Effects of mannanoligosaccharide and/or organic acid mixture on performance, blood parameters and intestinal microbiota of broiler chicks

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ABSTRACT

The experiment was conducted to evaluate the effects of organic acid mixture and/or mannanoligosaccharides (MOS) on growth performance, blood parameters and intestinal microbiota in 120 Ross 308 male broiler chicks, over a period of 21 days. Birds were maintained in battery brooders confined in an environmentally controlled experimental room. There were 4 dietary treatments, each consisting of 6 replicates. Dietary treatments were: (i) basal diet (as a control), (ii) basal diet + MOS 2 kg/ton feed, (iii) basal diet + organic acid mixture 3 kg/ton feed and (iv) basal diet + MOS 2 kg/ton feed + organic acid mixture 3 kg/ton feed. Weight gain of the broilers in this study was significantly influenced by the addition of organic acid mixture (P<0.01). The lowest feed intake and feed conversion ratio (FCR) were detected in the MOS supplemented groups (P<0.05). Erythrocyte length (EL) was significantly increased in MOS + organic acid mixture fed groups (P<0.05). In ileal digesta, lactic acid bacteria counts increased in MOS + organic acid mixture fed groups (P<0.001). Otherwise, E. coli counts decreased in MOS, organic acid mixture and MOS + organic acid mixture fed groups compared to control groups (P<0.001). In caecal digesta, lactic acid bacteria counts increased (P<0.001), whereas E. coli numbers decreased compared to control groups (P<0.001).

Key words: Organic acid, Mannanoligosaccharide, Blood morphology, Intestinal microbiota, Broiler.

RIASSUNTO

MANNANO-OLIGOSSACCHARIDI E ACIDI CARBOSSILICI ADDIZIONATI O USATI SINGOLARMENTE NELLA DIETA DI BROILER: EFFETTI SULL’ACCRESCIMENTO, SUI PARAMETRI EMATICI E SULLA FLORA MICROBICA INTESTINALE

La prova sperimentale è stata condotta al fine di valutare gli effetti di una miscela di acidi organici e/o mannanoligosaccaridi (MOS) sulle performance di accrescimento, sui parametri ematici e sulla flora microbica intestinale di 120 broiler Ross 308 per un periodo di 21 giorni. Gli animali sono stati mantenuti in gabbie all’interno di stabulari sperimentali sottoposti a controllo ambientale. Sono stati somministrati 4
trattamenti alimentari ognuno costituito da 6 replicazioni: (i) dieta di base (controllo), (ii) dieta di base + MOS 2 kg/ton, (iii) dieta di base + miscela di acidi organici 3 kg/ton, (iv) dieta di base + MOS 2 kg/ton + miscela di acidi organici 3 kg/ton. L’incremento ponderale degli animali durante la sperimentazione è stato influenzato significativamente dalla presenza degli acidi carbossilici all’interno della razione (P<0,01). La quota più bassa di sostanza secca ingerita dagli animali e il minore indice di conversione dell’alimento sono stati rilevati nel gruppo ii (dieta di base + MOS) (P<0,05). È stata registrata una lunghezza degli eritrociti (EL) significativamente maggiore negli animali del gruppo iv (dieta di base + MOS + miscela di acidi organici). La conta dei batteri lattici nel contenuto ileale è risultata significativamente maggiore negli animali del gruppo iv (dieta di base + MOS + miscela di acidi organici) (P<0,001). Tuttavia il numero di E. Coli è risultato minore nei gruppi trattati rispetto al gruppo di controllo (P<0,001). A livello cecale il contenuto di batteri lattici risulta aumentato (P<0,001), mentre il contenuto di E. Coli risulta diminuito (P<0,001), sempre rispetto ai gruppi di controllo.

Parole chiave: Acidi organici, Mannano-oligosaccaridi, Morfologia ematica, Flora intestinale, Broiler.

