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Effects of Dietary Nucleotide Supplementation on Growth Performance, Internal Organs, Blood Metabolites, and HIF-1α mRNA Expression in Ascites Induced Broiler Chickens

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Abstract

This experiment was conducted to study the effects of nucleotide supplementation on induced ascites and its effects on growth performance, blood metabolites, and expression of hypoxia-inducible factor 1α (HIF-1α) mRNA in Ross 308 broiler chickens. A total of 672 one-day-old Ross 308 broiler chicks were allocated to eight treatments with two levels of common salt in drinking water (0 and 2 g/lit) and four dietary supplemental levels of nucleotides (0, 0.5, 1, and 2 g/kg diet). Each treatment was included 7 replicates of 12 birds each. The experimental design was completely randomized in a factorial arrangement. In this study, 0.2% salt in drinking water induced ascites, decreased body weight (BW), and increased mortality, right ventricle (RV) weight as well as RV to total ventricles ratio ($P < 0.05$). Results showed that nucleotide levels of 0.05 and 0.1% significantly decreased RV weight and blood triiodothyronine (T3) concentration on 42 d. Interaction effects between salt and nucleotide supplement revealed that 0.1% nucleotide level in the salt group significantly reduced blood T3 concentration in comparison with non-nucleotide supplemented and normal water group. Feed intake, weight gain, feed conversion ratio, carcass characteristics, and the expression of HIF-1α mRNA in heart of broilers were not significantly affected by excess salt, nucleotide supplement, and their interaction. It was concluded that 0.5 g/kg dietary nucleotide supplementation decreased the ascites parameter of the RV/BW ratio in broiler chickens.

Introduction

Ascites syndrome (AS) is one of the major metabolic disorders in present broiler chickens and it is the main cause of their death, resulting in great economic losses (Druyan *et al.,* 2009; Gupta, 2011; Wideman *et al.,* 2013). The maximum incidence of AS is detected in fast-growing birds, because of insufficient supply and high requirement of oxygen in this chickens (Malan *et al.,* 2003; Arce-Menocal *et al.,* 2009). After increasing the metabolic rate, sufficient oxygen is not supplied, then the heart rate increases to supply more oxygen for the tissues. These factors increase the cardiac output and cause right ventricle hypertrophy and ascites in modern broiler chicks (Julian *et al.,* 1987; Decuypere *et al.,* 1994; Scheele *et al.,* 2003; Wideman *et al.,* 2013; Hassanzadeh *et al.,* 2014).

High altitude, low temperature, and excess salt in drinking water increase hypoxia (Julian *et al.,* 1987; Wideman *et al.,* 1995; Qiao *et al.,* 1999). Excess salt in broilers drinking water can induce ascites by increasing cardiac output and blood flow in the lungs and decreasing erythrocyte deformability (increased resistance to blood flow in the lung) (Julian *et al.,* 1987; Mirsalimi and Julian, 1993).

Zhang *et al.* (2013) found that the expression of HIF-1α mRNA in hearts of ascitic chickens is significantly higher than normal chicks. They also reported that excess salt could stimulate HIF-1α expression. In hypoxia conditions, HIF-1α mRNA expression increases in the brain, lungs, and kidneys (Wang *et al.,* 2006).

Nucleotides are the main constituents and precursors of nucleic acids. They have main roles in Nucleotides are the main constituents and precursors of the nucleic acids. They have main roles in many biological functions (Carver and Walker, 1995).

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Nucleotide supplemented diets decreased stress levels, particularly in birds having poor dietary status (Vasquez-Garibay *et al.,* 2006). New findings suggest that nucleotides decrease responses to the hormones associated with physiological stresses (Naughton *et al.,* 2007). The objective of this study was to evaluate the effect of dietary nucleotide supplementation in ascites induced broiler chickens.

