Histopathological assesment of the intestinal barrier in broilers treated with a phytogenic oregano rich essential oil and challenged with *Eimeria* spp

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Introduction

Maintaining and modulating the complex intestinal barrier composed of intestinal microbiota, enteric epithelial cells, and mucosal immunity is crucial for the poultry industry. The integrity of the intestinal barrier relies significantly on intercellular junctional complexes, which connect neighboring intestinal epithelial cells, affecting gut permeability. Histology, histomorphometry, and immunohistochemistry for evaluating the expression pattern of Claudin-3 and CD3 represent robust tools to investigate these parameters. It has been shown that Tight Junctions proteins (TJs) may also undergo regulation by modulating their cellular localization. This study evaluated the growth performance parameters, the oxidative status and the intestinal barrier profile in broilers infected with *Eimeria* spp. and treated with a phytogenic oregano-rich essential oil (OEO) compared to salinomycin.

Material and methods

288 male Ross-308 broilers were randomly allocated to four groups: Non-infected control (A), infected control (B), infected treated with salinomycin (C), and infected treated with OEO (D). Infection was done with 3.5×10^4 *E. acervulina*, 7.0×10^3 *E. maxima*, and 5.0×10^3 *E. tenella* oocysts, respectively. On day 35, muscle samples were analyzed for malondialdehyde (MDA) and protein carbonyl (PCO) levels. Additional assessments included body weight gain, anticoccidial index (ACI), and gross intestinal lesions. Tissue samples from duodenum, jejunum, and ileum were processed and stained with HE and immunohistochemistry for CD3 (rabbit, polyclonal antibody, A0452, Agilent Dako, dilution 1:300) and Claudin-3 (rabbit, polyclonal antibody, ab15102, Abcam, Cambridge, dilution 1:100. A scoring system by Ruggeri et al. (2014) was used for the histopathological evaluation. The number of CD3+ cells were counted in the following micro-compartments: villus (epithelium and lamina propria) and deep lamina propria (lamina propria between and below intestinal crypts). For each of these areas, lymphocytes were counted on 10 villi and 10 random non-overlapping areas of 17.600μm (110x160μm) of deep lamina propria using a 20x and 40x objective respectively. Claudin-3 expression pattern including distribution and intensity (by comparing mean pixel intensity values in images converted into 8-bit grayscale) was also evaluated morphometrically on 10 fields using a 10x objective and the QuPath 0.4.3 programme.

Results

Group A demonstrated optimal health parameters (Table 1-5, Figure 1). Groups C and D showed mirroring values and exhibited significantly improved performance (Table 1, 2), antioxidant status (Table 3, 4), Claudin-3 expression, and less severe intestinal lesions, including lower numbers of CD3+ cells than Group B (Figure 1).

