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Effects of pyrimidine nucleosides on growth performance, gut morphology, digestive enzymes, serum biochemical indices and immune response in broiler chickens

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ABSTRACT

The present study was carried out to evaluate the effects of pyrimidine nucleosides on growth, intestinal morphology, digestive enzymes and humoral immunity in broilers from 0 to 21 days of age. A total number of 360 day old chicks (Cobb 500) was randomly divided into 4 treatments with 6 replications. Treatments were comprised of a corn-soybean meal based control diet and diets containing 0.1% pure cytidine, 0.1% pure uridine and 0.1% equal amounts of pure cytidine (0.05%) and uridine (0.05%). On days 11 and 21, two birds per cage (12 birds per treatment) were euthanized to obtain samples of serum, intestine, bursa and spleen. The combination of cytidine plus uridine increased ($P < 0.05$) body weight and average daily gain of broilers. Supplementing cytidine plus uridine increased ($P < 0.05$) villus height and width along with activities of alkaline phosphatase and aminopeptidase; however, maltase was not affected by the experimental diets. The combination of cytidine and uridine increased ($P < 0.05$) the relative weight of the bursa of Fabricius and IgA activity in the jejunum, but there was no significant difference among treatments regarding the relative weight of the spleen. In conclusion, the study clearly indicated that the combination of cytidine and uridine could improve health status and performance of broilers.

1. Introduction

Nucleotides are ubiquitous low-molecular-weight compounds that play crucial roles in diverse biological processes such as encoding genetic information, mediating energy metabolism, and serving as coenzymes (Carver, 1999). *De novo* and salvage pathways are two biosynthetic pathways which provide nucleotides required by cells. Although these pathways can provide nucleotides for the maintenance of host cells, such pathways are metabolically costly processes requiring energy sources and amino acids (Zollner, 1982; Carver, 1999). On the other hand, some cells such as the epithelial cells of the intestine and the immune system cells have little ability to synthesize nucleotides through biosynthetic pathways and depend on exogenous sources (Grimble and Westwood, 2001). In addition, the nucleotide requirement of cells increases under special conditions such as rapid growth, limited nutrient intake, diseases, and enhancement of the immune responses (Grimble and Westwood, 2001; Sauer et al., 2011). In a previous study, Sauer et al. (2011) showed that fast growing animals require exogenous nucleotides (combination of pure nucleotides) to provide rapid growth rate of organs such as the intestine and immune system which have a low nucleotides biosynthesis capacity while playing important roles in rapid growth process during early life. Domeneghini et al. (2004) showed that dietary nucleotides (0.05% yeast commercial product) enhanced villus height and width and other morphometric characteristics of various segments of the intestine resulting in the development of entrocytes. Deng et al. (2005) evaluated the effects of dietary yeast RNA (0, 5, and 10 g/kg diet) on various immunological parameters in Leghorn layers and demonstrated that yeast RNA could improve humoral and cellular immune response during the experiment, but this result was unsustainable over longer periods.

Most previous studies investigating the effects of nucleotides on broiler performance and immunity were based on the supplementation of yeast extract as a source of nucleotides whereas Sauer et al. (2012) and Jung and Batal (2012) indicated that yeast extract (0.25% *Torula* yeast) contains various amounts of nucleotides along with other nutrients like amino acids, vitamins, viable cells and cell wall components which can confound the interpretation of the results obtained relative

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to the role of specific nucleotides. In fact, dietary nucleotides require to be enzymically hydrolyzed to nucleosides and bases which are the only forms absorbed in the intestine (Quan and Uauy, 1991). Nucleosides are absorbed into the enterocyte by facilitated diffusion through Na-dependent channels (Bronk and Hastewell, 1987). More recently, we examined the effects of purine and combinations of purine and pyrimidine nucleosides on various parameters in broilers (Daneshmand et al., 2017a, 2017b). The results of these experiments showed that adenosine (Daneshmand et al., 2017a) and combination of adenosine + cytidine + uridine (Daneshmand et al., 2017b) improved growth performance, intestinal morphology and enzyme secretion and immune function in broilers. Pyrimidine nucleosides are a group of non-protein nitrogenous compounds that primarily include cytidine and uridine which play important roles in the biochemical processes such as components of DNA and RNA as well as of coenzymes (D'Mello, 1982; Zollner, 1982). Although previous studies worked on the effects of yeast extract as a source of nucleotides in poultry, the effects of pure pyrimidine nucleosides have not been investigated, to the best of our knowledge. Therefore, the current study was carried out to assess the effects of pyrimidine nucleosides on the growth performance, morphology and maturity of intestinal cells, serum biochemical indices and immune response in broiler chickens.