Introduction

Increase in bacteria resistance against antibiotics and residues in animal products that might be harmful to consumers resulted in the ban of nontherapeutic antibiotics. A number of alternative products, such as probiotics, prebiotics, organic acids, essential oils, and oligosaccharides, are the subject of research to enhance health and growth performance.

One of the alternatives is supplementation of yeast products to poultry diets. Mannanoligosaccharides (MOS) are obtained from cell walls of *Saccharomyces cerevisiae*, with approximately 45% of the cell wall containing mannose residues (Tizard *et al.*, 1989). MOS have shown promising effects, such as reduction of pathogenic microflora of the gut, stimulating a strong immune response, and elevating the strength of the intestinal mucosa in studies with poultry (Tucker *et al.*, 2003; Hooge, 2004; Rosen, 2007). It appears that the response in growth performance to MOS is more pronounced in early life (Tucker *et al.*, 2003; Yang *et al.*, 2005). Decreased improvement in the growth performance of broiler chickens associated with age may be related to a less-balanced gut microflora in younger birds. Changes in the gut microflora of growing birds exist; for example, it takes about 2 weeks for lactobacilli to become the predominant bacteria (Barnes *et al.*, 1972).

Like antibiotics, short-chain organic acids also have a specific antimicrobial activity. Unlike antibiotics, the antimicrobial activity of organic acids is pH dependent. Most of the pathogens grow at a pH close to 7 or slightly higher. In contrast, beneficial microorganisms can persist at an acidic pH (5.8-6.2) and compete with pathogens (Ferd, 1974). Reductions in bacteria are associated with feeding organic acids, which are particularly effective against acid-intolerant species such as *E. coli*, *Salmonella* and *Campylobacter* (Dibner and Buttin, 2002).

Experiments conducted with MOS revealed inconsistent performance results in broilers. Moreover, the effects of MOS and organic acid mixture in combination on the intestinal microbiota have not been extensively investigated.

Therefore, the aim of this study was to evaluate the effects of MOS and organic acid mixture supplementation individually or in combination, on growth performance, blood parameters and intestinal microbiota in commercial broilers.

Material and methods

**Animals and housing**

Male Ross 308 broilers (1-day-old chicks, n=120) were obtained from a local parent
stock supplier and randomly transferred to compact-type three-tier cages, five chicks per cage. Battery cages were equipped with wire mesh, dropping trays, nipple drinkers and trough feeders.

The experiment was set up in a completely randomized design where 30 birds were randomly assigned to each of four treatments with six replicates. The battery cages were placed in a controlled temperature room. The experiment lasted for 21 days and the chicks were fed the experimental diets throughout the experimental period. Chicks had free access to feed and water. The lighting regime was 23h/d. Birds were weighed by pen at 21 days of age.

**Diets**

A mannanoligosaccharide (ExcelMOS, manufactured and supplied by GLOBAL NUTRITECH 41600 Kandira, Kocaeli-Turkey) and an organic acid mixture (Biotronic® SE, manufactured and supplied by Bimomin, International GmbH. Herzogenburg, Austria) were used. Organic acid mixture contains formic acid 17.4%, ammonium formate 14.1%, propionic acid 12.4%, ammonium propionate 8.4% and filler material 47.7% (Celik et al., 2003).

Dietary treatments were: (i) basal diet (as a control), (ii) basal diet + MOS 2 kg/ton feed, (iii) basal diet + organic acid mixture 3 kg/ton feed and (iv) basal diet + MOS 2 kg/ton feed + organic acid mixture 3 kg/ton feed. Experimental diets were formulated using ration-formulation software (UFFDA, University of Georgia, 1992, Athens, GA) to be isocaloric and isonitrogenous in accordance with National Research Council recommendations (NRC, 1994). The basal diet consisted mainly of corn, soybean meal, wheat, full fat soybean and fish meal, from a local feed market. Experimental diets were formulated to contain 22% crude protein and 3050 kcal/kg, and other essential nutrients (Table 1). Birds were fed the experimental diets *ad libitum* in mash form. Feed intake was recorded weekly. The feed conversion ratio (FCR) was calculated as grams of feed consumed per chick divided by grams of weight gain per chick.

