Microencapsulation allows slow release of organic acids in the GI tract of broilers

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Aim of the study was to investigate intestinal concentrations of citric and sorbic acid (OA) from a microencapsulated blend and the possible consequences on intestinal fermentations. Twelve male ROSS 508 broilers were selected from 2 dietary treatments: a control diet (CTR), or the control diet added with a microencapsulated blend of citric and sorbic acid at 400 ppm (TRT). Contents of gizzard, small intestine and ceca were collected to be analysed for pH, NH_3, VFA and OA concentration; coliforms and lactic acid bacteria were counted. All data were analysed with an unpaired t-student test, and considered statistically significant at $P<0.05$. Citric acid was detected all along the GI tract in both CTR and TRT group, but it was present in higher concentrations in TRT (gizzard:1.8 mmol/L for the CTR group vs 3.8 mmol/L; small intestine 0.58 mmol/L vs 1.16 mmol/L; ceca: 0.40 mmol/L vs 0.46). Sorbic acid was not detectable in birds fed the CTR diet whereas it decreased along the GI tract of TRT fed birds (gizzard: 60 µmol/L; small intestine: 6 µmol/L; ceca: 0 µmol/L). Data showed that the microencapulation allowed the supply of sorbic acid to the small intestine.

Keywords: microencapsulation, broilers, citric acid, sorbic acid.

Introduction

In light of the ban of antibiotics as growth promoters in the European Union, a significant amount of nutritional research has been re-focused to prevent diet malabsorption, unbalanced intestinal fermentation, microbial overgrowth and as a consequence diarrhoea. Organic acids (OA) have long been used as feed and food preservatives to prevent microbial spoilage (Frank, 1994). As such, feeding OA to farm animals is a widely accepted tool to control the microbial balance in the gut (Piva et al., 2007). Acidification with various weak organic acids to diets such as fumaric, propionic, lactic, and sorbic have been reported to decrease colonization of pathogen and production of toxic metabolites, and to improve digestibility of protein and of Ca, P, Mg and Zn and to serve as substrates in the intermediary metabolism (Kirchgessner and Roth, 1987). Because these natural compounds are classified as generally recognized as safe by the Food and Drug Administration (FDA, 2006), their use to prevent growth of foodborne pathogens or spoilage organisms has gained increasing interest. The inherent limitation of the effective dose of OA in modulating intestinal flora may reside in the prompt absorption, metabolisation, or both, that they undergo upon entering the duodenum. This could be overcome by microencapsulating the active compounds in a matrix that could dissolve and release active principles (Piva et al., 2007).

Aim of the present study was to investigate intestinal concentrations of citric and sorbic acid (OA) from a microencapsulated product and the possible consequences on intestinal fermentations.

Materials and Methods

Animals

Two-hundred and forty broilers ROSS 508 one day old (body weight d0 =37.7±2.7 g) were randomly allocated to 12 pens, 20 per pen. Chicks were divided into two experimental groups: the control diet (CTR) or the control diet added with a microencapsulated blend containing fumaric, malic, citric, sorbic acid and natural identical flavours at 400 ppm (TRT; EU patent 1391155B1).The individual weight of the chickens was recorded. The floor of each pen was covered with a 5 cm woods shaving.

Management

The room temperature was adjusted at 25°C at start, and it decreased to 22°C after the first two weeks of the study. Adequate ventilation was maintained to avoid any accumulation of ammonia and to control moisture. The lighting period over the study was light: dark 23:1 at 100-120 lux.

Feeding program and diets

Birds were fed ad libitum a vegetable diet without enzymes, antibiotic, containing 50% corn (Tab. 1). The feeding program was divided into three phases (0-10 days, starters; 11-28, growers; 29-42 finishers) and designed for ROSS 508 broilers. The diets did not differ for energy value and protein content. A coccidiostat (sodium monensin) was included.

Experimental procedure

At the end of the 42 days study 1 bird per pen from each treatment was randomly selected and slaughtered, and immediately after death the gizzard, the small intestine and the ceca were removed and the contents from each segment were collected. Samples for sorbic and citric acid, short chain fatty acids, and ammonia analyses were immediately stored at -20°C; samples for pH determination and microbial counts were immediately processed.