Materials and Methods

Supplemental nucleotide

Nucleotide supplement was a commercial product (AUGIC-15) which was derived from Saccharomyces cerevisiae yeast by ICC Brazil Company. It was a purified and concentrated source of nucleotides and its chemical composition was shown in Table 1 (ICC Brazil, 2017).

Table 1. Chemical composition of AUGIC-15 [g/kg].

Crude Protein	380.0
Free Nucleotides/Nucleosides	150.0
Moisture	70.0
Crude Fiber	10.0
Ash	40.0

Birds and management

The rearing conditions and standards used in this experiment were approved by the Ferdowsi University of Mashhad Animal Ethics Committee. This investigation was carried out using 672 one-day-old broiler chicks (Ross 308, mixed-sex) at the Ferdowsi University of Mashhad (Mashhad, Iran). The birds were distributed in floor pens (1.5 × 1.0 × 0.8 m) and reared up to 42 d of age. Each treatment was included 7 replicates of 12 chicks each. Feed and water were provided *ad libitum* throughout the experiment. The temperature was set at 32◦C on day 1, then was gradually decreased by 0.5◦C per day to reach 21◦C and it was maintained constant afterward. Birds received 23L: 1D lighting schedule during the experiment.

Dietary treatments

The experiment was conducted in a completely randomized design with 2×4 factorial combination, including two levels of common salt (NaCl) in drinking water (0 and 2 g/Lit) and four dietary supplementation levels of nucleotide (0, 0.5, 1, and 2 g/kg of diet) with 7 replicates each. The sodium concentration of the farm tap water was measured according to AOAC (2000) method, before the experiment, which was 1.95 mEq/L. For preparing experimental diets, a corn-soy basal diet (Table 2) was formulated to meet nutrient requirements recommended by Ross-308 company (Aviagen, 2014) for starter (1-10 d), grower (11-24 d), and finisher (25-42 d) periods as mash form and supplemented with appropriate levels of nucleotide supplement.

Table 2. Ingredient composition and calculated analysis of basal diet [g/kg, as fed basis].

Ingredients (g/kg)	Starter (1 to 10 d)	Grower (11 to 24 d)	Finisher (25 to 42 d)
Corn, grain	510.7	540.9	588.0
Soybean meal (440 g CP)	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
NaCl	2.9	2.9	2.9
Vitamin mix[1]	2.5	2.5	2.5
Mineral mix[2]	2.5	2.5	2.5
DL-Methionine	4.2	3.6	3.4
L- Lysine HCl	1.9	1.3	1.5
Calculated nutritional composition, g per kg (as-fed basis)			
Metabolizable Energy, Kcal/Kg	3000	3100	3200
Crude protein	230.0	215.0	195.0
Ca	9.6	8.7	7.9
Na	2.3	2.3	2.0
Available phosphorus	4.8	4.4	4.0
Methionine	7.7	6.9	6.5
Methionine + Cystine	10.8	9.9	9.1
Lysine	14.4	12.9	11.6

[1]Vitamin premix provided per kilogram of diet: vitamin A (retinyl acetate), 15,000 IU; vitamin D3, 5,000 IU; vitamin E (DL-α-tocopheryl acetate), 80 mg ;vitamin K, 5 mg; thiamin, 3 mg; riboflavin, 10 mg; pyridoxine, 5 mg; vitamin B12, 0.02 mg; niacin, 70 mg; choline chloride, 1800 mg; folic acid, 2 mg; biotin, 0.4 mg; pantothenic acid, 20 mg.
[2]Mineral premix provided per kilogram of diet: Mn (manganese sulfate), 100 mg; Zn (zinc sulphate), 65 mg; Cu (copper sulfate), 5 mg; Se (Sodium Selenite), 0.22 mg; I (calcium iodate), 0.5 mg; and Co, 0.5 mg.

Growth performance and mortality

The body weight (BW) and feed intake (FI) were recorded during feeding phases. Feed conversion ratio (FCR) was calculated for each rearing phase and

the whole experimental period (1-42 d), after adjusting for dead birds. The birds were monitored daily for total mortality and ascites-related mortality.