Pathology

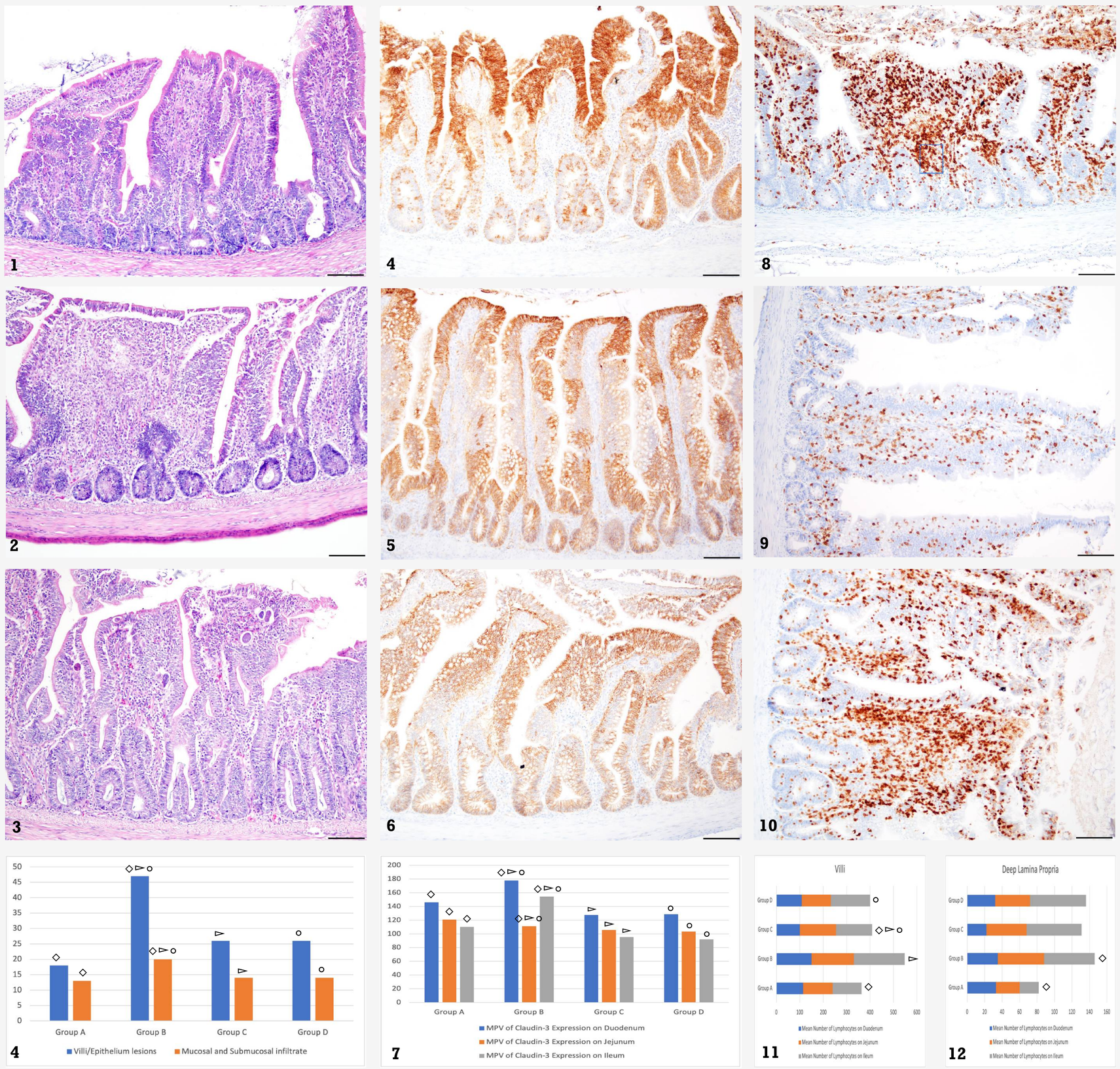

Fig.1: Left column: HE. Small intestine. Main histological parameters that were evaluated. Bar=100 μm. 1) Villus shortening and surface and glandular epithelial hyperplasia. 2) Villus blunting and fusion, surface and glandular epithelial hyperplasia, lymphoplasmacytic infiltrate in the lamina propria. 3) Multiple apicomplexan coccidian forms in various developmental stages in the mucosa.4) Summary results of the HE total score. Symbols show a significant difference among groups ($p<0.05$). Central column: Bar=100 μm. 4,5,6) The immunohistochemical expression of Claudin-3 (on apical and basal regions and the pericellular borders of the epithelial cells) showed continuousity or differences in the spatial distribution resulting in epithelial gaps. 7) Mean pixel intensity values (MPV) in each group's Claudin-3 immunohistochemical reaction. Symbols show a significant difference among groups ($p<0.05$). Groups A, C and D showed significantly lower mean pixel values, interpreting as higher intensity of claudin 3 expression. Right column: Bar=100 μm. 8,9,10) CD3+ positive cells. The rectangle represents an area of interest of deep lamina propria (AOI; 17,600μm2). 11) Mean number of lymphocytes in each group. Symbols show a significant difference among groups ($p<0.05$).

Performance and oxidative status

	Group A	Group B	Group C	Group D
BW_D1 (g)	44.5	44.17	44.33	43.83
BW_D14 (g)	327.33	314.33	312.33	303.83
BW_D35 (g)	1953.67^{B}	$1764^{C,D}$	1835.83B	1814^{B}

Table 1: Body weight (BW) on days 7, 14 and 35 of the trial. Means in each row with superscripts show a significant difference $P<0.05$.

	Group A	Group B	Group C	Group D
FCR1_7 (g/g)	1.07	1.12	1.11	1.06
FCR1_14 (g/g)	1.75	1.79	1.77	1.88
FCR1_35 (g/g)	1.47^{B}	$1.49^{A,C,D}$	1.5^{B}	1.62^{B}

Table 2: Feed Conversion Ratio (FCR) on days 7, 14 and 35 of the trial. Means in each row with superscripts show a significant difference $P<0.05$.

	Group A	Group B	Group C	Group D
MDA Breast	$6.71^{C,D}$	$6.23^{C,D}$	$10.09^{A,B}$	$10.21^{A,B}$
MDA Thigh	13.09^{B}	$11.92^{A,C,D}$	$14.85^{A,B}$	$13.22^{A,B}$
MDA Intestine	$56.49^{C,D}$	$56.57^{C,D}$	$72.15^{A,B}$	$72.48^{A,B}$

Table 3: Oxidative stability of intestinal tissue and breast meat (ngMDA/g of tissue). Means in each row with superscripts show a significant difference $P<0.05$.

	Group A	Group B	Group C	Group D
Protein Carb. Breast	4.49	4.42	4.24	4.58
Protein Carb. Thigh	3.84	3.83	3.86	3.84

Table 4: Protein Carbonyls concentration in Breast and Thigh meat representing the oxidative damage (nmol/mg).

	Group A	Group B	Group C	Group D
ACI d21	>200	93.96	136.91	188.45

Table 5: Impact of dietary supplements and a coccidial challenge on the anticoccidial index (ACI).

Conclusions

Our results indicate that OEO and salinomycin improve the intestinal barrier in broilers infected with *Eimeria* spp, but the modulation warrants further research.

References: SCAN ME