### Table 1. Chemical composition of the basal diet (as fed).

| Ingredients (g/kg):     |          |
|-------------------------|----------|
| Corn                    | 490      |
| Soybean meal            | 207      |
| Wheat                   | 100      |
| Full fat soybean        | 140.2    |
| Fish meal               | 10       |
| Sunflower oil           | 7.4      |
| Limestone               | 15.7     |
| MCP                     | 15.2     |
| Salt                    | 3.2      |
| DL-Methionine           | 2.7      |
| L-Lysine HCl            | 5.9      |
| Premix¹                 | 2.5      |

| Nutrient content²:     | kcal/kg |
|------------------------|---------|
| ME                     | 3050    |
| Crude protein          | 22.00   |
| Ether extract          | 5.62    |
| Crude fibre            | 2.90    |
| Lysine                 | 1.65    |
| Met + Cys              | 0.97    |
| Methionine             | 0.60    |
| Calcium                | 1.00    |
| Non phytate P          | 0.50    |

¹Provided the following per kg of diet: vitamin A (retinyl acetate), 14,000 U; vitamin D₃, 5000 U; vitamin E, 50 mg; vitamin K₃, 4 mg; vitamin B₁₂, 3 mg; vitamin B₂, 8 mg; vitamin B₆, 4 mg; vitamin B₁₂, 16 µg; niacin, 20 mg; iron, 80 mg; folic acid, 2 mg; pantothenic acid, 20 mg; biotin, 150 µg; choline, 1800 mg; copper, 5 mg; manganese, 100 mg; zinc, 80 mg; selenium, 150 µg.

²Based on NRC (1994) values for feed ingredients.
The birds were then weighed before the gastrointestinal tract of each bird was quickly removed and weighed. This was followed by weighing of empty proventiculus, gizzard, duodenum, jejunum and ileum. The weights of the organs were expressed relative to the respective body weight of the birds.

Microbiology of the intestine
At the end of experiment, six birds per treatment were killed by cervical dislocation. Samples of the ileal and caecal contents were immediately collected. The entire ileal and caecal contents were transferred under aseptic conditions into sterile glass bags kept at +4°C until subsequently being plated on agar. All of the samples were prepared before microbiological analysis by a previous dilution in sterile saline solution: 1 g of ileal and caecal contents in 9 mL of saline solution (8.5 g of NaCl L⁻¹ of distilled water). After shaking, 10 mL of the extract were taken for further dilutions. From diluted extracts (10⁻¹ to 10⁻⁶) plates were prepared with specific medium for each studied microorganism. Accordingly, the incubation medium, MRS agar (MERCK, 1.10660), was used for lactic acid bacteria (LAB) and malt extract agar (MERCK, 1.05398) was used for yeast. LAB and yeast counts of the ileum or caecum contents were obtained at 30°C degrees following 3-5 days of incubation. *E. coli* was detected on Violet Red Bile agar (MERCK, 1.01406) after inoculation aerobically at 37°C for 24-48 hours (Seale *et al.*, 1990). The LAB, yeast and *E. coli* counts were counted, and the average number of live bacteria was calculated per gram of original ileal and caecal contents. The LAB, yeast and *E. coli* counts of the samples were converted into logarithmic colony forming units (cfu/g).

Slide preparation and staining
Blood smears were prepared on pre-cleaned glass slides. A drop of blood was smeared over a slide and air dried. Then the smears were fixed in methanol and stained using Giemsa’s azur eosin methylene blue solution (MERCK, 1.09204) method. Blood smears were observed under a microscope (BX 51, Olympus, Japan) at 40x magnification. Afterwards, erythrocyte length (EL) and erythrocyte width (EW) were determined using an image processing and analysis system (Motic Images Plus 2.0).

