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ผลของการเสริมนิวคลีโอไทด์ในอาหารต่อสมรรถภาพการเจริญเติบโต ระบบภูมิคุ้มกัน และลักษณะทางจุลกายวิภาคของลำไส้เล็กในไก่เนื้อ

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บทคัดย่อ: การเสริมนิวคลีโอไทด์ในอาหารมีส่วนช่วยกระตุ้นการเจริญเติบโต ช่วยปรับปรุงประสิทธิภาพการใช้อาหาร และช่วยกระตุ้นระบบภูมิคุ้มกันในสัตว์อายุน้อยได้ การวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของการเสริมนิวคลีโอไทด์ในอาหารที่ระดับต่างๆ ต่อสมรรถภาพการเจริญเติบโตของไก่เนื้อการทดลองนี้ใช้ไก่เนื้อคละเพศ อายุ 1 วัน (Ross 308) จำนวน 180 ตัว แบ่งออกเป็น 3 กลุ่มๆ ละ 4 ซ้ำ จำนวนซ้ำละ 15 ตัว วางแผนการทดลองแบบสุ่มตลอดสมบูรณ์ (CRD) กลุ่มทดลองแบ่งออกเป็น กลุ่มที่ไม่เสริมนิวคลีโอไทด์กลุ่มที่เสริมนิวคลีโอไทด์ในอาหารที่ระดับ 1.5% ในช่วงอายุ 7 วันแรกและกลุ่มที่เสริมนิวคลีโอไทด์ในอาหารที่ระดับ 1.5% ในช่วงอายุ 14 วันแรกอาหารทดลองแบ่งออกเป็น 2 ระยะ ได้แก่ อาหารสำหรับไก่เล็ก (1-21 วัน) และอาหารสำหรับไก่ใหญ่ (21-42 วัน) ผลการทดลองพบว่าเมื่อสิ้นสุดการทดลองที่ 42 วัน การเสริมนิวคลีโอไทด์ในอาหารไม่มีผลต่อน้ำหนักตัวที่เพิ่มปริมาณอาหารที่กินประสิทธิภาพการเปลี่ยนอาหารเป็นน้ำหนักตัว และระดับภูมิคุ้มกันต่อโรคนิวคาสเซิล อย่างไรก็ดีพบว่าที่อายุ 28 วัน ไก่เนื้อในกลุ่มที่เสริมนิวคลีโอไทด์ในอาหารมีความสูงของวิลไลสูงกว่าไก่เนื้อในกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) จากผลการทดลองในครั้งนี้สรุปได้ว่าการเสริมนิวคลีโอไทด์ในอาหารที่ระดับ 1.5% มีแนวโน้มช่วยปรับปรุงสมรรถภาพการเจริญเติบโตและลักษณะทางจุลกายวิภาคของลำไส้เล็กในไก่เนื้อ

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Effect of Nucleotides Supplementation on Growth Performance, Humoral Immunity, and Intestinal Morphological Structure of Broiler Chickens

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Abstract: Feeding nucleotides in young animals is believed to have benefit effects such as improving growth rate, enhancing gut health and immune system. Thus, the aim of this present study was to evaluate the effect of nucleotides supplementation in diets at difference periods on growth performance of broiler chickens. A total of 180 Ross 308 (mixed sex), one-day-old broiler chickens were randomly distributed in completely randomized experimental design (CRD) into 3 treatments with 4 replicates of 15 birds in each. The experimental treatments consisted of: birds fed control diet, birds fed 1.5% nucleotides for the first 7 days of age and birds fed 1.5% nucleotides for the first 14 days of age. The dietary period was divided in two phases: starter diet (1 to 21 days) and grower diet (21 to 42 days). The result showed that, at 42 days of ages, there were no significantly difference (P>0.05) in body weight gain, feed intake, feed conversion ratio and HA-HI titer of broiler serum antibodies specific to ND virus between treatments. However, broilers fed with nucleotides had a significant higher (P<0.05) villus height than those fed with control diet. In conclusion, the current study demonstrated that feeding broiler chickens with 1.5%nucleotides in the diet tended to improve growth performance and intestinal morphology of broiler chickens.

