



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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INTRODUCTION

Necrotic enteritis (NE) is an enteric disease of poultry, caused by *Clostridium perfringens*. Several predisposing factors to the disease have been identified, including diet and *Eimeria spp.* infestations. Coccidiosis vaccines are indicated to decrease the intestinal lesions caused by specific *Eimeria* species which are a known predisposing factor to NE (3, 4). Feed additives have gained special attention from the poultry industry as one of the alternative solutions to antimicrobials. The combination of vaccination against coccidiosis and the supplementation of the diet with 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 a loss of production performance and intestinal lesions in broilers challenged with necrotic enteritis.

MATERIALS & METHODS

960 healthy day-old Ross 308 male broiler chicks were equally allocated depending on body weight in 48 cages and distributed among 6 groups (Table 1). Feed and water were supplied *ad libitum*. The birds were reared with a starter (0-14 days post-vaccination – dpv), grower (14-28 dpv) and finisher (28-42 dpv) pellet diet. The standard diets were formulated without coccidiostats and antimicrobial growth promoters. Moreover, the grower 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. Tested diets were prepared by supplementing (150 ppm) the standard diet with Short Chain Fatty Acids (SCFA), Medium Chain Fatty Acids (MCFA), or Phytogenic Feed additives (PFA). Vaccination with EVANT® (LABORATORIOS HIPRA, Spain) was carried following manufacturer's instructions. NE was triggered with a primary infestation with *Eimeria maxima* (4,500 sporulated oocysts) at 14 dpv and a secondary infection with *Clostridium perfringens* (2.5 x 108 cfu) at 20 dpv.

Table 1. Experimental design

Group (G)	Vaccination	NE challenge	Feed additive	
1	No	No	No	
2	No	Yes	No	
3	Yes	Yes	No	
4	Yes	Yes	SCFA	
5	Yes	Yes	MCFA	
6	Yes	Yes	PFA	

Productive parameters were recorded during the study. Intestinal lesions were scored at 21 and 22 dpv according to different methods depending on whether it was for *E. maxima* (1) or for *C. perfringens* (2). Data were analysed by ANOVA using the Fisher's post-hoc Least Significant Difference (LSD). Values with $P \le 0.05$ were considered statistically significant. Statistical analyses were based on two-sided tests implemented with GenStat® (18th edition for Windows, VSNI).

RESULTS

BIRD HEALTH

The experimental NE challenge in G2 showed a slight but not significant increase in mortality, no clinical signs and a significant increase in intestinal lesions associated with *E. maxima* and *C. perfringens* compared to G1 (Table 2). Vaccine administered alone (G3) or in combination with feed additives in the diet (G4-6) significantly decreased the intestinal lesion score associated with *C. perfringens* and slightly but not significantly the mortality compared to G2. G4 was the only one among those fed with additives in the diet, which showed a positive effect with the vaccine; thus, a further statistically significant decrease in the intestinal lesion score associated with *C. perfringens* was observed in comparison with G3 at 22 dpv. No statistically significant effect on the intestinal lesion scores associated with *E. maxima* of the vaccination alone or combined with feed additives in the diet was observed, with the exception of group 4.

Table 2. Mean intestinal lesion score and mortality

Group	21 dpv		22 dpv		Mortality (%)
	Em	Ср	Em	Ср	
1	O ^a	O ^a	0	0 ^a	3.75
2	0.63 ^c	2.16 ^d	0.13	2.13 ^c	7.50
3	0.53 ^{bc}	0.81 ^{bc}	0.16	0.91 ^b	6.25
4	0.28 ^{ab}	0.38 ^{ab}	0.09	0.41 ^a	5.63
5	0.44 ^{bc}	1.03 ^c	0.03	0.97 ^b	6.25
6	0.66 ^c	1.03 ^c	0.03	0.91 ^b	6.88

a-d Values without a common superscript within a column are significantly different (P ≤ 0.05). *Em: E. maxima; Cp: C. perfringens.*

