

## Efficacy of an attenuated coccidiosis vaccine in combination with different feed additives on production performance and intestinal lesions in broilers challenged with necrotic enteritis

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### 1 INTRODUCTION

Coccidiosis is one of the most common and widespread diseases in commercial poultry. Coccidiostats continue to be the most widely used prevention tool in broilers. Reduced sensitivity of *Eimeria* parasites due to prolonged exposure to these drugs together with the ever-increasing need to reduce antibiotic use have drawn the attention of poultry producers towards the use of anticoccidial vaccines. The objective of this study was to evaluate the efficacy of an attenuated coccidiosis vaccine for broilers (EVANT<sup>®</sup>), in combination with different in-feed additives in broilers challenged with necrotic enteritis (NE).

Coccidiosis vaccines are indicated to decrease the intestinal lesions caused by specific *Eimeria* species which are a known predisposing factor to NE (8, 9) and, consequently, these vaccines could be a holistic approach to the control of NE disease and an alternative solution to coccidiostats.

Over the last few years, feed additives have gained special attention from the poultry industry as tool to improve zootechnical performance (1, 2) and as an alternative solution to antimicrobials. This latter is the case of organic acids like Short-chain fatty acids (SCFA) and Medium-chain fatty acids (MCFA) (6, 7, 10), or phytogenic feed additives (PFA) (5, 7). The combination of vaccination against coccidiosis and the supplementation of the diet with the above-mentioned feed additives could be a composite approach to the control of NE problems triggered by *Eimeria* spp. infestation in broilers.

Therefore, the objective of this study was to test the efficacy of an attenuated coccidiosis vaccine in combination with different feed additives in preventing loss of production performance and intestinal lesions in broilers challenged with NE.

### 2 MATERIALS AND METHODS

#### Birds and diets

Broiler chicks were supplied from a commercial source and housed at the Schothorst Feed Research facilities.

The standard diets were formulated without coccidiostats and antimicrobial growth promoters. Moreover, the grower feed diet was formulated to provide predisposing factors for NE development. Therefore, feedstuffs rich in non-starch polysaccharides were provided, whereas enzymes like glucanase and xylanase were omitted. The tested diets were prepared by supplementing the standard diet with SCFA, MCFA, or Phytogenic Feed additives (PFA) throughout the study.

#### Necrotic enteritis challenge

**DAY 15:** chicken inoculated with 1 ml PBS containing 4,500 sporulated oocysts of *Eimeria maxima*.

**DAY 20:** chicken inoculated with 1 ml liver broth containing 2.5 x 10<sup>8</sup> CFU *Clostridium perfringens*

#### Experimental design

Chickens were distributed between 6 experimental groups (Table 1). Groups 3, 4, 5 and 6 were vaccinated at one day of life against coccidiosis with EVANT<sup>®</sup>. The rest of the groups were not vaccinated. Groups 1, 2 and 3 were then reared without feed additives in the diet, whereas groups 4, 5, 6 were reared with MCFA, SCFA or PFA respectively in the diet. NE was then experimentally induced in groups 2 to 6, whereas group 1 was sham-infected. Body weights (BW) and feed intake



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Table 1. Trial Groups

Group	Number of cages (20 birds each one)	Description	Feed Additive	Treatment Days	NE Challenge
1	8	Negative Control	NO	NO	NO
2	8	Positive Control	NO	NO	YES
3	8	EVANT®	NO	1 d	YES
4	8	EVANT®+ MCFA	47% acid lauric (53% MCFAs: capric, caprylic). 94% total free fatty acids (myristic, oleic, palmitic, stearic, linoleic)	1d +42 d	YES
5	8	EVANT®+ SCFA	Sodium butyrate, gradual release	1 d+42 d	YES
6	8	EVANT®+ PFA	Phytogenics (thymol, eugenol, piperine) + benzoic acid	1 d+42 d	YES

(FI) were recorded during the study. Intestinal lesions were scored according to different methods depending on whether it was for *E. maxima* (3) or for *C. perfringens* (4). Finally, excreta samples were periodically collected to evaluate the dynamics of oocyst excretion after vaccination and after challenge (Table 2).

Table 2. Summary of recorded productive parameters

Parameters	Days of age (D)								
	0	5	6	7	14	21	22	28	42
Clinical signs, mortality					Daily				
Body weight	+				+			+	+
Body weight gain, Feed Intake and Feed conversion ratio					+			+	+
Oocysts per gram (OPG) in the faeces		+	+	+	+	+		+	
Lesion scoring						+	+		