Keywords: Nucleotides, Growth performance, Humoral immunity, Intestinal morphology, Broiler

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Introduction

Antibiotic growth promoters (AGP) in animal diets have been banned in the recent years due to the public concern over possible antibiotic residual effects and the development of drug resistant bacteria

(Butaye *et al*., 2003). Animal fed AGP free diets are more susceptible to infections caused by pathogens that cause enteric diseases, which may compromise nutrient absorption, growth performance and immune systems (Chiofalo *et al*., 2011; Pelicia *et al*., 2010). Nutritional strategies are being developed to overcome these problems. One possibility is supplementing young animal with bioactive components such as nucleotides (Sauer *et al*., 2009).

Nucleotides are low-molecular-weight intracellular compounds. They are composed of a nitrogenous base (pyrimidine and purine) linked to a pentose (ribose or deoxyribose) sugar (nucleoside) to which at least one, two or three phosphate groups are attached. The pyrimidine consists of cytosine (C), uridine (U) or thymine (T). The purine consists of adenine (A), guanine (G) and hypoxanthine (I) (Alberts *et al*., 2008; Sauer *et al*., 2009). Nucleotides participate in many intracellular biochemical processes as carries of activated metabolites for biosynthesis, as structural moieties of co-enzymes, and as metabolic regulators and signal molecules (Mathews and van Holde, 1990). The major nucleotides include adenosine 5´monophosphate (AMP), cytosine 5´monophosphate (CMP), guanosine 5´monophosphate (GMP), inosine 5´monophosphate (IMP) and uridine 5´monophosphate (UMP) (Mateo *et al*., 2004).

Nucleotides present in colostrum and milk, and the concentration of most nucleotides change during the lactation period (Mateo *et al*., 2004; Sauer *et al*., 2009). Particularly, IMP is mainly found in food with high protein content such as organ meat, poultry, and seafood. Yeast protein sources (baker's or brewer's yeast and yeast extract) are ingredients that also a relatively high concentration of nucleotides (Sauer *et al*., 2009).

Nucleotides are substances that are synthesized endogenously. They have important effects on growth and development of cells which have rapid turnover rate such as in the immune or gastrointestinal system (Sauer *et al*., 2009). However, under certain circumstances (e.g. rapid growth, deficient diet, stress and immune suppression) exogenous nucleotides may be semi-essential, optimizing the function of the immune system and energy utilization when the endogenous supply may limit the synthesis of nucleotides (Jung and Batal, 2012; Sauer *et al*., 2009; Sauer *et al*., 2012).

Young birds have a gastrointestinal tract with a limited capacity (Leeson and Summers, 2005; Kleyn, 2013). Thus, feeding nucleotides in young animals is believed to have benefit effects such as improving growth rate, enhancing gut health and immune system

(Pelícia *et al*., 2010; Chiofalo *et al*., 2011). The objective of this present study is to evaluate the effect of nucleotides supplementation at difference periods on growth performance, humoral immunity and intestinal morphological structure of broiler chickens.

Materials and Methods

Animals and experimental diets

A total of 180 Ross 308 (mixed sex), one-day-old broiler chickens (average body weight 48.43 ± 0.81 g) were randomly distributed in completely randomized experimental design (CRD) into 3 treatments with 4 replicates of 15 birds in each, totaling 60 birds per treatment. The experimental treatments consisted of: basal diet (control), birds fed 1.5% nucleotides (Hilyses®) in basal diet for the first 7 days of age (1.5% N; 7 d), and birds fed 1.5% (Hilyses®) nucleotides in basal diet for the first 14 days of age (1.5% N; 14 d). The dietary period was divided in two phases: starter (1-21 days) and grower (21-42 days) which are 22% CP; 3,100 Kcal ME/kg and 20% CP; 3,150 Kcal ME/kg, respectively. Feeds based on corn and soybean meal, and were formulated to meet the requirements according to the (NRC, 1994). All diets were pelleted with steam; starter diet was fed as crumbles.

All birds were given *ad libitum* access to feed and water. Routine medication, vaccination and husbandry practices were administered. Growth performance data were collected and analyzed for accumulated periods of 1 to 21, 21 to 42, and 1 to 42 days of age. The body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were analyzed.