Statistical analyses
Collected data were recorded on a weekly basis and statistically subjected to ANOVA using a statistical package program (Statistica, 1999). The differences between group means were separated by Duncan’s multiple range test.

Results and discussion
The impacts of MOS and organic acid mixture supplementation on performance of male broiler chicks are shown in Table 2. Weight gain (P<0.01) of broilers was significantly influenced by the addition of organic acid. The lowest feed intake and FCR were detected in the MOS supplemented groups (P<0.05).

Table 3 shows the effects of MOS and organic acid mixture supplementation on digestive organ weights (g/100 g body weight). No significant differences were found for relative organ weights (P>0.05).

Blood parameters are presented in Table 4. Erythrocyte length (EL) significantly increased in MOS + organic acid mixture fed groups compared to the control fed groups (P<0.05). No significant difference was found for the erythrocyte width (EW) (P>0.05).

The effects of dietary treatments on ileal microbiota (log cfu/g ileal content) are shown in Table 5. In ileal digesta, lactic acid bacteria counts increased in MOS + organic acid mixture fed groups (P<0.001). Otherwise,
Feed additives in broiler chicks

Table 2.  Effects of MOS and organic acid supplementation on broiler performance (0-21 d)

| Treatments                  | Weight Gain (g) | Feed Intake (g) | FCR    |
|-----------------------------|-----------------|-----------------|--------|
| Control                     | 491.6<sup>bc</sup> | 587.3<sup>ab</sup> | 1.215<sup>ab</sup> |
| MOS                         | 532.7<sup>ab</sup> | 528.5<sup>b</sup>  | 0.942<sup>c</sup>  |
| Organic acid mixture        | 574.2<sup>a</sup> | 590.1<sup>ab</sup> | 1.032<sup>bc</sup> |
| MOS + Organic acid mixture  | 467.9<sup>c</sup> | 660.4<sup>a</sup>  | 1.318<sup>a</sup>  |
| SEM                         | 13.67           | 16.88           | 0.049           |
| P levels                    | 0.005           | 0.088           | 0.019           |

<sup>a, b</sup>: Means in row with different letters differ significantly.

Table 3.  Effects of MOS and organic acid supplementation on digestive organ weights (21 d).

| Treatments                  | Proventriculus | Gizzard | Duodenum | Jejunum | Ileum  |
|-----------------------------|----------------|---------|----------|---------|--------|
| Control                     | 0.64           | 2.75    | 1.36     | 2.24    | 1.46   |
| MOS                         | 0.59           | 2.57    | 1.33     | 2.13    | 1.37   |
| Organic acid mixture        | 0.66           | 2.97    | 1.32     | 2.20    | 1.49   |
| MOS + Organic acid mixture  | 0.65           | 2.65    | 1.30     | 2.10    | 1.45   |
| SEM                         | 0.023          | 0.100   | 0.045    | 0.047   | 0.034  |
| P levels                    | 0.724          | 0.543   | 0.968    | 0.753   | 0.683  |

Table 4.  Effects of dietary treatments on erythrocyte length (EL) and erythrocyte width (EW) of broiler (21 d).

| Treatments                  | EL (µm) | EW (µm) |
|-----------------------------|---------|---------|
| Control                     | 13.53<sup>b</sup> | 7.95    |
| MOS                         | 14.04<sup>ab</sup> | 7.88    |
| Organic acid mixture        | 13.73<sup>ab</sup> | 7.88    |
| MOS + organic acid mixture  | 14.25<sup>a</sup> | 7.75    |
| SEM                         | 0.103   | 0.076   |
| P levels                    | 0.069   | 0.857   |

<sup>a, b</sup>: Means in row with different letters differ significantly.