Ascites-related parameters

Chickens who died during the experiment were inspected to specify the cause of death. Index of ascites usually depends on this features a) clinical signs in chickens: ascites birds show bluish discoloration of the skin; veins are loose and protuberant; combs and wattles are often shrunk; abdominal cavity filled with fluid; b) yellow and colloidal fluid in the abdomen or a plasma clot is attached to the surface of the liver; and c) ascites heart index is measured by dividing right ventricle (RV) weight to total ventricles (TV) weight (Julian, 2005).

Carcass characteristics

After 8-h feed withdrawal at 42 d of the experiment, one male bird per pen was randomly selected and slaughtered for organ sampling of heart, liver, lungs, proventriculus, gizzard, abdominal fat, bursa of Fabricius, spleen, thighs, and breast yields. The organs were weighed and the carcasses were processed as described by Akbari Moghaddam Kakhki *et al.* (2017).

Blood parameters

Blood samples (approximately 2 mL) from 5 birds per treatment were randomly taken from the wing vein at the end of the experiment (d 42). The whole blood samples were collected by venipuncture into EDTA-K3 anticoagulation tubes for hematological parameters consisting of hematocrit (HCT), hemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC) counts. The blood samples were centrifuged at 400 g for 15 min to separate sera to measure the concentrations of lactate dehydrogenase (LDH), alkaline transaminase (ALT), and aspartate transaminase (AST). Plasma T3 and thyroxine (T4) concentrations were analyzed by the ELISA test (Wang *et al.,* 2012).

HIF-1α mRNA expression

Heart samples of the broilers were taken at 42 days of age, moved into liquid nitrogen, and stored at –80°C for RNA extraction to measure the quantitative expression of the HIF-1α gene. Total RNA was extracted using RNeasy Mini Kit (QIAGEN), and RNA samples were treated with RNaseout (Invitrogen) and DNaseI (Deoxyribonuclease I). RNA concentration was measured at 260 nm using a spectrophotometer. Quantitative real-time PCR was achieved as denaturation at 95°C for 5 min, then 40 cycles of amplification with 30 s of denaturation at 95°C, 30 s annealing at 56°C, and extension at 72°C. Quantitative real-time PCR was done by the Agilent Mx3005P sequence detection system. Reactions were performed using the SYBR Green Real-time PCR Master Mix in a total volume of 25 µL. Gene expression was normalized for RNA loading using β-action as the internal control. According to the standard curves for each gene (HIF-1α and β-action), the cDNA copy numbers of the samples were calculated based on the mean value of each sample (Table 3).

Table 3. Primers used for RT-PCR analysis of chicken mRNAs.

Gene	sense primers	antisense primers	Size of PCR	Accession no.
β-actin	5′-TGAGAGAAATGCTTACACACAG-3′	5′-TGATGGGTGAGGAATTGGTTCAC-3′	184 bp	NM204297
HIF-1α	5′-CAGTGCCAGCCTCGTCTCAT-3	5′-AGGGGCCATCCACAGTCTTC-3′	341 bp	NM205518

Statistical analysis

Data were analyzed as a completely randomized design in a factorial arrangement using the General Linear Model (GLM) procedure in SAS software (SAS Institute, 2009). Statistical significance of differences among treatments was assessed using Tukey's test when the F-test from the ANOVA was declared significant ($P \leq 0.05$). Orthogonal comparisons were tested using polynomial regression to determine the linear and quadratic effects of increasing nucleotide supplementation levels and considered significant at $P \leq 0.05$ (SAS Institute, 2009).

