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Role of Nucleotides Supplementation in Reduce Rate of Pulmonary Hypertension Syndrome in Broiler Chickens

[1] Mohammad Safaei, *[2] Ahmad Hassanabadi and [2] Mojtaba Tahmoorespur

[1] Ph. D Student of Poultry Nutrition, Ferdowsi University of Mashhad, International campus, Mashhad, Iran
[2] Professor, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

Background: Ascites is a major cause of mortality and morbidity in modern broiler production. The aim of this study was to determine the effects of different levels of dietary Nucleotide Supplementation (NS) on pulmonary hypertension syndrome (also known as ascites syndrome, AS).
Materials and methods: In the trial, 308 Ross 308 (mixed sex) broiler chickens were distributed in a completely randomized experimental design into 4 treatments with 7 replicates of 11 birds each. Experimental groups were defined by the inclusion of 0 (control), 0.5, 1 and 2 g/kg NS in the diets. Ascites was induced in broiler chickens by excess salt (receiving 0.2 % NaCl) in drinking water. Growth performance, mortality, blood parameters as well as ascites indices (right ventricle [RV], total ventricle [TV] weights, and RV/TV) were evaluated.
Results: The results of this study showed that feed intake, body weight gain, feed conversion ratio of broilers were not affected by treatments ($P > 0.05$). Ascites-related mortality, plasma triiodothyronine (T3) concentrations were significantly decreased in treatment with 1 g/Kg nucleotide in diets ($P < 0.05$). RV weight was significantly heavier and RV/TV ratios was significantly higher in treatment with 0 g/Kg nucleotide diets, and decreased in treatment with 1 g/Kg nucleotide in diets ($P < 0.05$).
Conclusion: Our findings showed that excess salt in drinking water induced pulmonary hypertension in broiler chickens. It is concluded that the inclusion of 1 g/kg nucleotide in susceptible broiler chicken diets has a systemic anti-hypertensive effect and could decrease ascites incidence.

Keywords: Ascites, broiler performance, blood parameters, nucleotide, excess salt.

* Corresponding author: Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. hassanabadi@um.ac.ir

Introduction

Ascites or Pulmonary Hypertension Syndrome (PHS) in broilers is one of the main causes for their death, resulting in great economic losses. PHS is one of the major metabolic disorders in present broiler chickens (Gupta, 2011, Wideman *et al*, 2013). The maximum incidence of PHS has been detected in fast growing birds (Arce-Menocal *et al*, 2009). Because ambient temperatures, dietary energy levels, and growth rate directly influence the basal metabolic rate and thus the amount of oxygen required by the animal, it should be possible to reduce the oxygen requirements and lower the incidence of ascites by reducing broilers' growth rates and rearing them under thermo neutral conditions (Kamely *et al*, 2015). Excess salt can stimulate PHS in chickens by increasing the blood volume that cause to the raise of cardiac output and blood flow through the lung (Mirsalimi and Julian, 1993). Ascetic chickens from the excess salt group indicated severe right ventricular hypertrophy, assessed by ascites heart index (AHI) compared with age-matched healthy broilers. These information recommended that broilers with the excess salt treatment were in the progression of PHS (Zhang *et al.*, 2013). Nucleotides play key roles in numerous biological processes, they are nucleic acid precursors, components of coenzymes, sources of cellular energy, involved in the synthesis of DNA and RNA, and mediate hormonal action (Carver and Walker, 1995). Nucleotide adequate diets are used to decrease stress level particularly those having poor dietary status (Vasquez-Garibay *et al.*, 2006). New finding suggested that nucleotides decrease response of the hormones associated with physiological stress (Naughton *et al.*, 2007). There are few studies in literature using nucleotides as additives in broiler diets. Therefore, the objective of the present study was to evaluate the effect of different levels of dietary nucleotide supplementation (NS) on ascites incidence in broiler chickens reared under salt conditions.

Materials and Methods

Experimental Design

The experiments were conducted in Poultry Research Station of Ferdowsi University Mashhad, Iran. The birds were subjected to 0.2% NaCl in drinking water to induced ascites. Zhang *et al.* (2013) reported the incidence rate (20%) of Ascites syndrome in the excess salt group was prominently higher than that in the control group (1.67%), then suggested that an Ascites syndrome model was successfully achieved by excess salt in drinking water. A total of 308 one-day-old mixed two sex broiler chickens were allocated to 4 experimental diets with 7 replicates of 11 birds each. Treatments consisted of the inclusion of the Nucleotide Supplementation (NS) at levels of 0 g/kg (N0), 0.5 g/kg (N0.5), 1 g/kg (N1), or 2 g/kg (N2) based on DM in diets. Tested NS was with Augic-15 brand (38% crude protein, 1% crude fiber, 4% ash and 7% moisture) that were prepared International Chamber of Commerce (ICC) in Brazil.

