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Effects of dry whey powder alone or combined with calcium butyrate on productive performance, duodenal morphometry, nutrient digestibility, and ceca bacteria counts of broiler chickens

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ABSTRACT

Prebiotics and organic acids have been proposed as safe additives in poultry feeding to promote performance and health. The purpose of this study was to assess the influence of supplementing corn-soybean diets of broiler chickens with dry whey powder (WP), fat-coated calcium butyrate (CaB), and a mixture of both on apparent ileal digestibility (AID), pH of gastrointestinal content at various segments, productive performance, duodenal histomorphometry, and ceca microbial counts. The experiment consisted of a 2 × 2 factorial arrangement, with 2 WP inclusion rates (0 and 60 g/kg of diet) and 2 CaB rates (0 and 1 g/kg of diet). One-day-old male broiler chickens were randomly allocated to floor pens and assigned to 1 of 4 treatments. In Trial 1, 120 broiler chickens were allocated to 4 treatments with 3 pens per treatment and 10 broiler chickens per pen during 21 d. With the addition of WP, the AID of dry matter, crude protein, Ca, and P increased, and cecum pH decreased only when CaB was also added (CaB × WP, $P < 0.046$). In Trial 2, 1200 broiler chickens were allocated to the 4 treatments with 10 pens per treatment and 30 broiler chickens per pen during 42 d. With the dietary supplementation of WP, average daily gain and feed intake of broiler chickens increased during starter, grower-finisher periods, and the entire feeding period only when CaB was also added ($P < 0.047$). However, with addition of WP, feed conversion ratio (FCR) decreased in broiler chickens fed the diet without CaB, but it increased in those fed with CaB during the grower-finisher and entire feeding periods ($P < 0.001$). Duodenal histomorphometry measurements were evaluated using hematoxylin and eosin stains, and cecal microbial counts were determined by selective culture media. With the addition of WP, villus height, villus height to crypt depth ratio, and villus surface area were increased only when CaB was also added (CaB × WP, $P < 0.017$), while the supplementation of WP increased *Bifidobacterium* spp. counts only when CaB was not added (CaB × WP, $P = 0.049$). Results obtained in the present study indicate that the supplementation of WP without CaB addition improved the FCR of broiler chickens. However, the supplementation of WP together with CaB improve duodenal development, increases nutrient AID, and the weight and ingestion of broiler chickens.

1. Introduction

The use of prebiotics and organic acids in poultry feeding are in force as a result of the banning on the use of in-feed antibiotics as growth promoters in the EU (EC, 2003), and their restricted use in other countries (Huyghebaert et al., 2011). Prebiotics are defined as a non-digestible dietary compounds that modulate the composition, activity or both of gut microbiota, conferring a beneficial physiological effect on the host (Bindels et al., 2015). They promote the growth of specific species such as bifidobacteria and lactobacilli at the expenses of potentially pathogenic bacteria (Macfarlane et al., 2006; Vicente et al., 2007), and generate positive changes in gut morphology and digestive enzymes secretion in broiler chickens (Xu et al., 2003). Dry whey is a co-product of cheese industry, with lactose being its major component (about 70% of dry matter). Because of negligible lactase activity in the gastrointestinal tract of broiler chickens (Denbow, 2000), lactose can be used as a prebiotic. Most of non-digested lactose reaches the ceca, becoming an available substrate for beneficial bacteria such as *Bifidobacterium* spp. (Goodfellow et al., 2012). Moreover, lactose fermentation can lead to a decrease of cecum pH, and to a reduction of potentially pathogenic bacteria such as *Salmonella enteriditis* (Stringfellow et al., 2009; Tellez et al., 1993). However, to our knowledge, the effect of dry whey powder on the duodenal histomorphometry, pH of digestive

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organs, and nutrient ileal digestibility of broiler chickens has not been studied so far.

Organic acids and their salts are considered as safe feed additives, being their use in animal diets approved by most EU member states (Adil et al., 2010). Their general mode of action relates to their antimicrobial activity, their ability to reduce gastrointestinal tract pH (Dibner and Buttin, 2002), and to improve nutrient digestion (Dibner and Buttin, 2002). Butyric acid, a short chain organic acid, has been used in poultry diets in its free form (Adil et al., 2010), as glyceride, or as lipid coated sodium butyrate (Lesson et al., 2005). Although benefits of butyric acid on broiler chickens' performance (Adil et al., 2010), pH of digestive organs (Mahdavi and Torki, 2009), and intestinal morphometry (Lesson et al., 2005) have been reported, little is known about their effect on ceca microbial counts and nutrient ileal digestibility. Similarly, results concerning the use of fat-coated calcium butyrate in broiler chickens' diets are still lacking.

Studies about the joint utilization of prebiotics and organic acids in poultry feeding are scarce (Bozkurt et al., 2009; Taherpour et al., 2012). Furthermore, we are not aware of any study assessing the combined effect of dry whey powder and fat-coated calcium butyrate. We hypothesized that butyric acid could reduce the gastrointestinal load of potentially pathogenic bacteria. Under these conditions, lactose could be selectively used as a substrate for the growth of beneficial ceca bacteria in detriment of pathogenic bacteria, thus improving the sanitary status of broiler chickens. In addition, given the reported benefits of both additives, we expected a synergistic activity that would improve gut development and nutrient digestibility. Thus, this study was conducted to assess the effect of supplementing broiler chickens diets with dry whey powder, fat-coated butyric acid, and the combination of both on apparent ileal digestibility, duodenal histomorphometry, productive performance, pH of gastrointestinal content at various segments, and ceca bacterial populations.