Results

Mortality and growth performance

Ascites in the current study were induced by excess salt in the drinking water of broiler chickens. As shown in Table 4, the total incidence of ascites in the salt group (71.6%) was significantly higher ($P < 0.05$) than the normal group (29.4%) during the whole experimental period (1-42 d). The ascitic birds showed ascites symptoms like a yellow fluid with clots in the abdominal cavity, edematous lungs, hypertrophy in RVs, and congestions in veins. The body weight of salt and normal groups showed a significant difference in 42 d. BW of birds in the salt group was significantly lower ($P < 0.05$) than the normal group (Table 4). Growth performance indices of broiler chickens presented in Table 4 shows that nucleotide supplementation, salt in drinking water and their interaction did not significantly affect WG,

FI, and FCR during 1-42 d ($P > 0.05$). However, birds receiving normal water exhibited significantly more BW in comparison to the salt group. There were not any linear and quadratic relationships between increasing dietary supplementation levels of nucleotides and growth performance parameters ($P < 0.05$).

Table 4. Effects of salt, nucleotide supplementation, and their combination on the performance of broiler chickens during the whole experimental period (1 - 42 d).

Treatment	Body weight (g)	Body weight gain (g/bird/day)	Feed intake (g/bird/day)	Feed conversion ratio (g:g)	Mortality (No. of dead birds / No. of Total birds∗100)		
					Ascites	other cases	Total
NG	2516[a]	55.84	97.29	1.72	2.92[b]	6.16	9.09[b]
SG	2415[b]	55.57	95.41	1.70	12.33[a]	4.87	17.20[a]
SEM	27.71	0.68	1.14	1.01	1.59	1.35	2.07
P-Value	0.01	0.78	0.25	0.31	0.001	0.50	0.008
Dietary nucleotide, g/kg							
0	2498	56.20	97.24	1.73	7.79	5.19	12.98
0.5	2447	56.04	95.85	1.71	7.14	3.89	11.03
1	2455	55.35	95.44	1.72	7.14	6.49	13.63
2	2463	55.22	96.87	1.75	8.44	6.49	14.93
SEM	39.19	0.96	1.61	0.02	2.25	1.91	2.94
			P-Value				
ANOVA	0.80	0.85	0.84	0.61	0.97	0.74	0.82
Linear	0.69	0.65	0.46	0.28	0.31	0.55	0.10
Quadratic	0.76	0.63	0.36	0.21	0.19	0.50	0.48
Group ×dietary nucleotide level							
NG × N0	2416	56.39	99.65	1.76	1.29	9.09	10.38
NG × N0.5	2373	54.81	94.34	1.72	3.89	3.89	7.79
NG × N1	2404	55.38	96.48	1.74	2.59	6.49	9.09
NG × N2	2468	56.76	98.70	1.73	3.89	5.19	9.09
SG × N0	2580	56.01	94.84	1.69	14.28	1.29	15.58
SG × N0.5	2522	57.26	97.37	1.70	10.38	3.89	14.28
SG × N1	2507	55.31	94.40	1.70	11.68	6.49	18.18
SG × N2	2457	53.68	95.04	1.77	12.98	7.79	20.77
SEM	55.42	1.36	2.28	0.03	3.19	2.70	4.15
P-Value	0.39	0.26	0.34	0.44	0.79	0.25	0.86

NG (normal group) and SG (salt group) received tap water and 2 g/Lit common salt in drinking water, respectively.
N0, N0.5, N1, and N2 diets contained 0 (control), 0.5, 1 and 2 g nucleotide/kg diet, respectively.
[a,b] Values with uncommon superscripts within each column are significantly different ($P < 0.05$).

Carcass characteristics

The effects of nucleotide supplementation on carcass cuts and internal organs (thighs, breast yield, lungs, proventriculus, gizzard, abdominal fat, heart, and liver) and lymphoid organs (bursa of Fabricius and spleen) are shown in Table 5. Different dietary levels of nucleotides, salt levels in drinking water, and their interaction had no significant effects on carcass traits. As shown in Table 5, the orthogonal contrasts did not show significant linear and quadratic effects between nucleotide supplementation levels and carcass characteristics of broiler chickens ($P < 0.05$).

