens* type A (ATCC® 13124, 10^7 cfu per bird).

At day 20, 15 birds out of each isolator were euthanized and a section of about 10 cm of their small intestine, cranial to Meckel's diverticulum, was examined for macroscopic lesions and scored according to Prescott et al. (1978). Prescott's scores 0 and 1 were considered "no lesions"; 1 and 2 "minor lesions" and scores higher than 2 "severe lesions". *Clostridium perfringens* colonies were counted (Keyburn et al., 2006) and the count figures converted to $\log_{10}$ in order to compute them statistically. At day 25 the other 15 birds were sacrificed and exactly the same examination measurements were made.

The categories "lesions" vs. "no lesions" and "severe lesions" vs. "no and minor lesions" were compared by means of the χ^2 test. The logarithms of *Clostridium* counts figures were used to compute regression equations between cfu's and levels of tannin.

Results and discussion

The number and severity of the gut lesions, both at 20 and at 25 days are clearly depicted by the two bar graphs of *figure 1* and *figure 2*.

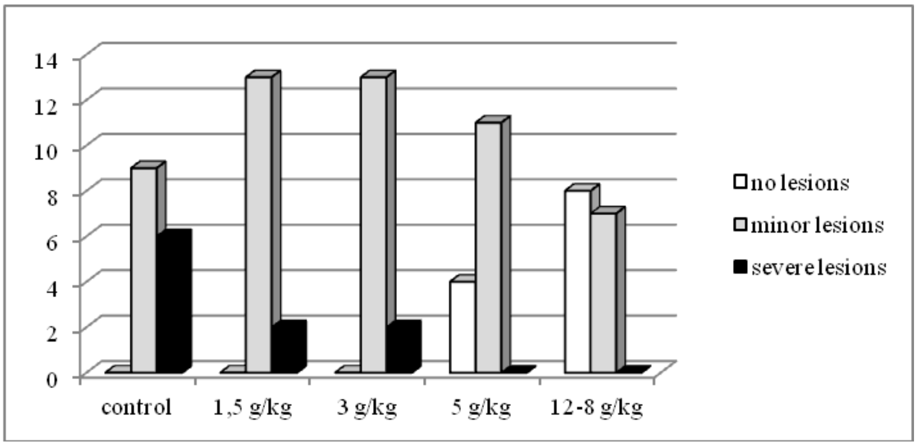

Figure 1 Number of birds with more or less severe gut lesions at day 20

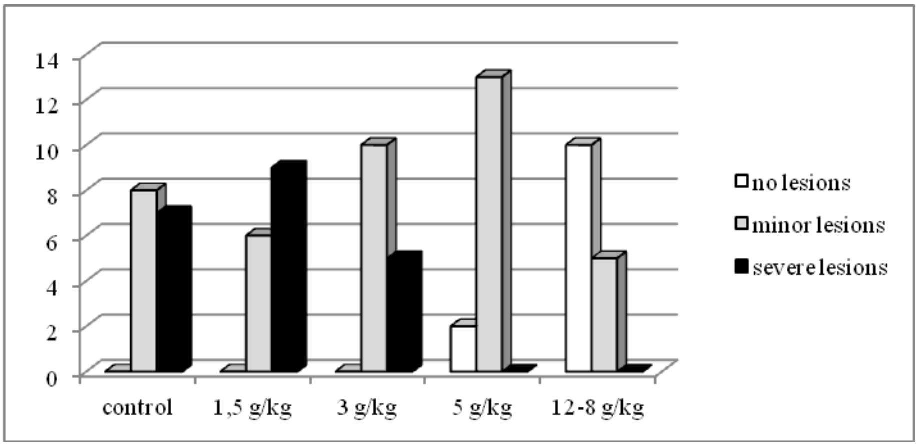

Figure 2 Number of birds with more or less severe gut lesions at day 25

The interpretation of the two graphs is quite clear: both at day 20 and at day 25 all the 15 control birds resulted positive for gut lesions, minor and severe. The same situation was observed in treated animals both with 1.5 and 3 g/kg tannin. When the level of tannin was increased up to 5 g/kg feed and, even better, at the highest level (12 down to 8 g/kg feed), the severe lesions disappeared completely and the number of birds with minor lesions was reduced.

In *table 3* the levels of statistical significance of the χ^2 test demonstrate that, in order to completely avoid lesions, the level of 5 g/kg tannin was not enough, whereas, if the severe lesions (scores 4 up to 6) only are to be avoided, 5 g/kg were sufficient.

Table 3 Effect of increasing amounts of chestnut tannin on the onset of gut lesions: levels of statistical probability

Lesions vs. no lesions	**1.5 g/kg**	**3 g/kg**	**5 g/kg**	**12-8 g/kg**
20 days	n.s.	n.s.	n.s.	0.005
25 days	n.s.	n.s.	n.s.	0.001
Severe lesions vs. no or minor lesions	**1.5 g/kg**	**3 g/kg**	**5 g/kg**	**12-8 g/kg**
20 days	n.s.	n.s.	0.025	0.01
25 days	n.s.	n.s.	0.025	0.01

The graph of figure 3 depicts the survival behaviour of *Clostridium perfringens* colonies in the gut content as a response to the treatment with the hydrolysable tannin. In particular, at 25 days the count of colonies in the untreated birds was nearly 10^8, but went down to a little higher than 10^3 with the highest level of tannin, with a linear regression characterized by an R^2 very close to 0.99. Five days earlier, the situation of untreated or lightly treated birds was slightly better, as it was to be expected.

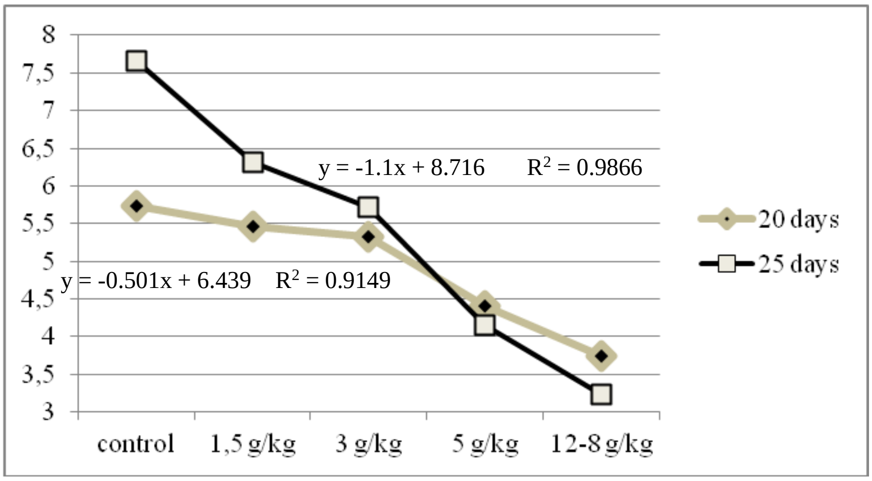

Figure 3 Counts of *Clostridium perfringens* (log_{10} of cfu/g gut content)

Conclusions

The extreme pathological conditions created in the birds' gut by challenging them must be considered exceptionally uncommon in practice. Yet, also under these conditions, the addition of chestnut tannin in the diet resulted capable of limiting the infection even at the lowest level and resulted efficient in healing the disease starting from the level of 5 g/kg feed. The level of 12 g/feed in the first week succeeded in completely avoiding the presence of lesions in the gut mucosa.

It is concluded that levels of chestnut tannin as low as 3 g/kg in the chickens' diet are sufficient to prevent the onset of the disease in mild infection conditions. In more severe conditions of infection, higher levels should be adopted.

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