J. BIOL. ENVIRON. SCI.,
2015, 9(27), 151-158

Original Research Article

The Effects of Different Diet Total Volatile Nitrogen Levels on Blood Biochemical Parameters in Broilers

Mohammad Ghasemi-Sadabadi, Yahya Ebrahimnezhad[*], Behrad Eshratkhah and Naser Maheri-Sis
Department of Animal Science, Faculty of Animal Science and Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, IRAN

Received: 23.01.2016; Accepted: 17.02.2016; Published Online: 19.04.2016

ABSTRACT

A study was conducted to determine the effects of different diet total volatile nitrogen levels on blood biochemical parameters in broilers. A total of 320 one day old, Ross 308 broiler chicks, were randomly allocated to one of the 20 floor pens in a completely randomized design with five treatments and four replicate groups and sixteen chicks in each group. The aim of using different levels of urea in this study due to created various levels of total volatile nitrogen in the diet. Dietary treatments consisted five different levels of urea including zero (control), 0.5, 1, 1.5 and 2 percentages of urea in the diets. The total content of volatile nitrogen (TVN) measured after adding different levels of urea in diet and found as 13.30, 14.95, 17.26, 23.26 and 27.47 mg per 100 g respectively in starter diets and 16.66, 15.02, 17.81, 24.66 and 26.25 TVN mg per 100 g growing diets, respectively. The chicks were fed without adding urea during the first week. At 42 days of age, four chickens (two male and two female) from each pen were selected and blood samples collected from taken wing vein. Glucose, triglyceride (TG), cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), phosphorus, calcium, albumin, total protein, globulin, uric acid and urea were measured. The results indicated that high level of total volatile nitrogen in diet significantly increased blood urea, uric acid, TG, cholesterol, LDL, phosphorus, total protein and globulin levels, while significantly decreased blood glucose level in our study (P<0.05). In conclusion, the results of this study concluded that, increasing TVN in diet had deleterious effect on blood biochemical parameters in broilers.

Keywords: Total volatile nitrogen, Urea, Blood biochemical parameter, Broiler

Farklı Uçucu Azot Seviyelerindeki Besinlerin Broiler Tavuklarının Kan Parametreleri Üzerine Etkileri

Farklı uçucu azot seviyelerindeki besinlerin broiler tavuklarının kan parametreleri üzerindeki etkilerini belirlemek amacıyla bir çalışma yapılmıştır. Toplamda 320 adet Ross 308 civciv 4 tekerrürlü 5 uygulama için uygulama başına 16 civciv gelecek şekilde rastgele olarak dağıtılmıştır. Çalışmada üre kullanılarak besinlerdeki farklı azot seviyeleri ayarlanmıştır. Besin uygulamaları 0 (kontrol), 0.5, 1, 1.5 ve 2 olmak üzere beş farklı dozda üre içermektedir. Toplam uçucu azot miktarları besinlere üre eklemesi yapıldıktan sonra ölçülmüş ve başlangıç besinleri için sırasıyla 13.30, 14.95, 17.26, 23.26 ve 27.47 mg/100 g, büyüme besinleri için ise sırasıyla 16.66, 15.02, 17.81, 24.66 and 26.25 TVN mg/100 g olarak tespit edilmiştir. Civcivler ilk hafta üre eklenmeden beslenmiştir. 42 günlük olduktan sonra 4 piliç (2 dişi 2 erkek) seçilerek kanat damarlarından kan örnekleri alınmıştır. Glukoz, trigliserid, kolesterol, LDL, HDL, fosfor, kalsiyum, albumin, toplam protein, globulin, ürik asit ve üre ölçümleri yapılmıştır. Araştırma sonuçlarına göre yüksek seviyelerideki uçucu azot içeriği kandaki üre, ürik asit, TG, kolesterol, LDL, fosfor, toplam protein ve globulin miktarını önemli ölçüde artırırken, glukoz seviyesi önemli ölçüde azalmıştır. Genel olarak incelendiğinde besindeki toplam uçucu azot seviyesinin artırılması, kan paramterlerinde olumsuz etkiler ortaya çıkarmaktadır.