2. Material and methods

2.1. Animals, housing, and experimental diets

The experiment followed the European Union (2010/63/EU) and Spanish regulations (RD 53/2013) for the care and use of animals for experimental, and was conducted at the experimental facilities of Neiker-Tecnalia (Vitoria-Gasteiz, Spain). One-day old male broiler chickens (Ross 308 strain) were obtained from a local commercial hatchery (AN Avícola Melida, S.A., Zumaia, Spain), and were randomly allocated to floor pens, at a stock density of 30 kg/m^2. Pens were equipped with a manual feeder, nipple drinkers, and wood shavings as litter material. Room temperature and lighting program were implemented according to the strain guidelines (Aviagen, 2014).

Diets were corn-soybean based, and were formulated to meet broiler chickens' requirements during the starter and grower-finisher stages (FEDNA, 2008). Starter diets (from d 0 to 21) were offered in a crumbles form, and grower-finisher diets (from d 21 to 42) in a pelleted form. Chromium oxide (5 g Cr_2O_3/kg) was added to starter diets as external indigestible marker. The ingredient composition and the analyzed nutritional value of experimental diets are shown in Table 1. Animals had ad libitum access to one of the following experimental diets: no supplementation of dry whey powder (WP) or fat-coated calcium butyrate (CaB), inclusion of 60 g/kg of WP, inclusion of 1 g/kg of CaB, or inclusion of a mixture of 60 g/kg of WP and 1 g/kg of CaB. The WP was a commercial sweet powder (703 g/kg lactose; Sueromancha S.L, Toledo, Spain). The CaB was a commercial product composed by 860 g/kg of salt (160 g Ca and 700 g butyric acid) and 140 g/kg of lipids (Globamax Performant, Global Nutrition International, Fougères, France).

2.2. Experimental design, measurements, and sampling

2.2.1. Trial 1: apparent ileal digestibility (AID) and pH of gastrointestinal content at various segments

In this trial, 120 one-day-old broiler chickens were randomly allocated to 1.0 × 0.83 m floor pens, and assigned to receive 1 of the 4 experimental diets formulated for the starter period (Table 1). Each treatment comprised 3 pens with 10 broiler chickens each. At 21 d of feeding, 6 broiler chickens per pen were slaughtered by CO_2 inhalation. For nutrient AID determination, ileal digesta was collected. Ileum was considered to be the portion of the small intestine from the Meckel's diverticulum to the ileocecal junction, and the digesta of the last two-thirds of this section were gently collected as described by (Kluth, 2005). Ileal samples from broiler chickens within the same pen were pooled, stored in plastic containers, frozen at −20 °C, and lyophilized. Dry ileal digesta samples were ground to pass through a 0.5-mm screen, and stored in airtight containers at room temperature until chemical analyses. The AID of dry matter (DM), crude protein (CP), starch, Ca, and P were estimated using Cr_2O_3 as indigestible external marker.

For pH measurements, crop, proventriculus, gizzard, ileum, and cecum were carefully ligated and removed. Approximately 0.4 g of digesta from each section were collected, diluted in 1.6 mL of distilled water and gently agitated. Measurements were made using a calibrated electronic pH meter (Basic 20, Crison, Barcelona, Spain).

2.2.2. Trial 2: growth performance, duodenal histomorphometry, and ceca bacteria counts

For this trial, 1200 one-day-old broiler chickens were used during 42 d. They were randomly allocated to 2.5 × 1.0 m floor pens, and assigned to 1 of the 4 experimental diets (Table 1). Starter diets were offered from d 0 to 21, and grower-finisher diets from 21 d to 42. Each treatment consisted of 10 replicate pens, and 30 broiler chickens each. To determine productive performance, all broiler chickens and feeders in each pen were weighted weekly. Body weight (BW), average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR) were recorded on a pen basis. Mortality was recorded daily.

To determine ceca microbial counts, 3 broiler chickens from each treatment were randomly selected on d 21 and slaughtered by CO_2 inhalation, as previously was done in Pineda-Quiroga et al. (2017). The gastrointestinal tract was dissected, cecum was collected under sterile conditions, and 1 g of digesta content, resulting from the mixture of both ceca pouches, was diluted in 9 mL of buffered peptone water (BioMérieux, Marcy-L'etoile, France) and immediately 10-fold serially diluted in sterile saline solution. Dilutions were tested in duplicate for enumeration of *E. coli*, *Clostridium perfringens*, *Bifidobacterium* spp., and *Lactobacillus* spp. For *E. coli*, dilutions to 10^{-9} were plated on a selective chromogenic agar medium (ChromID Coli, BioMérieux, France) and incubated at 37 ± SD 1 °C for 24 h; only glucuronidase-positive red-pink colonies were counted. For *C. perfringens*, dilutions to 10^{-7} were plated on tryptone sulphite neomycine agar (Scharlab, Barcelona, Spain) and incubated at 45 ± SD 1 °C for 24 h. For *Lactobacillus* spp., the selective medium Lactobacilli man rogosa and sharpe (MRS) agar (BD, Franklin Lakes, New Jersey, US) with 50 U/mL of nystatin (Sigma-Aldrich, St. Louis, Missouri, US) was used. The same MRS Agar (BD, US) supplemented with L-Cysteine hydrochloride (0.5 g/kg) (Oxoid, Basingstoke, UK), 100 μg/mL mupirocin (Oxoid, UK), and 50 U/mL nystatin (Sigma-Aldrich, US) was used for *Bifidobacterium* spp. In both cases, dilutions to 10^{-11} were tested and plates were incubated at 37 ± SD 1 °C for 48 h; 5 colonies from the last dilutions with 30–150 colony-forming units (cfu) were then selected for Gram staining and catalase test to identify catalase-negative, Gram-positive bacilli. Plates for *E. coli* were incubated aerobically, whereas all other incubations were carried out under anaerobic conditions (Genbox Anaer, BioMérieux, France). To confirm the species, selected colonies from each