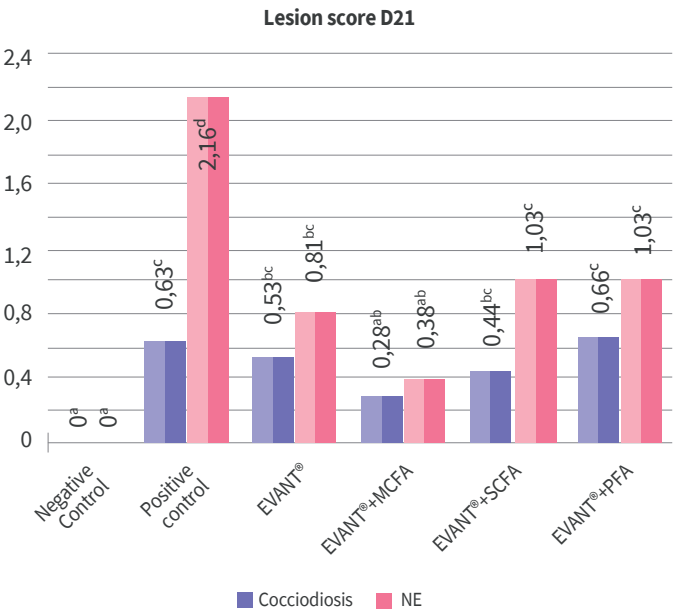
Weighing of vaccinated chicks before allocation

## 3 RESULTS

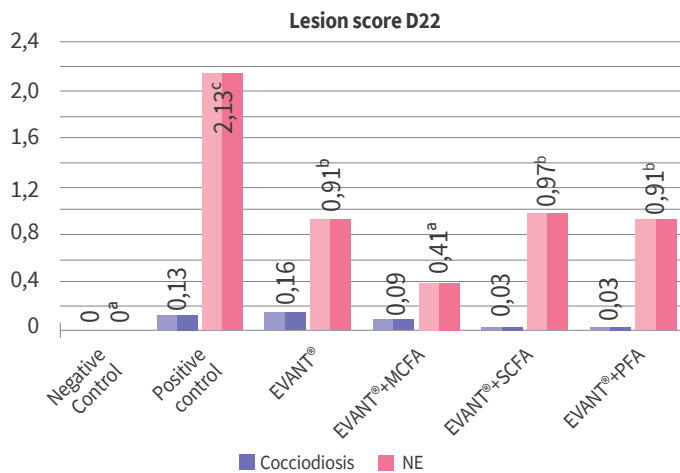
### Bird health

The experimental NE challenge in the non-vaccinated group of birds showed a slight but not significant increase in mortality and a significant increase in intestinal lesions associated with *E. maxima* and *C. perfringens* (Graphic 1). Vaccine administered alone or in combination with feed additives in the diet significantly decreased the intestinal lesion score associated with *C. perfringens* and slightly but not significantly the mortality compared to the untreated group. MCFA supplemented group was the only one which showed a synergistic effect with the vaccine; thus, a further statistically significant decrease in the intestinal lesion score associated with *C. perfringens* was observed.

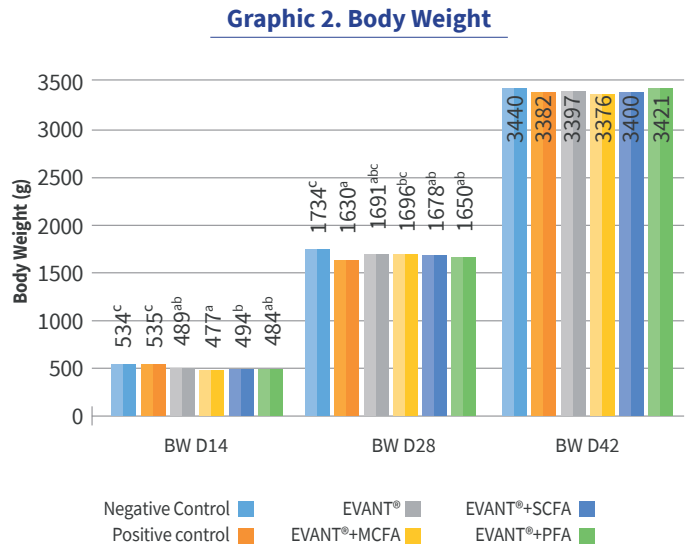
Graphic 1. Mean intestinal lesion score



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a-c Values without a common superscript within a column are significantly different ( $P \leq 0.05$ ).



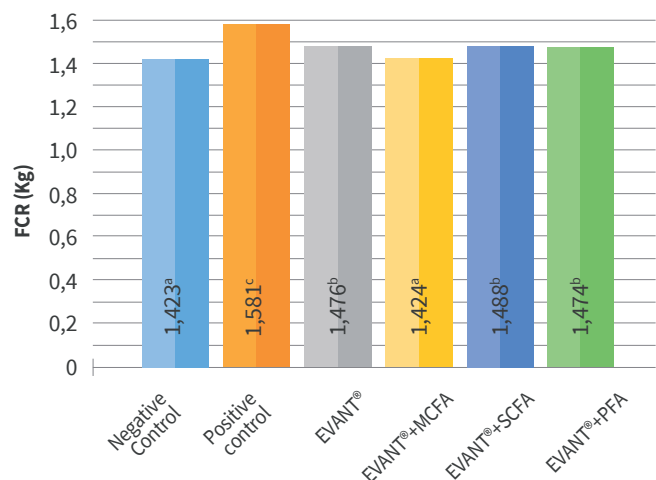
NE lesions in the positive control group after challenge