Anahtar Kelimeler: Toplam uçucu azot, Üre, Kan biyokimyasal parametresi, Broiler

INTRODUCTION

Total volatile nitrogen (TVN) show the amount of nitrogen compounds in the diet, which is determines the quality of the diet and large part of TVN containing non protein nitrogen (NPN) of protein, and it can also be used as indicators to determine the true protein (Ariyawansa 2000). TVN count depends on source as dimethyl amine and ammonia; these are two important parts of total volatile nitrogen combination (Ariyawansa 2000). However, TVN counts related to protein breakdown in diet (Egan *et al.* 1981) and increasing of TVN count attributed to ammonia or other compounds (Banwart *et al*. 1981).

Sahraei *et al*. (2012) showed that the use of poultry by-product meal (PBPM) containing 209 mg per 100 g of TVN reduced feed intake, body weight gain and feed conversion ratio. Supplementation of NPN can reduce the cost of poultry diets, but utilization of high level of NPN sources had deleterious effects on poultry health (Pervaz *et al* 1996). Urea is a NPN substance commonly used to replace some of the true protein in diets of ruminant (Shahzad *et al*. 2012, Nolan and Leng 1972). Murray *et al*. (1988) stated that urea is non-protein compound. Recently, many researchers have done more experiment to determine the use of urea in poultry diets (Davis and Martindale 1973, Kazemi and balloon, 1973, Kobayashi 1981). Some researchers have shown that urea and NPN may replace the non-essential amino acids in the poultry diets (Okumura and Kio 1984, Brukental and Nitsan 1981, Lee and Blaier 1972). Javed *et al*. (2002), Pervaz *et al*. (1996) reported that urea is a source of

[*] Corresponding author: Ebrahimnezhad@iaushab.ac.ir

nonessential nitrogen (ammonia), a product of urease activity Also the toxicity of urea is related with the release of ammonia.

Chandra *et al.* (1984b) indicated that the use of urea at higher levels has shown effects in broilers including poor growth rate, altered blood parameters and untoward changes in different organs/tissues of the body. Also, Lee and Blaire (1972) and Featherston *et al*. (1962) reported that use of low level urea in diet has also been documented. Abdou *et al*. (2006) reported a reduction in RBC counts, WBC counts, hematocrits and hemoglobin, while elevation in ALP, LDH and all the treated birds lost a weight. In similar cases, Chandra *et al*. (1984c) showed that supplementation of urea had significantly affected on blood parameter. Itoh *et al*. (1979) indicated that blood ammonia and uric acid did not differ in among amounts of urea in the diet. Ammonia tended to be less when urea level increased and uric acid was more. Pervaz (1994) and Chandra *et al*. (1983) who also indicated significantly increase in serum enzymes with the high level urea in diet. The higher levels of SAST and SALT in the blood serum are suggestive of continuous liver damage at cellular level as these enzymes are released from degenerating liver cells and blood cells (Anderson 1971).

Pervaz (1994) studied the hematological and enzymological changes produced by urea feeding who showed serum enzymes were significantly higher in chicks fed one percent urea. The histological findings were described with reference to uric acid granulation and calcinosis. Also, Nagalakashmi *et al*. (1999) reported that the urea concentrations increased in blood when the concentration of urea increased in the diet. Javed *et al*. (2002) conducted that the concentrations of serum total proteins, albumin and fibrinogen were similar among the treatment groups. On the other hand, concentrations of serum globulins revealed significantly high values in broilers supplemented with 1% urea + 20 ml formalin than the control. Due to these facts, recently, in animal industry, the utilization of NPN sources such as urea, fish meal and etc. for reduces the cost of feed used. So this study was conducted to determine the effects of different diet total volatile nitrogen levels on blood biochemical parameters in broilers.

MATERIALS AND METHODS

Animals, management and nutrient

A total of 320 one day old, male and female, from the Ross 308 strain broiler chicks, were randomly allocated to 20 floor pens in a completely randomized design with five treatments, four replicates and sixteen chicks in each replicate (eight male and eight female). Dietary treatments consisted five different levels of urea including zero, 0.5, 1, 1.5 and 2% urea based on the diet. The chicks were fed without adding urea during the first week of the experiment. All chicks in this experiment were fed on starter diet (1-21 days) and grower diet (22-42 days) and the experimental period was 42 days. After addition of urea to the diet, TVN content was measured (AOAC, 1992). The amounts of TVN were found as 13.30, 14.95, 17.26, 23.26 and 24.47 in the starter diet and 14.66, 15.02, 17.81, 24.66 and 26.25 mg per 100 g, in grower diet, respectively (Table 1 and 2). During the experimental period the feed and water available *ad libitum* to broiler were placed on floor pens (1×2 m^2). All the chicks were kept under similar management conditions according to Ross 308 strain catalogue (Aviagen 2009). Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory animals by the National Institutes of Health (USA) and the current laws of the Iranian government for animal care.