2. Materials and methods

2.1. Animals, treatments, housing and experimental design

Three hundred and sixty one-day-old male chicks (Cobb 500) were obtained from a local commercial hatchery (Foster Farms, Ellenwood, CA, USA). All birds were raised in brooder battery cages with woven wire floors (Petersime Inc., Gettysburg, OH, USA). The temperature was set at 35 °C for the first 3 d and then gradually decreased by 3 °C per week until it reached 29 °C and was maintained at that level until the end of the experiment (d 21). Feed and water were provided ad libitum throughout the experiment and a 23L:1D lighting program was applied. Birds were weighed on arrival and assigned to 4 treatments of 6 replications with 15 birds in each replication. Briefly, from d 0 to 21, birds were fed diets as follows: 1) a corn-soybean meal control diet; 2) control diet supplemented with 0.1% pure uridine; 3) control diet supplemented with 0.1% pure cytidine; 4) control diet supplemented with a 0.1% equal combination of pure uridine and cytidine. Pure pyrimidine nucleosides were donated by Chemoforma AG (Augst, Switzerland) and added to the diets at the expense of corn. All diets were in mash form and formulated to be isonitrogenous and isoenergetic and also meet or exceed the minimum requirements of Cobb 500 (Cobb-Vantress, 2012; Table 1). All experimental procedures were approved by the University of California-Davis Institutional Animal Care and Use Committee.

Table 1
Composition of diets (as fed basis).[a]

Ingredients (g/kg)	Starter (0–10 d)	Grower (11–21 d)
Corn	598.0	629.2
Soybean meal (441 g/kg CP)	338.3	299.9
Soybean Oil	22.3	32.4
Dicalcium phosphate[b]	17.8	16.6
Limestone[c]	8.0	7.4
Salt	3.7	3.7
DL-Methionine	3.3	2.8
L-Lysine	2.8	2.4
L-Threonine	0.8	0.6
Vitamin Premix[d]	2.5	2.5
Mineral Premix[e]	2.5	2.5
Calculated value		
Metabolizable energy (MJ/kg)	12.6	12.9
Crude protein (g/kg)	210.6	190.0
Available Phosphorus (g/kg)	4.5	4.2
Calcium (g/kg)	9.0	8.4
Sodium (g/kg)	1.6	1.6
Methionine (g/kg)	6.4	5.7
Methionine + Cysteine (g/kg)	9.8	8.9
Lysine (g/kg)	13.2	11.9
Arginine (g/kg)	13.8	12.6
Threonine (g/kg)	8.6	7.8
Analyzed value		
Crude protein (g/kg)	209.8	190.6
Calcium (g/kg)	8.8	8.6
Total phosphorous (g/kg)	6.2	5.7

[a] Pure nucleosides (1 g/kg) were added as a replacement for maize.
[b] Dicalcium phosphate contained 210 g/kg calcium and 180 g/kg phosphorus.
[c] Limestone contained 380 g/kg calcium.
[d] Vitamin premix per kilogram contained vitamin A, 9000 IU; vitamin D3, 2000 IU; vitamin E, 18 IU; vitamin K3, 2 mg; vitamin B1, 1.8 mg; vitamin B2, 6.6 mg; vitamin B3, 10 mg; vitamin B5, 30 mg; vitamin B6, 3 mg; vitamin B9, 1 mg; vitamin B12, 0.015 mg; biotin, 0.1 mg; choline chloride, 250 mg; antioxidant, 100 mg.
[e] Mineral premix per kilogram contained Mn, 100 mg; Zn, 84.7 mg; Fe, 50 mg; Cu, 10 mg; I, 1 mg; Se, 0.2 mg.

2.2. Growth parameters

On days 10 and 21, BW and feed consumption of each cage were recorded to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Mortality for each cage was recorded daily in order to modify the abovementioned parameters accordingly. The average daily mortality was less than 1% (2 from 360 chicks).

2.3. Feed analysis and sample collection

The basal diet was analyzed for CP, calcium, and total phosphorus according to AOAC (2003) procedures.

Two birds from each cage (12 birds per treatment) were randomly selected at 11 and 21 days of age. Blood samples (5 ml) were collected from a cardiac puncture of selected birds (without applying fasting) using Vacutainer tubes and a One-Use Holder (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 2000 × *g* for 15 min at 4 °C to obtain serum samples in the same day. The serum samples were stored at − 20 °C prior to analysis. Selected birds were then euthanized by CO_2 asphyxiation, the viscera was excised, and the intestine was removed and carefully cleaned of adherent materials. The jejunal segment was separated and gently squeezed to empty digesta content. The bursa of Fabricius and spleen were collected to obtain relative weights of these organs as the immunological indices.

2.4. Intestinal morphology

About 2 cm of the same position of mid-jejunal samples were stored in a 10% formaldehyde phosphate buffer for 48 h. The sample was then embedded in paraffin, fixed on microtome (Leica HI1210, Leica Microsystems Ltd., Wetzlar, Germany), sliced to a thickness of 3 μm, mounted on a slide and dehydrated on a hotplate (Leica ASP300S, Leica Microsystems Ltd., Wetzlar, Germany). Then, the prepared sample was dyed with hematoxylin and eosin (Leica Autostain BRXL, Leica Microsystems Ltd., Wetzlar, Germany), and examined under a microscope (Olympus BX41, Olympus Corporation, Tokyo, Japan). A total of 9 slides were prepared from each jejunal segment per bird, and 10 individual well-oriented villi were measured per prepared slide (90 villi per bird). The average of slide measurements per sample was expressed as a mean for each bird. Villus width (VW) was measured at the base of each villus; villus height (VH) was measured from the top of the villus to the villus-crypt junction, crypt depth (CD) was measured from the base of the adjacent villus to the sub-mucosa, and the ratio of VH to CD was calculated.