E. coli counts decreased by MOS, organic acid mixture and MOS + organic acid mixture fed groups compared to control groups (P<0.001).

Table 6 reveals the effects of dietary treatments on caecal microbiota (log cfu/g caecal content). In caecal digesta, lactic acid bacteria counts significantly increased (P<0.001),
whereas *E. coli* numbers decreased compared to control groups (P<0.001).

Effects of MOS and/or organic acid mixture supplementation on performance, morphology of blood erythrocyte and intestinal microbiota in broiler chicks were evaluated in this study.

Organic acid mixture in the diet has been shown to improve bird performance and decrease mortality. Significant improvement was induced in weight gain and FCR by organic acid mixture supplementation alone (Table 2). These results are in line with the findings of Langhout, 2000; Denli et al., 2003; Senkoylu et al., 2007; Owens et al., 2008. In contrast to our findings, several researchers (Cave, 1984; Alp et al., 1999; Gunal et al., 2006) reported that no significant differences were observed in weight gain and feed conversion. Waldroup et al. (1995) suggested that organic acids may improve bird performance by decreasing the pH of the intestinal tract and therefore reducing bacterial numbers.

The present study revealed that the effects of MOS and organic acid mixture supplementation did not affect digestive organ weights (Table 3). Some other researchers (Denli et al., 2003; Owens et al., 2008) reported similar results.

Blood erythrocytes are of an elongated and elliptical shape in chickens (Ji et al.,

### Table 5. Effects of dietary treatments on ileum microbiota (log cfu/g ileal content).

| Treatments                  | Lab  | Yeast | E. Coli |
|----------------------------|------|-------|---------|
| Control                    | 5.80c| 4.26b | 4.92a   |
| MOS                        | 6.64b| 4.53a | 3.55c   |
| Organic acid mixture       | 5.65d| 3.82c | 3.33d   |
| MOS + Organic acid mixture | 7.14a| 4.22b | 3.75b   |
| SEM                        | 0.140| 0.062 | 0.145   |
| P levels                   | <0.001| <0.001| <0.001  |

*a, d: Means in row with different letters differ significantly.*

### Table 6. Effects of dietary treatments on caecum microbiota (log cfu/g caecal content).

| Treatments                  | Lab  | Yeast | E. Coli |
|----------------------------|------|-------|---------|
| Control                    | 6.23d| 4.90b | 5.53a   |
| MOS                        | 7.29b| 5.05a | 4.11b   |
| Organic acid mixture       | 6.34c| 4.47d | 3.80c   |
| MOS + Organic acid mixture | 7.71a| 4.78c | 4.14b   |
| SEM                        | 0.145| 0.052 | 0.156   |
| P levels                   | <0.001| <0.001| <0.001  |

*a, d: Means in row with different letters differ significantly.*
The erythrocyte length increased in MOS + organic acid mixture (P<0.05; Table 4).

The results of the present study showed that the supplementation of MOS, organic acid mixture and MOS + organic acid combination fed groups positively affected the ileal and caecal microbiota compared to the control groups. Counts of LAB significantly (P<0.001) increased in MOS + organic acid mixture (Table 5 and 6). Counts of E. coli, significantly (P<0.001) decreased in organic acid mixture treatments (Table 5 and 6). These findings are in line with Alp et al. (1999) and Yang et al. (2008). LAB, yeast and E. coli counts were lower in the ileum compared to counts from the caecum.

Conclusions

Consequently, the results of the present study indicate that the MOS + organic acid mixture decreased weight gain the 21 day old broiler chicks, whereas, feed intake and FCR increased in the group fed the MOS + organic acid mixture. In addition, it was found that MOS and organic acid mixture individually or in combination significantly decreased E. coli count in ileum and caecum. This effect might aid in improved performance when they are fed individually. However, the results of the present study indicated that these feed additives (MOS and organic acid mixture) did not positively affect broiler chick performance when they are used in combination.

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