Feed and water were provided for ad libitum consumption and lighting was continuous. Diets were formulated to meet or exceed National Research Council recommendation for all nutrient requirements (NRC, 1994).

Table 1: Ingredient composition and calculated analysis of diets (g/kg, as fed basis).

Ingredients (g/kg)	Starter (1–10 day of age)	Grower (11–24 day of age)	Finisher (25–42 day of age)
Corn grain	510.7	540.9	588.0
Soybean meal (44%)	421.9	384.7	331.1
Vegetable oil	23.8	35.0	44.0
CaCo3	14.4	13.2	12.2
Dicalcium phosphate	15.2	13.4	11.9
Na Cl	2.9	2.9	2.9
Vitamin mix[1]	5	5	5
Mineral mix[2]	5	5	5
DL-Methionine	4.2	3.6	3.4
L- Lysine HCl	1.9	1.3	1.5
Total (g)	1000	1000	1000
Calculated values[3] (as fed basis)			
Metabolizable energy (Kcal/kg)	3000	3100	3200
Crude protein (%)	23	21.5	19.5
Ca (%)	0.96	0.87	0.79
Available phosphorus (%)	0.48	0.44	0.40
Methionine (%)	0.77	0.69	0.65
Methionine + Cystine (%)	1.08	0.99	0.91
Lysine (%)	1.44	1.29	1.16

[1]Vitamin mix provided the followings (per kg of diet): Thiamin mononitrate, 24 mg, nicotinic acid, 44 mg, D-Ca pantothenate, 12 mg, vitamin B12 (cobalamin), 120 mg, pyridoxine HCL, 47 mg, D-biotin, 110 mg, folic acid, 55 mg, menadione sodium bisulfate complex, 334 mg, choline chloride, 220 mg, cholecalciferol, 275 mg, trans-retinyl acetate, 1,892 mg, all-rac tocopheryl acetate, 11 mg, ethozyquin, 125 mg, [2]Trace mineral mix provided the followings (per kg of diet): manganese (MnSO4 H2O), 60 mg, iron (FeSO4 7H2O), 30 mg, zinc (ZnO), 50 mg, copper (CuSO4 5H2O), 5 mg, iodine (ethylene diamine dihydroiodide), 015 mg, selenium (Na2SeO3), 03 mg, [3]The values were calculated from NRC (1994).

The starter, grower and finisher diets are shown in Table 1. Birds were warm – room - brooded at 32°C during the first week, and the temperature decreased by 1 to 2°C every day from 8 d of age until 22°C at 15 d of age, and then was maintained until the end of experiment.

Measurement of Mortality and Performance

The birds were monitored daily for mortality. The criteria for diagnosis of Pulmonary Hypertension Syndrome were water belly and RV/TV weight ratio in the dead broilers. Feed intake was determined as the difference between the amount of feed offered and the amount remaining uneaten per of each phase (starter, grower and finisher). Individual BW were recorded at 42 d. Feed conversion ratio (FCR, feed g/ gain g) was calculated by dividing feed intake by BW gain.

Ascites-Related Parameters

All the chickens that died from d 1 onward were necropsied and examined to determine the cause of death. Diagnosis of AS generally depends on the ascites heart index (AHI) > 0.25, assessed by the ratio of right ventricle (RV) weight to total ventricle (TV) weight. To calculate the RV/TV, the hearts were collected, and the pericardium, peripheral adipose tissues, and atria were trimmed. The left ventricle and right ventricle were separated and their individual weights were measured on an analytical balance (Scaltec SBA41, Germany, precision 10−3 g), and the RV/TV was calculated (Julian, 2005).

Blood Parameters

The blood samples (approximately 2 mL) from 7 birds per treatment (28 birds) were randomly taken from a wing vein at the end of the experiments at 42 days of age. Whole blood samples were collected by venipuncture into EDTA-K3 anticoagulation tubes for hematological parameters consisting of haematocrit (HCT), hemoglobin (Hb), red blood cell counts (RBC), white blood cells (WBC), The non-fixed blood samples were centrifuged for 15 minutes at 400 g and separated sera were used to determine the levels of the following factors: such as Lactate dehydrogenase (LDH), Alkaline transaminase (ALT) and Aspartate transaminase (AST). Plasma triiodothyronine (T3) and thyroxin (T4) concentrations were analyzed by ELISA (Wang *et al*, 2012).

