Modulation of intestinal mucin composition and mucosal morphology of broiler chickens by the dietary inclusion of a blend of SCFA, MCFA and essential oils

M. MAZZONI¹ and D.A. QUADRELLI²*

¹Dept. of Veterinary Med. Science, Alma Mater Studiorum, University of Bologna, IT-40064 Ozzano dell’Emilia (Bologna), Italy, ²Unione Commerciale Lombarda, 36 V. G. Di Vittorio, Brescia, Italy

daniel.quadrelli@uclspa.it

Consumer pressure to reduce antibiotic use in animal feeding has resulted in the development of nutraceuticals to promote good health and performances. Ent-oil is a blend of SCFA, MCFA and essential oils, with known benefits for microflora growth, modulation and gut morphology and function. Aim of this research was to prove the effect of Ent-oil inclusion on the jejunum mucosae of chickens. A total of 1170 1-day old Ross 308 males were divided into 2 groups of 9 floor-pen boxes of 65 birds each. Both groups received 4-phases commercial meal diets from 0 till 42 days without coccidiostatics. The difference between groups was Ent-oil inclusion: 0,00% for Control (CTR) vs 0,15% in starter diet followed by 0,10% thereafter for Ent-oil (EO). At the end of the trial a total of 18 (9+9) chicken guts were analysed. In the jejunum was observed a significant increase in villus height (836 ± 127.9) in EO compared to CTR group (690.9 ± 153.6, CTR vs. E0 P <0.05), and an increase of the mean values of the mucosal surface area in the EO group (12.2 ± 1.8) compared with CTR (9.5 ± 1.9, CTR vs. E0 P <0.01). By means the combined alcian blue (AB)-periodic acid Schiff’s (PAS) staining we observed, in the villi jejunum, a significant increase in the number of goblet cells in the EO (1625 ± 23.7) compared to the CTR group (1182 ± 19). In addition, the EO group showed a high number of PAS goblet cells (69 ± 9.7) respect to the CTR group (54.8 ± 16.6, P < 0.05); moreover, Ent-oil group has a high number of AB goblet cells (111.7 ± 8.6) than that of CTR group (76.5 ± 15.4, P < 0.01).

In conclusion, dietary inclusion with Ent-Oil modulates broiler intestinal mucin composition and morphology.

Keywords: broiler; nutraceutics; villus; mucosa; goblet

Introduction

As an answer to the increasingly need to reduce the use of antibiotics in animal feeding many attempts to develop functional feeding strategies have been recently done, in order to promote good intestinal health and performances (Langhout, 2000; Mellor, 2000a; Mellor, 2000b; Taylor, 2001; Wenk, 2000). Possible work tools are represented by short chain fatty acids (SCFA), medium chain fatty acids (MCFA) and essential oils supplemented in the diet (Lee et al., 2004; Kabara, 1984; Sevcik et al., 2004; Van Immerseel et al., 2000). The purpose of this research was to test the effect of the dietary inclusion of Ent-oil, wich is a synergistic combination of free and esterified SCFA, MCFA plus special essential oils, on the intestinal morphology of broiler chickens.
Materials and method

A total of 1170 1-day old Ross 308 males were divided into 2 groups of 9 floor-pen boxes of 65 birds each (stocking density 0.091 m2/bird). Both groups received 4-phases commercial meal diets from 0 till 42 days without coccidiostatics. Feeding phases and diets were 0-10, 11-21, 22-35 and 36-42 days of age. Diets were whitening vegetable type and following nutritional specifications ranging data as recommended by Ross.

The difference between groups was Ent-oil inclusion: 0.00% for Control (CTR) vs 0.15% in starter diet (0-10 days) followed by 0.10% thereafter for Ent-oil (EO).

At the end of the trial one chicken per box (9 replicate chickens for 2 treatments for a total of 18 animals) was submitted to jejunum collection at about 5 cm from the Meckel's diverticulum. Tissue samples were fixed in formalin 10 fresh buffer, dehydrated, cleared, and embedded in paraffin.

For each sample, sections were cut slices of 5 µm thickness, placed on glass slides, stained with hematoxylin-eosin and combined alcian blue-periodic acid Schiff’s (AB- PAS) technique.

Intestinal morphology

In several sections of each slide stained with hematoxylin and eosin, villus height and width and crypts depth and width were randomly evaluated in 10 villi and 10 crypts using a Zeiss Axioplan microscope (10x objective) connected to KS 300 image analysis software (Kontron Elektronic). Villus height, were measured as the distance from the crypt opening to the top of the villus, whereas crypt depth were measured from the base of the crypt to the level of the crypt opening. To estimate the mucosal surface area, the mucosal-to-serosal amplification ratio M were calculated as indicated by Kisielinski et al. (2002) and subsequently adapted in for piglets by Trevisi et al. (2009).

Mucin staining

The sections were stained with combined alcian blue pH 2.5 (AB) plus periodic acid Schiff’s (PAS) (AB+PAS). The AB detects acidic mucopolysaccharides and the PAS detects neutral mucopolysaccharides. The goblet cells that produce acidic mucins result blue stained, while the goblet cells that produce neutral mucins result purple-magenta stained (Figure 1). The number of goblet cells stained with PAS and AB was evaluated in 10 randomly selected villi using a Zeiss Axioplan microscope (20x objective) connected to KS 300 image analysis software (Kontron Elektronic).

Statistical analysis

Data were analyzed using the Student’s t-test (Graph Prism 4, GraphPad Software, Inc., La Jolla, Ca, USA). A $P < 0.05$ was considered as statistically significant. Values were expressed as mean ± standard deviation (SD).

Results

The villus height in the EO group (836 ± 127.9) was greater and statistically significant compared to the control (CTR) group (690.9 ± 153.6) (CTR vs EO $P < 0.05$, graph 1). In addition, the average values referred to the mucosal surface area (M index) showed a statistically significant difference in the EO group (12.2 ± 1.8) compared to the CTR group (9.5 ± 1.9) (CTR vs EO $P < 0.01$, graph 2).
The analysis of the sections stained with combined alcian blue (AB) plus periodic acid Schiff’s (PAS) (Figure 1) revealed an increase of total number of the goblet cells in EO group (1625 ± 23.7) respect to CTR group (1182 ± 19) (CTR vs EO P <0.01, graph 3). The same trend was observed in both PAS and AB positive cells. In details, in the EO group a high number of PAS goblet cells (69 ± 9.7) were counted respect to the CTR group (54.8 ± 16.6, P < 0.05); moreover, EO group has a high number of AB goblet cells (111.7 ± 8.6) than that of CTR group (76.5 ± 15.4, P < 0.01, graph 4).
Graph 3. Total number of goblet cells in the CTR and EO groups.

Graph 4. Mean number of neutral goblet cells (PAS positive) and acid ones (AB positive) in the jejunum villi.
**Figure 1.** (A) and (B): combined alcian blue-periodic acid Schiff’s (AB-PAS) stain of the chicken jejunum villi. In the (B) magnification the black arrow indicate a AB stained goblet cell, while red arrow indicate a PAS stained goblet cell.

**Conclusions**

Dietary inclusion of SCFA, MCFA and essential oils significantly increases villus height and mucosal surface area at the jejunum section of small intestine.
Moreover, there is an increase of the goblet cells whether as total number or PAS and AB positive mucin types in the jejunum villi.
References