## Productive performances

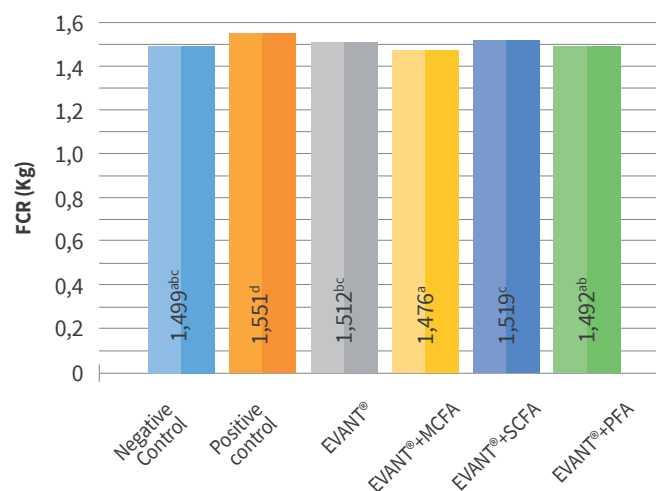
The effect on body weight (BW) due to NE was seen shortly after the challenge (Graphic 2). The disease reduced the growth of the birds as demonstrated by the lower body weight (BW) and higher feed conversion ratio (FCR) (Graphic 3). The main effect of the disease on the productive performances was observed at 28 days post-vaccination (dpv) and growth losses were compensated by birds afterwards but with less efficiency.

After the NE challenge, vaccinated groups grew and converted feed better than the positive control as shown by the higher BW and lower FCR (Graphics 2-3). A compensatory growth after the challenge (28-42 dpv) was probably the cause of the observed lack of significant benefits on this parameter at 42 dpv (Graphic 2). The supplementation of the diet with feed additives did not further improve the body weights of the vaccinated birds after the NE challenge (28 dpv) compared to the vaccine alone; thus, no statistically significant differences in those parameters were found (Graphic 2). However, the group with MCFA was the only one among those vaccinated and with a diet supplemented with additives which converted the feed more efficiently, as shown by the lower FCR in the overall period (Graphic 4).

## Graphic 3. Feed Conversion Ratio D14-D28



## Graphic 4. Feed Conversion Ratio D0-D42

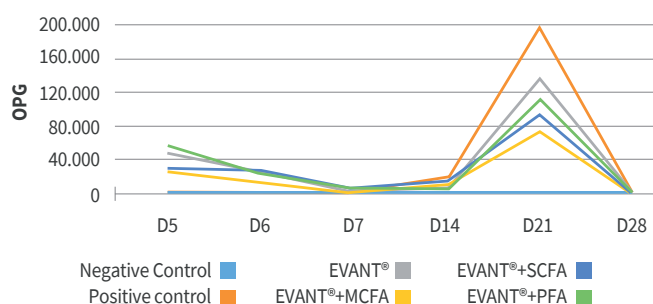


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## Oocyst excretion in the faeces

The number of *Eimeria* spp. oocysts excreted in fresh faeces was counted to check the vaccination as well as the challenge performances (Graphic 5). The period between 5, 7 dpv and 14 dpv generally corresponded to the excretion of the vaccine's attenuated strains during the first and second replication cycles respectively. Vaccinated groups showed excretion of certain levels of oocysts during that period, indicating a successful vaccination process. After the challenge (21 dpv), the oocyst excretion greatly increased in challenged groups, which was consistent with the replication of the inoculated *E. maxima*. Non-vaccinated and challenged group showed the highest levels of oocyst excretion. On 28 dpv, the oocyst excretion was low in all treatments and without significant treatment effects.

**Graphic 5. *Eimeria* spp. oocyst excretion in faeces.**  
Results are reported as number of oocysts per gram (OPG).



## 4 DISCUSSION & CONCLUSION

In this study, vaccination with EVANT®, an attenuated coccidiosis vaccine for broilers, was shown to be able to reduce intestinal lesions associated with NE. Intestinal lesions associated with *C. perfringens* were reduced compared to non-vaccinated birds and, as a consequence, benefits in productive performances were observed as well. Feed conversion was improved compared to non-vaccinated birds during the critical period of the disease. The supplementation of SCFA- and PFA- based feed additives in the diet of vaccinated birds did not provide more benefits than the vaccine alone. MCFA were shown to be able to further decrease intestinal lesion scores associated with *C. perfringens* than vaccination alone, and a significant improvement in the feed conversion efficacy was observed.

In conclusion, the vaccination with EVANT® reduced the intestinal lesions caused by a NE disease triggered by *E. maxima*, a predisposing diet and *C. perfringens* infection; therefore, it also prevented some of the productivity losses associated with the disease. Supplementation of MCFA to the diet of vaccinated birds further reduced the intestinal lesions caused by the same NE challenge model compared to vaccination alone and improved the feed conversion of birds regardless of the NE challenge.

## 5

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