Quantitative assessment of humoral immunity

Blood samples were collected from the wing veins of 2 birds selected randomly from each replication of the following ages: 14, 28 and 42 days. Serum was separated from the blood sample and stored individually for each bird at -20°C to allow for analysis at the same time. Haemagglutination-Haemagglutination inhibition (HA-HI) titers of serum antibodies specific to the Newcastle disease (ND) virus were quantities in the serum samples (OIE Terrestrial Manual, 2012).

Examination of intestinal morphological structure

At 14, 28 and 42 days of age, two birds of each replication were randomly dissected to collect small intestinal samples. All samples were fixed in 10% formalin. After fixation, the samples were dehydrated by increasing concentration of alcohol and embedded in paraffin. The middle of jejunum samples were sectioned horizontally in 2 cm length (Hu and Guo, 2000). The villus height and crypt depth were determined in cross section using inverted microscope by using image-analysis software. The measurements were examined on eight villi and eight crypts for each segment. The villus height-to-crypt depth ratios were calculated.

Statistical analysis

The data were analyzed by ANOVA using the General Linear Models (GLM) procedure of the statistical software program. The means were separated by means of the Least Significant Difference (LSD) test when a significant ($P \leq 0.05$) treatment was observed.

Results and Discussion

Growth performance

The effect of nucleotides (Hilyses®) supplementation at different feeding periods on growth performance of broilers was shown in Table 1. There was no significant different ($P>0.05$) among dietary treatments on growth performance parameters analyzed at 1-21, 21-42 and 1-42 days of age. Previous studies showed regarding results of nucleotides supplementation in broilers. (Pelicia *et al*., 2010) reported that no effect on performance parameters of broilers fed diet supplemented with 0.00%, 0.00% + AGP, 0.04%, 0.05%, 0.06% and 0.07% nucleotides. Whereas (Rutz *et al*., 2004) observed in broilers fed 2% NuPro® (yeast extract) from 1-7 days and 38-42 days of age resulted in improvement growth performance. Furthermore, (Wang *et al*., 2009) reported that birds fed diets with 2% NuPro® for the first 7 days or for the first 7 days followed by feeding from 35-42 days had significantly lower feed conversion than those fed the control.

The present study, however, supplementation of 1.5%nucleotides for the first 7 and 14 days of age helped to slightly improve growth performance of broilers. (Pelicia *et al*., 2010) suggested that yeast extract contains other nutrients in addition to nucleotides, such as amino acids, vitamins and minerals, which may contribute to increase growth performance.

Humoral Immunity

The effect of nucleotides supplementation at different feeding periods on humoral immunity of broilers was shown in Table 2.There was no significant different ($P>0.05$) among dietary treatments on HA-HI titer of broiler serum antibodies specific to ND virus analyzed at day 14, 28 and 42 of age. Various studies investigated the effect of supplemented nucleotides on the immune function of several species such as pigs and infants. The study of (Sauer *et al*., 2012) indicated that plasma IgA concentrations of newly weaned pigs increased with age and were greater in the nucleotide ($P<0.05$)

Table 1 Mean of body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of broiler in periods of 1 to 21, 21 to 42, and 1 to 42 days of age.

Parameter	Group			Pool SEM	P-value
	Control	1.5% N; 7 d	1.5% N; 14 d		
1 to 21 days of age					
Initial weight, g	48.50	48.34	48.46	0.23	0.97
BW, g/bird	901.31	916.48	960.85	13.05	0.15
BWG, g/bird	852.81	868.14	912.39	12.09	0.16
FI, g/bird	1,305.97	1,329.83	1,295.35	18.09	0.77
FCR	1.53	1.53	1.42	0.03	0.17
21 to 42 days of age					
BW, g/bird	2,576.73	2,721.18	2,859.88	58.93	0.14
BWG, g/bird	1,675.42	1,804.69	1,899.03	55.61	0.28
FI, g/bird	3,389.42	3,465.59	3,580.62	47.74	0.28
FCR	2.04	1.92	1.89	0.04	0.45
1 to 42 days of age					
BW, g/bird	2,576.73	2,721.18	2,859.88	58.93	0.14
BWG, g/bird	2,528.18	2,672.84	2,811.42	58.92	0.14
FI, g/bird	4,695.39	4,795.42	4,875.97	52.92	0.42
FCR	1.86	1.80	1.73	0.03	0.29