Chemical analysis of feed and intestinal content

Feed composition analyses (DM, ash, and starch; Table 1) were performed according to the methods of the Italian Ministry of Agriculture and Forest; CP according to G.U. Series General n. 92 21.04.96; ether extract according directive CEE n. 84/4/CEE 20.12.83; G.U. CE n. L15 18.01.84; and crude fiber according to directive CEE n. 92/89 03.11.92.

The analyses of sorbic, citric acid and short chain fatty acids (SCFA) concentrations, and pH were performed on the intestinal contents. Sorbic, citric acid and SCFA were analyzed according to Piva et al. (2007).

Ammonia in intestinal contents was measured with an enzymatic kit for ammonia analysis (Biosystem, Spain).

Bacterial Counts

Serial 10-fold dilutions of 1 g of samples from were serially diluted and plated onto Rogosa agar plates for lactic acid bacteria, and Violet Red Bile agar (Oxoid Ltd., Basingstoke, Hampshire, UK) plates for coliforms. Rogosa agar plates were incubated for 48 h at 37°C under anaerobic conditions (BBL GasPak Plus Anaerobic System Envelopes). Violet Red Bile agar plates were incubated for 24 h at 37°C under aerobic conditions.

Statistical analysis

Ammonia and short chain fatty acid concentrations, pH, and microbial plate counts within the same gastrointestinal site from the 2 dietary treatments (CTR and TRT) were compared by unpaired t-test. Data were analyzed using the program GraphPad Prism (GraphPad Software 4.00, San Diego, CA).

Table 1: Diets composition (% as fed basis)

Ingredients	Starters (0-10d)	Growers (11-28 d)	Finisher (29-42d)
Corn meal	51.09	52.26	57.63
Soybean meal	42.70	40.50	35.00
Vegetable oil	2.00	3.50	4.00

Vitamin premix[1]	0.5	0.5	0.5
L-Lys HCl	0.11	0.09	-
DL-Met	0.35	0.23	0.15
Calcium carbonate	0.25	0.22	0.22
Bicalcium phosphate	2.50	2.20	2.10
NaCl	0.30	0.25	0.37
Sodium bicarbonate	0.15	0.20	0.03
Sodium monensin 20%	0.05	0.05	-

[1] supplying per kg: vit. A: 2,500,000 UI; vit. D3: 600,000 UI; vit, E: 15,000 mg; vit. B1: 400 mg; vit. B2: 1,600 mg; vit.B6: 1200 mg; D-pantotenic acid: 2,500 mg; vit. H: 30 mg; vit. C: 20,000 mg; vit. K: 612 mg; vit. PP: 8,000 mg; vit. B12: 6 mg; folic acid: 250 mg; coline: 100,000 mg; Co: 40 mg; Fe: 10,000 mg; I: 200 mg; Mn: 26,000 mg; Cu: 1,000 mg; Se: 40 mg; Zn: 15,000 mg.

Results and discussions

Sorbic acid was not detected in CTR fed animals, while it was found in the gizzard and in the small intestine contents of TRT fed animals, and it was under the detection limit (0.45 nmol L^{-1}) in ceca contents (gizzard: CTR vs TRT, 0 µmol L^{-1} and 60 µmol L^{-1} , respectively; small intestine: CTR vs TRT, 0 µmol L^{-1} and 6.2 µmol L^{-1} , respectively). Citric acid was found both in the CTR and TRT fed group along the GI tract. Citric acid tended to be higher in the TRT animals than in the CTR fed birds in the gizzard and in the small intestine (gizzard: CTR vs TRT, 1.83 and 3.77 mmol L^{-1}, respectively, $P = 0.15$; small intestine: CTR vs TRT, 0.58 and 1.16 mmol L^{-1}, respectively, $P = 0.06$), while it was numerically higher, but not statistically different in ceca (CTR vs TRT, 0.40 vs 0.44, respectively) (Fig. 1).