Statistical Analysis

Data from all experiments were analyzed using the ANOVA procedure of SAS software (SAS Institute, 2005) for completely randomized designs. All results are represented as mean ± SEM. Statistical significance of differences among treatments was assessed using Duncan's new multiple range test ($P \leq 0.05$).

Results

Growth Performance

The growth performance of the broiler chickens is presented in Table 2. The initial live body weight was similar in all treatments, also the final live body weight and body weight gain at 1 to 42 d don't change in treatments with increasing NS levels in the diets ($P > 0.05$). Feeding of NS did not change feed intake and feed conversion ratio in treatments on day 42 ($P > 0.05$). The feed conversion ratio was linearly increased ($P > 0.05$) until treatment with 2 g/kg NS at 1 to 42 days of age. In addition, no significant differences were found in the total mortality and mortality due to other cases between groups. Lowest ascites-related mortality was in broilers fed diets containing 1 g/kg NS ($P < 0.05$).

Table 2: Broiler chicken performance at 42 days of age

Parameter	Treatments					
	N 0	**N 0.5**	**N 1**	**N 2**	**SEM**	**P-Value**
Body weight (g)						
42 day of age	2352.8	2405.1	2323.3	2254.7	63.18	0.416
Body weight gain (g/bird/day						
1–42 day of age	56.017	57.266	55.319	53.684	1.504	0.416
Feed intake (g/bird/day)						
1–42 day of age	94.849	97.373	94.400	95.049	2.314	0.803
Feed conversion ratio						
1–42 day of age	1.69	1.70	1.70	1.77	0.029	0.229
Mortality (No. of dead birds / No. of Total birds*100)						
Ascites	13.35[a]	8.89[ab]	3.90[b]	9.16[ab]	2.174	0.042
Other cases	4.94	5.44	5.17	7.80	1.511	0.523
Total	18.29	14.33	9.07	16.96	3.605	0.304

[1]N0, N0.5, N1, and N2 diets contained 0 (control) 0.5, 1, and 2 g nucleotide/kg DM, respectively, [a–b] Means with different superscripts in each row are significantly ($P \leq 0.05$).

Blood Parameters and Ascites-Related Parameters

Table 3 shows that the RV and RV/TV were significantly decreased in treatment with 1 g/kg NS compared to the other groups ($P < 0.05$). HCT, Hb, RBC, WBC, LDH, AST, ALT and T4 did not significantly change among treatments (Table 3). The increase in NS levels up to 0.5 g/kg increased the WBC and further NS level (1 g/kg) numerically decreased these value. The concentration of Hb for the diets containing 0, 0.5, 1 and 2 g/kg NS was 10.87, 10.77, 11.15 and 11.30 g/dl, respectively. The T3 was decreased in the N1 group compared to other groups ($P < 0.05$), while the T4 was not influenced by the NS levels. Dietary 1 g/kg NS numerically decreased amount of LDH and AST.

Table 3: Blood parameters, and ascites-related index of broiler chickens at 42 days of age

Parameter	Treatments					
	N 0	N 0.5	N 1	N 2	SEM	P-Value
Hematology						
HCT (%)	33.05	32.07	32.47	34.22	1.146	0.588
Hb (g/dL)	10.87	10.77	11.15	11.30	0.396	0.774
RBC(10^{12}/L)	2.492	2.422	2.502	2.567	0.078	0.645
WBC(10^{9}/L)	12.00	12.60	11.90	11.97	0.445	0.672
LDH(U/L)	1665	1633	1326	1957	199.0	0.223
AST(U/L)	344.5	315.2	281.5	299.2	21.55	0.254
ALT(U/L)	6.50	5.25	7.75	5.00	1.735	0.668
Thyroid hormones (ng/ml)						
T3(ng/ml)	2.52^{a}	2.52^{a}	1.95^{b}	2.42^{a}	0.130	0.025
T4(μg/dl)	2.02	1.90	2.05	2.02	0.127	0.838
Ascites-related parameters						
RV (g)	2.63^{a}	1.57^{ab}	1.36^{b}	1.9^{ab}	0.329	0.001
RV/TV	0.34^{a}	0.29^{b}	0.18^{c}	0.26^{b}	0.013	0.001

[1]N0, N0.5, N1, and N2 diets contained 0 (control) 0.5, 1, or 2 g nucleotide/kg DM, respectively. HCT: Haematocrit Hb: Haemoglobin, RBC: Red blood cell,WBC: white blood cell, T3: Triiodothyronine, T4: Thyroxine, RV: Right ventricle weight and TV: Right+left ventricle weight, [a–b-c] Means with different superscripts in each row are significantly ($P \leq 0.05$).