PRODUCTIVE PERFORMANCES

The effect on body weight (BW) due to NE was seen shortly after the challenge; birds in G2 were lighter compared to G1 at 28 dpv (Table 3). The disease reduced the growth of the birds as demonstrated by the lower body weight gain (BWG) and higher feed conversion ratio (FCR) of G2 compared to the G1 during the grower period. The differences observed in terms of BW and BWG disappeared afterwards; however, the effect on FCR persisted until 42 dpv. After the NE challenge, G3-6 showed average BW similar to the G2. However, the former grew and converted feed better than the latter as shown by the higher BWG and lower FCR (Table 3). The supplementation of the diet with feed additives did not further improve the BW and BWG of the vaccinated birds after the NE challenge; thus, no statistically significant differences in those parameters were found between G3 and 4-6. However, G4 was the only one among those with a supplemented diet which converted the feed more efficiently, as shown by the lower FCR in the overall period (0-42 dpv) in comparison to G3 (Table 3). The observed FCR of G4 was associated with a lowest feed intake (FI) by the birds in the overall period (0-42 dpv) compared to all other tested groups.

Table 3. Productive parameters

Group (G)	1	2	3	4	5	6
0-14 dpv						
0 dpv BW	43.0	43.0	42.8	42.8	42.8	42.8
14 dpv BW	534 ^c	535 ^c	489 ^{ab}	477 ^a	494 ^b	484 ^{ab}
BWG	491 ^c	492 ^c	446 ^{ab}	434 ^a	455 ^b	441 ^a
FI, g/bird	550 ^c	554 ^c	520 ^b	495 ^a	528 ^b	513 ^b
FCR	1.121 ^a	1.126 ^a	1.167 ^c	1.141 ^b	1.160 ^c	1.162 ^c
14-28 dpv						
28 dpv BW	1743 ^c	1630 ^a	1691 ^{abc}	1696 ^{bc}	1678 ^{ab}	1650 ^{ab}
BWG	1210 ^b	1095ª	1203 ^b	1219 ^b	1184 ^b	1167 ^b
FI	1721	1727	1776	1737	1762	1720
FCR	1.423 ^a	1.581 ^c	1.476 ^b	1.424 ^a	1.488 ^b	1.474 ^b
28-42 dpv						
42 dpv BW	3440	3382	3397	3376	3400	3421
BWG	1718	1752	1750	1680	1721	1771
FI	2817 ^b	2899 ^b	2843 ^b	2688a	2817 ^b	2792 ^{ab}
FCR	1.640 ^{bc}	1.654 ^c	1.625 ^{abc}	1.602 ^{ab}	1.637 ^{bc}	1.578a
0-42 dpv						
BWG	3397	3340	3354	3333	3357	3378
FI	5092	5181	5072	4919	5100	5040
FCR	1.499 ^{abc}	1.551 ^d	1.512 ^{bc}	1.476 ^a	1.519 ^c	1.492ab

a-d Values without a common superscript in a raw differ significantly (P < 0.05). BW: body weight (g); BWG: body weight gain (g); FI: feed intake (g/bird); FCR: feed conversion rate.

DISCUSSION

In vaccinated animals, intestinal lesions associated with *C. perfringens* were reduced compared to non-vaccinated birds and, as a consequence, benefits in productive performances were observed. Although benefits in body weight were not observed, feed conversion improved compared to non-vaccinated birds during the critical period of the disease. The mechanism behind these effects is not clear. Vaccination of birds reduced the lesions induced by *E. maxima*, although not significantly. Therefore, the vaccine produced an indirect effect on 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 inclusion in the diet, together with the vaccine application, was shown to further decrease intestinal lesion scores associated with *C. perfringens* than vaccination alone. Despite observing an improvement in intestinal health due to MCFA supplementation in the diet, no benefits in body weight were observed. Instead, a significant improvement in the feed conversion efficacy was observed. This was associated with a lower feed intake.

In conclusion, EVANT® reduced the intestinal lesions caused by a NE disease triggered by *E. maxima*, a predisposing diet and *C. perfringens* infection; therefore, the vaccine 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 NE challenge model compared to vaccination alone and improved the feed conversion of birds regardless of the NE challenge.

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