Nutrient requirements of broilers based on nutritional requirements proposed by the National Research Council (NRC 1994) were prepared. The basal diets were corn and soybean meal so the different percentages of urea were added to the basal diets. Urea available in the market as nitrogenous fertilizer with 46% nitrogen was used in this study. The experimental diets were shown on table 1 and 2.

Diet total volatile nitrogen

Before was began the experiment and after adding of urea into diets, five sample from each diets were taken then the TVN level was measured (AOAC 1992). The diet was examined by Kjeldahl method by methods for measurement of total volatile nitrogen in the diet. Ten grams of the each sample were obtained and to was placed in the Kjeldahl distillation system, then volatile nitrogen in a glass balloon (to contain Boric acid 2%, methyl red, bromocresol green, was collected and titration with sulfuric acid (0.1 N) for measurement of total volatile nitrogen by mg/100 g of diet.

Blood biochemical parameters

Four chicks (two male and two female) from each group were selected on days 42 and blood samples were collected from the wing vein. All the samples were properly labeled and stored for further studies at -20°C. The plasma biochemical parameters including total protein (TP), glucose, triglyceride (TG), cholesterol, LDL, HDL,

phosphorus, calcium, albumin, globulin, urea and uric acid were determined by Technicon RA-1000 Auto-analyzer (Technicon Instruments Corporation, Tarrytown, New York, USA).

Statistical analysis

The data were analyzed by ANOVA was performed to assess the effects of the different level of TVN in diets and gender, also interaction between TVN level × gender (Snedecor and Cochran, 1989). When the ANOVA indicated significant treatment effects, means were separated using turkey's (Tukey 1949) multiple range test.
The general linear models procedure of the SAS system (SAS, 2003) was applied, fitting the following nonlinear equation:

$$Yij_1 = \mu + Wi + Sj + (WS)\ ij + Eij_1$$

Where μ is the population mean; Wi is the effect of TVN levels (i = 1 to 2); Sj is the effect of gender (j = 1 to 2); (WS) ij and (WSA) ij are the interactions of the main effects; and Eij_1 is the overall error term.

Table 1. Composition and calculated nutrient content of broilers at starter period (7-21 days).

	Treatments				
Ingredients (%)	Control	0.5% urea	1% urea	1.5% urea	2% urea
Corn	54.66	58.12	62.68	66.32	70.13
Soybean meal (44% CP)	38.00	33.54	28.78	24.30	19.73
Soybean oil	3.47	3.39	2.55	2.35	2.05
Urea	0.00	0.50	1.00	1.50	2.00
Di calcium phosphate (DCP)	1.50	1.61	1.66	1.75	1.87
$CaCO_3$	1.35	1.34	1.35	1.34	1.25
Common salt	0.30	0.30	0.30	0.30	0.30
Vitamin premix [a]	0.25	0.25	0.25	0.25	0.25
Mineral premix [b]	0.25	0.25	0.25	0.25	0.25
DL- Methionine	0.12	0.17	0.20	0.23	0.25
L-Lysine mono hydro chloride	0.11	0.25	0.43	0.59	0.78
L-Threonine	0.00	0.08	0.17	0.24	0.32
K_2So_4	0.00	0.20	0.38	0.58	0.82
Calculated Analysis					
ME (Kcal/kg)	3000	3000	3000	3000	3000
Crud Protein (%)	21.56	21.56	21.56	21.56	21.56
Calcium (%)	0.97	0.97	0.97	0.97	0.97
Available Phosphorus (%)	0.44	0.44	0.44	0.44	0.44
Sodium (%)	0.14	0.14	0.14	0.14	0.14
Potassium (%)	0.93	0.93	0.93	0.93	0.93
Chlorine (%)	0.22	0.22	0.22	0.22	0.22
Lysine (%)	1.35	1.35	1.35	1.35	1.35
Methionine (%)	0.48	0.48	0.48	0.48	0.48
Met + Cys (%)	0.84	0.84	0.82	0.80	0.77
Threonine (%)	0.89	0.89	0.89	0.89	0.89
Tryptophan (%)	0.31	0.28	0.25	0.22	0.19
TVN (mg/100 g)	13.3	14.95	17.26	23.26	24.47
Cation-Anion balance (meq/kg)	237.35	237.35	237.35	237.35	237.35