Total short chain fatty acids tended to be decreased by TRT in the cecum content (-14%, $P = 0.14$). In particular acetic and nor-butyric acids tended to be reduced in the treated group (-20%, $P = 0.09$ and -40%, $P = 0.07$, respectively). In the small intestine there were a tendency by the treatment to reduce iso-butyric concentration (CTR vs TRT, 0.56 mmol L^{-1} and 0.54 mmol L^{-1}, $P = 0.06$). There were no differences for the other acids.

Ammonia concentration, pH, and microbiological counts were not different between treatments.

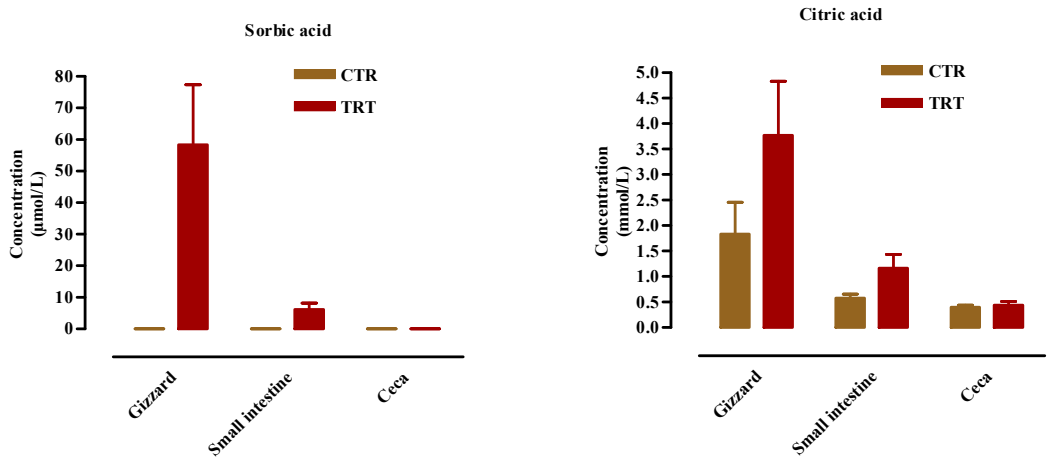

Fig. 1: concentration of sorbic and citric acid along the GI tract of sampled birds.

The debate over the risks associated with the use of AGP in feed, and the consequent ban of their use in Europe has focused research attention to alternative means to prevent disease and maintain market standard production levels. Especially in poultry production, concern about the rise of necrotic enteritis outbreaks forced to find precautionary measures that do not represent a threat to human health.

Organic acids have been evaluated as a possible strategy because of their antibacterial properties through diffusing across the bacterial cells in the gizzard, small intestine and cecum of animals (Lueck, 1980; Partanen and Mroz, 1999). To most effectively achieved this goal, OA need to reach the microbes in the gut in their undissociated form and appreciable concentrations.

In the present work we studied the slow release of microencapsulated sorbic and citric acid in intestinal sections of birds, and the possible consequences on intestinal metabolism. This study followed a previous slow-release study conducted in piglets (Piva et al., 2007), where sorbic acid was measured along the GI tract of animals fed a control diet, or a control diet with a protected blend of organic acids (PB), or the same blend not protected (NPB). Animals fed PB diet had progressively lower ($P < 0.01$) sorbic acid concentrations in cranial and caudal jejunum, ileum, cecum, and colon, whereas in pigs fed NPB, sorbic acid concentration declined immediately after the stomach. Furthermore, coliforms population was significantly reduced by PB in caudal jejunum and cecum compared with NPB and control groups.

The results of the present study showed that sorbic acid has been recovered in the upper intestine, as well as citric acid supplied with the blend, but not in the ceca. The possible reason of the progressively low recovery of organic acids along the GI tract could be due to the action of digestive enzymes (Piva et al., 2007), which degrade the lipid protective matrix and subsequent metabolization of released substances. However, the protective matrix prevented sorbic acid from totally disappearing after it entered the stomach, allowing a 10% recovery in the small intestine. Even though the organic acids did not reach the large intestine as suggested by SCFA concentrations in large amount, they tended to influence the microflora metabolism and feed digestion.

In conclusion, although the lipid matrix used in this study allowed a partial recovery of organic acids, further studies are needed to better understand the mechanisms underlying organic acids metabolism in the intestine of chickens.

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