Ascites-related parameters

As shown in Table 6, the RV and RV/TV were measured as an indicator of AS in broiler chickens. To measure RV and RV to TV ratio in the hearts, the left and right ventricles of each sample were separated and individually weighted, then the RV/TV ratio was calculated and recorded (Julian, 2005). At 42 d of age, RV and RV/TV in the excess salt group were significantly higher than the normal group ($P < 0.05$). These results indicated that excess salt induces AS in broiler chickens. There were significant differences in the RV/BW ratio among different levels of nucleotide supplementation (NS). The RV weight was significantly decreased in treatment 1 g/kg NS ($P < 0.05$) (Table 6).

Blood parameters

Blood parameters results are shown in Table 6. The HCT, Hb, RBC, WBC, LDH, ALT, AST, and serum T4 concentration were not affected either in normal group (NG) or salt group (SG) as well as their interactions (Table 6). As shown in Table 6, 1 g/kg dietary NS in the salt group significantly decreased T3 concentration ($P < 0.05$).

Table 5. Effects of salt, nucleotide supplementation, and their combination on carcass traits and lymphoid organ weights (%of live body weight) of broiler chickens at 42 d of age.

Treatment	Carcass[1]	Thighs[1]	Breast[1]	Lungs	Proventriculus	Gizzard	Abdominal fat	Heart	Liver	Lymphoid organ weights	
										Bursa	Spleen
NG	62.30	17.62	27.38	0.44	0.43	2.24	1.15	0.55	2.40	0.22	0.16
SG	63.45	18.00	27.34	0.45	0.39	2.28	1.14	0.55	2.34	0.21	0.13
SEM	10.05	0.52	0.34	0.01	0.02	0.07	0.07	0.02	0.06	0.021	0.008
P-Value	0.443	0.62	0.93	0.66	0.27	0.44	0.98	0.85	0.48	0.74	0.065
Dietary nucleotide, g/kg											
0	63.16	18.12	27.21	0.41	0.42	2.31	1.09	0.50	2.26	0.20	0.14
0.5	63.56	17.62	28.24	0.47	0.38	2.12	0.99	0.59	2.33	0.22	0.16
1	60.96	17.37	26.65	0.46	0.42	2.27	1.29	0.53	2.48	0.22	0.12
2	63.83	18.12	27.35	0.43	0.43	2.34	1.20	0.58	2.41	0.23	0.15
SEM	1.485	0.74	0.49	0.02	0.02	0.11	0.10	0.03	0.08	0.03	0.01
					P-Value						
ANOVA	0.518	0.85	0.17	0.22	0.76	0.51	0.22	0.24	0.27	0.91	0.26
Linear	0.41	0.59	0.61	0.19	0.67	0.67	0.64	0.19	0.72	0.86	0.46
Quadratic	0.35	0.44	0.47	0.14	0.56	0.48	0.21	0.15	0.49	0.48	0.72
Group ×dietary nucleotide level											
NG × N0	64.48	19.00	27.08	0.42	0.42	2.18	1.01	0.52	2.33	0.20	0.13
NG × N0.5	64.27	17.75	28.88	0.44	0.41	2.07	0.98	0.53	2.62	0.21	0.16
NG × N1	56.90	15.50	26.33	0.52	0.44	2.23	1.45	0.59	2.67	0.28	0.17
NG × N2	63.55	18.25	27.23	0.38	0.45	2.09	1.14	0.56	2.34	0.26	0.16
SG × N0	61.85	17.25	27.34	0.40	0.43	2.45	1.17	0.48	2.18	0.21	0.14
SG × N0.5	62.85	17.50	27.59	0.51	0.36	2.18	1.01	0.66	2.41	0.22	0.16
SG × N1	65.02	19.25	26.96	0.40	0.40	2.31	1.13	0.46	2.30	0.22	0.09
SG × N2	64.11	18.00	27.46	0.48	0.36	2.59	1.27	0.60	2.48	0.21	0.14
SEM	2.100	1.05	0.69	0.03	0.04	0.15	0.14	0.01	0.11	0.04	0.02
P-Value	0.072	0.08	0.53	0.08	0.82	0.53	0.37	0.10	0.10	0.87	0.11

[1] Peeled

NG and SG received tap water and 2 g/Lit common salt in drinking water, respectively.