[a] Provided the following per kilogram of diet: vitamin A, 9000 IU; vitamin D_3, 2000 IU; vitamin E, 18 IU; vitamin K_3, 2 mg; riboflavin, 6.6 mg; pantothenic acid, 9.8 mg; niacin, 29.7 mg; vitamin B_{12}, 0.015 mg; biotin, 0.1 mg; folic acid, 1 mg; pyridoxine, 2.94 mg; thiamin, 1.75 mg; Choline chloride, 250 mg; Anti-oxidant, 1 mg.
[b] Provided the following per kilogram of diet: Mn, 99.2 mg; Fe, 50 mg; Zn, 84.7 mg; Cu, 10 mg; I, 0.99 mg; Se, 0.2 mg; Choline chloride, 250 mg.

Table 2. Composition and calculated nutrient content of broilers at grower period (22-42 days).

	Treatments				
Ingredient (%)	Control	0.5% urea	1% urea	1.5% urea	2% urea
Corn	59.42	63.5	67.58	71.78	75.65
Soybean meal (44% CP)	32.60	28	23.42	18.76	14.23
Soybean oil	4.41	3.90	3.40	2.83	2.49
Urea	0.00	0.50	1.00	1.50	2.00
Di calcium phosphate (DCP)	1.23	1.29	1.34	1.40	1.41
CaCO3	1.29	1.29	1.30	1.30	1.29
Common salt	0.30	0.30	0.30	0.30	0.30
Vitamin premix [a]	0.25	0.25	0.25	0.25	0.25
Mineral premix [b]	0.25	0.25	0.25	0.25	0.25
DL- Methionine	0.10	0.12	0.14	0.17	0.20
L-Lysine mono hydro chloride	0.13	0.30	0.47	0.64	0.81
L-Threonine	0.00	0.08	0.15	0.23	0.31
K2So4	0.00	0.20	0.38	0.57	0.79
Salinomycin	0.02	0.02	0.02	0.02	0.02
Calculated Analysis					
ME (Kcal/kg)	3120	3120	3120	3120	3120
Crud Protein (%)	19.61	19.61	19.61	19.61	19.61
Calcium (%)	0.87	0.87	0.87	0.87	0.87
Available Phosphorus (%)	0.37	0.37	0.37	0.37	0.37
Sodium (%)	0.14	0.14	0.14	0.14	0.14
Potassium (%)	0.83	0.83	0.83	0.83	0.83
Chlorine (%)	0.22	0.22	0.22	0.22	0.22
Lysine (%)	1.22	1.22	1.22	1.22	1.22
Methionine (%)	0.42	0.42	0.42	0.42	0.42
Met + Cys (%)	0.77	0.74	0.71	0.69	0.66
Threonine (%)	0.79	0.79	0.79	0.79	0.79
Tryptophan (%)	0.25	0.23	0.21	0.18	0.15
TVN (mg/100 g)	14.66	15.02	17.81	24.66	26.25
Cation-Anion balance (meq/kg)	211.71	211.71	211.71	211.71	211.71

[a] Provided the following per kilogram of diet: vitamin A, 9000 IU; vitamin D_3, 2000 IU; vitamin E, 18 IU; vitamin K_3, 2 mg; riboflavin, 6.6 mg; pantothenic acid, 9.8 mg; niacin, 29.7 mg; vitamin B_{12}, 0.015 mg; biotin, 0.1 mg; folic acid, 1 mg; pyridoxine, 2.94 mg; thiamin, 1.75 mg; Choline chloride, 250 mg; Anti-oxidant, 1 mg.
[b] Provided the following per kilogram of diet: Mn, 99.2 mg; Fe, 50 mg; Zn, 84.7 mg; Cu, 10 mg; I, 0.99 mg; Se, 0.2 mg; Choline chloride, 250 mg.

Table 3. The effects of different levels of diet total volatile nitrogen blood biochemical parameters in broiler chickens at 42 day of age.