Discussion

Growth Performance

The inclusion of different levels of NS in diets did not have an effect on final body weight, body weight gain, feed intake and feed conversion ratio during 1 to 42 days of age ($P > 0.05$). Zavarize *et al.* (2007) also did not observe any performance improvement in broilers fed a diet supplemented with 0.05% nucleotides as compared to those fed a non-supplemented diet. Pelícia *et al.* (2010) reported that There was no effect of treatment with 0.04%, 0.05%, 0.06%, and 0.07% nucleotides on the performance parameters analyzed at 7, 21, and 42 days of age. Jung and Betal (2012) reported that there were no difference in Body weight (BW) gain, feed intake, and feed conversion ratios among all treatments in birds were fed on diets supplemented with nucleotides under normal environmental conditions.

Lowest ascites-related mortality was in broilers fed diets containing 1 g/kg NS ($P < 0.05$). Higher (numerically) total mortality and other cases mortality were not associated with lower body weight in broilers fed diets containing 2 g/kg NS. During the entire experimental period, the highest body weight gain was observed by inclusion of 0.5 g/kg NS. Scheele *et al.* (2003) reported that body weight gain was lower in broiler chicken flocks that had high mortality rates. These results were not so consistent with the findings of the present study. A marked reduction in mortality was observed in broiler chickens fed a diet supplemented with NS (Pelícia and Sartori, 2010), a result in agreement with the current study.

Blood Parameters and Ascites-Related Parameters

The RV and RV/TV were decreased in treatment with 1 g/kg NS (P< 0.05) (Table 3). The RV/TV ratio has been considered to be strongly related to ascites incidence (Daneshyar *et al*, 2009). An RV/TV ratio higher than 0.25 to 0.30 was considered to be ascites diagnostic criteria in broilers (Kamely *et al*, 2015). Decreased RV/TV ratios in the N1 group were associated with reductions in total mortality and ascites-related mortality (Table 2 and 3). The results of the current study is in agreement with the results of the other study.

RBC and Hb were linearly increased in treatments with NS in the diets. (Table 3). Quinn and *et al*. (2016) reported positive correlation between mean RBC survival and percent Hb. Dietary 1 g/kg NS significantly decreased T3 concentration (P < 0.05) and numerically decreased amount of LDH. A strong relationship between thyroid hormone activity and ascites incidence was reported previously. Thyroid hormones are involved in controlling metabolic rate, and the concentration of circulating T3 is positively correlated with oxygen consumption in broilers (Kamely *et al*, 2015). Changes in the cardiovascular system to accommodate oxygen needs have been observed in birds adapted to low T3 (Yahav *et al*, 1995). Kamely *et al*. (2015) reported that exposure to low ambient temperature enhanced plasma T3 and T4, as well as T3:T4 ratio, which might be due to the need for a higher metabolic rate to maintain body temperature in the cold environment. The highest amount of LDH is possibly due to the increase of liver metabolism and to the significant muscle development that usually happens during this period, as observed by Szabo *et al*. (2005) in turkeys of commercial strains. Also, the LDH test is used to detect tissue alterations and as an aid in the diagnosis of anemia, gill and liver disease (Banaee *et al*, 2008). In addition, AST were decreased numerically in treatment with 1 g/Kg nucleotide. The amount of AST is directly related to the number of cells affected by the disease. AST is considered to be one of the most important test to detect liver injury from damage to heart or muscle. Systemic arterial hypertension triggers the onset of key hematological parameters such as increased erythrocyte number, blood hemoglobin, and mean erythrocyte cell volume (Quinn and *et al*, 2016).

Conclusion

The results of the present study suggest that Salt-induced ascites creates clinical and pathological changes that are like those of naturally happening ascites. The inclusion of 1 g/kg NS in broiler chicken diets reduced the RV and RV/TV ratio, total mortality, ascites-related mortality and the plasma triiodothyronine (T3) concentration. It can be concluded that the inclusion of 1 g/kg NS in broiler chicken diets could help to prevent ascites in susceptible broilers.

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