N0, N0.5, N1, and N2 diets contained 0 (control), 0.5, 1 and 2 g nucleotide/kg diet, respectively.

Table 6. Effects of salt, nucleotide supplementation, and their combination on blood parameters, ascites-related index, and HIF-1α gene expression in heart of broiler chickens at 42 d of age.

Treatment	Hematology							Thyroid hormones (ng/ml)		Ascites-related parameters		Gene Expression
	HCT (%)	Hb (g/dl)	RBC (10^{12}/L)	WBC (10^{9}/L)	LDH (U/L)	AST (U/L)	ALT (U/L)	T3	T4	RV/BW	RV/TV	HIF-1α
NG	32.95	11.21	2.49	12.11	1645	285.06	4.31	2.35	1.84	0.055^{b}	0.198^{b}	4.13
SG	33.38	11.02	2.51	12.20	1897	310.13	6.12	2.42	2.00	0.075^{a}	0.254^{a}	5.02
SEM	0.57	0.19	0.04	0.19	94.15	14.11	0.79	0.07	0.06	0.005	0.01	0.37
P-Value	0.60	0.51	0.75	0.75	0.07	0.22	0.11	0.51	0.10	0.004	0.02	0.09
Dietary nucleotide, g/kg												
0	33.16	10.95	2.50	12.12	1866	321.25	5.00	2.62^{a}	1.88	0.084^{a}	0.265	5.33
0.5	32.81	11.26	2.47	12.31	1641	289.00	5.37	2.28^{b}	1.86	0.055^{b}	0.183	4.15
1	32.77	11.02	2.49	11.95	1634	276.25	6.62	2.23^{b}	1.93	0.066^{ab}	0.209	4.63
2	33.92	11.23	2.55	12.26	1945	303.88	3.87	2.41^{ab}	2.00	0.065^{ab}	0.235	4.19
SEM	0.81	0.28	0.05	0.27	133.15	19.96	1.11	0.10	0.09	0.006	0.02	0.52
						P-Value						
ANOVA	0.73	0.82	0.82	0.79	0.26	0.43	0.39	0.05	0.74	0.018	0.08	0.37
Linear	0.10	0.76	0.39	0.75	0.74	0.53	0.12	0.14	0.09	0.34	0.25	0.61
Quadratic	0.75	0.40	0.66	0.80	0.11	0.11	0.08	0.45	0.84	0.27	0.37	0.48
Group ×dietary nucleotide level												
NG × N_0	33.27	11.02	2.50	12.25	1603	298.00	3.50	2.72^{a}	1.75	0.066^{ab}	0.215	4.83
NG × $N_{0.5}$	33.55	11.75	2.53	12.02	2099	262.75	5.50	2.05^{ab}	1.82	0.0544^{b}	0.173	3.73
NG × N_1	33.07	10.90	2.48	12.00	1955	271.00	5.50	2.52^{ab}	1.82	0.045^{b}	0.168	4.25
NG × N_2	33.62	11.17	2.53	12.55	1933	308.50	2.75	2.40^{ab}	1.97	0.064^{ab}	0.221	3.72
SG × N_0	33.05	10.87	2.49	12.00	1665	344.50	6.50	2.52^{ab}	2.02	0.105^{a}	0.292	5.82
SG × $N_{0.5}$	32.07	10.77	2.42	12.60	1633	315.25	5.25	2.52^{ab}	1.90	0.077^{ab}	0.233	4.58
SG × N_1	32.47	11.15	2.50	11.90	1326	281.50	7.75	1.95^{b}	2.05	0.056^{b}	0.205	5.01
SG × N_2	34.22	11.30	2.56	11.97	1957	299.25	5.00	2.42^{ab}	2.02	0.084^{ab}	0.244	4.66
SEM	1.14	0.39	0.07	0.39	188.31	28.22	1.58	0.14	0.13	0.009	0.030	0.74
P-Value	0.83	0.43	0.79	0.52	0.19	0.65	0.75	0.01	0.79	0.01	0.88	0.99

NG and SG received tap water and 2 g/Lit common salt in drinking water, respectively.