compared with the control group, but there were no treatment differences in plasma IgG and IgM. Greater plasma IgA concentrations indicate that adding nucleotides in the weaning diet supported humoral immunity. In contrast, the study of (Hawkes *et al*., 2006), nucleotides supplementation did not influence the growth of formula fed infants or any markers of immunity measured at 7 weeks of age. In that way, however, (Kulkarni *et al*., 1994) reported that nucleotides were not needed for normal growth and development, but there need for nucleotides in the response to immunological challenges.

Intestinal Morphological Structure

The effect of nucleotides supplementation at different feeding periods on intestinal morphological structure of broilers was shown in Table 3. There was no significant different (P>0.05) among dietary treatments on intestinal morphology structure at day 14

Table 2 Mean HA-HI titers (log^2) of broiler[Ψ] serum antibodies specific to ND virus in days 14, 28 and 42.

Days	Group			Pool SEM	P-value
	Control	1.5% N; 7 d	1.5% N; 14 d		
14	3.50	2.75	2.83	6.29	0.10
28	7.08	6.75	7.08	3.42	0.66
42	7.50	7.46	7.92	1.23	0.09

[Ψ]Broilers were administered killed ND and live ND & IB at day 14 of age.

Table 3 Mean of villus height, crypt depth and villus height-to-crypt depth (V : C) ratios of broiler in days 14, 28 and 42 of age.

Parameter	Group			Pool SEM	P-value
	Control	1.5% N; 7 d	1.5% N; 14 d		
Day 14 of age					
Villus (µm)	499.60	520.76	547.51	16.78	0.51
Crypt (µm)	58.57	66.93	67.72	2.93	0.38
V : C ratio	9.36	9.35	8.51	0.61	0.82
Day 28 of age					
Villus (µm)	$1,090.17^a$	$1,307.85^b$	$1,473.01^b$	51.13	<0.01
Crypt (µm)	198.52	201.66	192.81	8.00	0.91
V : C ratio	5.92	7.15	8.07	0.38	0.06
Day 42 of age					
Villus (µm)	1,743.50	1,931.76	1,881.74	52.47	0.32
Crypt (µm)	233.95	255.85	259.77	8.67	0.44
V : C ratio	7.98	7.76	7.35	0.29	0.69

[a,b]Means in the same row with different superscripts are significantly different ($P \leq 0.05$)

and 42 of age. At day 21 of age, broilers fed diet with 1.5%nucleotides for the first 7 and 14 days of age had significantly higher ($P<0.05$) villus height than those fed control diet. Whereas, no significant different ($P>0.05$) on crypt depth and villus height-to-crypt depth ratios between the treatments. Similarly result, (Sauer *et al.*, 2012) observed that no differences in gut morphology of newly weaned pigs fed diet with and without mixture of nucleotides. In the present study, supplementation of nucleotides in the diet at 21 day of age showed beneficial effects. It might be due to the depressed conditions such as stress and climate change may enhance the effect of nucleotide on intestinal morphology.

Several studies reviewed of how nucleotides stimulate development of broiler gastrointestinal tract. Within a few hours post-hatch, villi height and area increase rapidly. Development is complete at 6-8 days in duodenum and 10 days in the jejunum and ileum. Crypts increase in number and size, proliferating rapidly during the first day post-hatch. Alterations in the enterocytes, villi and crypts are influenced by diet (Geyra *et al*., 2001; Rutz *et al*., 2007; Uni *et al*., 1998). Feeding nucleotides stimulate the development of cell and protein synthesis involves digestion and absorption of the animals. (Rutz *et al*., 2007) reported that the synthesis of rRNA in the crypts of jejunum dependent on dietary pyrimidine.

Conclusion

In summary, the current study demonstrated that supplementing the diet of broiler chickens with 1.5% nucleotides resulted in improve growth performance and intestinal morphology, but did not influence broiler humoral immunity.

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