N0, N0.5, N1, and N2 diets contained 0 (control), 0.5, 1 and 2 g nucleotide/kg diet, respectively.

PCV: Packed cell volume, HCT: HaematocritHb: Haemoglobin, RBC: Red blood cell, WBC: white blood cell, T3: Triiodothyronine, T4: Thyroxine, RV: Right ventricle weight, and TV: Right+left ventricles weight.

RV/BW = right ventricular to body weight ratio.

[a,b] Values with uncommon superscripts within each column are significantly different ($P < 0.05$).

Expression of HIF-1α mRNA in the heart

Table 6 shows that salty water increased (P = 0.09) expression level of HIF-1α mRNA in the hearts of the birds compared to broilers received tap water (5.02 vs. 4.13). There was no significant interaction effect between NG and SG regarding HIF-1α gene expression. The orthogonal contrasts did not show significant linear and quadratic effects between nucleotide supplementation levels and blood parameters, ascites-related indices, and HIF-1α gene expression in heart of broiler chickens at 42 d of age (Table 6).

Discussion

Mortality and growth performance

In some previous studies, ascites syndrome (AS) has been induced by the addition of salt in the drinking water of broiler chickens (Julian *et al.,* 1987; Julian, 2005; Zhang *et al.,* 2013). Excess salt intake increases cardiac output and blood flow through the lungs of the birds (Julian *et al.,* 1987; Mirsalimi and Julian, 1993). In this study, the incidence rate of AS (71.6%) in the salt group was higher than the normal group (29.4%), Therefore, AS was induced by adding salt to drinking water. Birds in SG showed more mortality and lower BW. Scheele *et al.* (2003) reported that AS causes more mortality and lower WG in broiler chickens.

Similar to the current experiment, Pelícia and Sartori (2010) as well as Jung and Batal (2012) reported that nucleotide supplementation in the diets had no significant effects on WG, FI, and FCR in broiler chickens. This finding showed that the nucleotide supply in the basal diets is sufficient to meet the broiler's requirements. Maribo (2003) observed that supplementing broiler chickens diet with a yeast product containing high nucleotides have no positive effect on growth performance. Therefore, he stated that nucleotide supplementation may be beneficial under challenges and infectious conditions.

Ascites-related parameters

Ascitic chickens due to excess salt showed severe right ventricular hypertrophy compared to the normal group. These data suggested that broilers received excess salt in drinking water are affected by AS. There were significant differences in RV weight and RV/TV ratio among different levels of nucleotide supplementation. The RV was significantly decreased in the treatment of 0.5 g/kg NS. Also, RV/TV of the birds fed diet without nucleotide supplementation was higher than nucleotide receiving groups.

The RV and RV/TV ratio are strongly related to ascites incidence (Daneshyar *et al.,* 2009), and the RV/TV ratio higher than 0.25 to 0.30 is the important ascites diagnostic criteria in broilers (Julian *et al.,* 1987). Sato *et al.* (2005) reported that nucleoside adenosine-induced vasodilation in human coronary arterioles from patients with heart disease. Also, Feoktistov *et al.* (2004) reported that adenosine has an important role in the adjustment of vascular tone in hypoxia conditions, and adenosine receptor activation motivates angiogenesis. Ryzhov *et al.* (2007) stated that adenosine actions do not have any additional application but complement the direct effects of hypoxia. The stimulation of adenosine receptors not only contributes to the hypoxia effect but also additional actions in regulating angiogenic elements. Therefore, adenosine receptors represent a potential therapeutic target for vascular remodeling.

Carcass characteristics

Dietary treatments did not alter the carcass characteristics (carcass yield, heart, liver, lung, proventriculus, gizzard, abdominal fat, thighs, and breast yield). Similarly, there were no differences in lymphoid organ weights (bursa and spleen) due to different dietary treatments. It may be mentioned that the carcass characteristics were not affected, due to adequate levels of nucleotides in the N0 diet. Sánchez-Pozo and Gil (2002) stated that nucleotides are common components of the diet and the body provides mechanisms for their absorption and synthesis in tissues. However, during periods of rapid growth, disease-specific states, restricted nutrient intake, or insufficient endogenous synthesis of nucleotides (Lerner and Shamir, 2000), their availability may be limited for rapid division tissues (Van Buren and Rudolph, 1997; Lerner and Shamir, 2000). In the present study, it was found that the lymphoid tissues (bursa and spleen) did not significantly affect by nucleotide supplementation.

However, Van Buren *et al.* (1985) suggested that the effects of dietary nucleotides on cellular immune responses may be due to acting on the T-helper cell-mediated effects by increasing antigen processing or lymphocyte proliferation. Even though the mechanism of immunostimulation by dietary nucleotides is not fully understood; dietary nucleotides may be required for optimal cellular immune response function including increased INF-γ, decreased IL-2 production and natural killer cells, and increased resistance to bacterial or fungal infection.

Blood parameters

Thyroid hormones are involved in controlling the metabolic rate, and the concentration of blood T3 is positively related to oxygen consumption in broiler chickens. Hassanzadeh *et al.* (2014) in a review article indicated that thyroid hormone insufficiency decreases growth performance and impairs oxygen supply when trying to sustain a fast growth rate. Changes in the cardiovascular system to accommodate oxygen needs have been observed in birds adapted to low T3 (Kamely *et al*., 2015). In this study, the activity of LDH in NG birds was lower

than SG (P=0.07). The higher concentration of LDH is probably due to increased liver and hypoxemia metabolism that usually occurs during AS, as observed by Szabo *et al*. (2005) in turkey commercial strains. LDH testing is also used to detect tissue changes and to aid in the diagnosis of anemia, gill, and liver disease (Banaee *et al*., 2008).

Expression of HIF-1α mRNA in the heart

Increasing the expression of HIF-1α mRNA in the hearts of birds in the salt group suggests that excess salt in drinking water induces HIF-1α mRNA expression in broiler hearts. Zhang *et al*. (2013) showed that excess salt in drinking water induces HIF-1α mRNA expression in broiler hearts. They also showed that ascitic birds from over-salted in terms of HIF-1α mRNA expression were more than the control group. HIF-1α mRNA expression gradually increases with increasing pulmonary arterial pressure. Hypoxia-induced factor-1 α is involved in the pathogenesis of pulmonary hypertension (PH) in humans and animals (Jiang *et al*., 2007). Current results confirmed that HIF-1α may be associated with the development of AS in broilers. It is generally accepted that hypoxia may be the major cause of increased HIF-1α mRNA expression. Increased expression levels may be a long-term adaptation to conditions associated with hypoxia or pathological changes, including PH. It is well known that ascites in broilers is a hypoxia-related disease, especially at high altitudes and cold temperatures (Julian, 2000).

Conclusion

Our findings showed that salt in drinking water induced the ascites in broiler chickens. HIF-1α might be associated with the development of AS in broilers. The inclusion of 0.5g/kg nucleotide in broiler chickens diet reduced the RV/BW ratio, and also 0.5 and 1 g/kg NS in the diets reduced plasma T3 concentration. It can be concluded that the supplementation of 0.5 g/kg nucleotide in broiler chickens diet could alleviate ascites in susceptible broilers.

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