TVN levels (TVN mg/100 g)	Gender	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Ca (mg/dl)	P (mg/dl)
Total Volatile Nitrogen (TVN mg/100 g)													
14.66 (TVN mg/100 g)		213.00^{a}	133.68^{ab}	23.62^{c}	88.56	33.31^{c}	17.31^{c}	4.21^{b}	6.00^{b}	3.60	2.40^{b}	7.31	5.59^{ab}
15.02 (TVN mg/100 g)		207.37^{ab}	121.12^{b}	26.81^{bc}	82.43	35.12^{bc}	26.56^{bc}	4.26^{b}	6.15^{ab}	3.64	2.51^{ab}	7.65	5.13^{b}
17.81 (TVN mg/100 g)		205.25^{ab}	130.75^{ab}	25.70^{bc}	85.50	42.31^{ab}	33.43^{b}	3.58^{b}	6.26^{ab}	3.57	2.69^{ab}	7.61	5.05^{b}
24.66 (TVN mg/100 g)		197.06^{ab}	129.87^{ab}	31.43^{ab}	85.00	37.00^{bc}	59.18^{a}	6.74^{a}	6.39^{ab}	3.36	3.03^{ab}	8.01	5.18^{b}
26.25 (TVN mg/100 g)		194.93^{b}	141.37^{a}	32.18^{a}	83.68	47.68^{a}	67.00^{a}	8.12^{a}	6.67^{a}	3.50	3.16^{a}	7.83	6.03^{a}
SEM		4.02	4.24	2.86	2.00	2.40	0.59	2.00	0.13	0.10	0.18	0.24	0.20
	Female	204.42	123.25^{b}	27.77	82.87	36.17^{b}	40.92	5.33	6.27	3.48	2.79	7.71	5.51
	Male	202.62	193.47^{a}	28.50	87.20	42.00^{a}	40.47	5.55	6.31	3.55	2.76	7.66	5.28
SEM		2.54	2.68	0.97	1.81	1.26	1.40	0.37	0.08	0.06	0.11	0.15	0.12
Total Volatile Nitrogen (TVN mg/100 g) × Gender													
14.66 (TVN mg/100 g)	Female	209.50	126.12	24.50	83.37	28.62	17.25	4.30	6.21	3.78	2.43	7.63	5.65
14.66 (TVN mg/100 g)	Male	216.50	141.25	22.75	92.75	38.00	17.37	4.13	5.78	3.42	2.36	6.98	5.53
15.02 (TVN mg/100 g)	Female	205.12	110.50	23.87	84.37	30.62	24.62	3.48	5.85	3.43	2.41	7.62	5.31
15.02 (TVN mg/100 g)	Male	209.62	131.75	29.75	80.50	39.62	28.50	5.05	6.45	3.67	2.77	7.69	4.95
17.81 (TVN mg/100 g)	Female	207.25	128.00	26.12	83.75	41.37	31.75	3.42	6.20	3.53	2.66	7.74	5.31
17.81 (TVN mg/100 g)	Male	203.25	133.50	25.37	87.25	43.25	35.12	4.27	6.31	3.60	2.71	7.49	4.78
24.66 (TVN mg/100 g)	Female	194.25	120.37	33.25	77.87	34.12	60.75	6.88	6.54	3.19	3.35	7.94	5.10
24.66 (TVN mg/100 g)	Male	199.87	139.37	29.62	92.12	39.87	57.62	6.60	6.23	3.52	2.70	8.08	5.27
26.25 (TVN mg/100 g)	Female	199.00	131.25	31.12	84.00	46.12	70.25	8.56	6.55	3.48	3.07	7.60	6.18
26.25 (TVN mg/100 g)	Male	190.87	151.50	31.12	83.37	49.26	63.75	7.68	6.79	3.53	3.26	8.06	5.87
SEM		5.70	6.14	2.76	4.06	2.82	3.14	0.71	0.19	0.14	0.25	0.35	0.28
Probability													
Total Volatile Nitrogen		0.0126	0.0004	0.0040	0.6320	0.0001	0.0001	0.0001	0.0109	0.5156	0.0229	0.3516	0.0044
Gender		0.6180	0.0001	0.7860	0.0962	0.0017	0.8350	0.6860	0.7230	0.4744	0.8551	0.8340	0.2110
TVN level × Gender		0.6330	0.7020	0.2270	0.1902	0.1902	0.5070	0.6210	0.6210	0.1757	0.3563	0.5720	0.7750

There are significant differences between groups with different codes (a, b, c) in a column ($P < 0.05$).

RESULTS

The effects of different levels of diet total volatile nitrogen on glucose, triglyceride (TG), cholesterol, LDL, HDL, phosphorus, calcium, total protein (TP), albumin, globulin, urea and uric acid in broiler chickens were shown on table 3.

This study results showed that the use of high TVN levels in the diet linearly reduced the blood glucose level compared to control group ($P<0.05$). In addition, the use of TVN in diets higher than 15.02 mg per 100 g decreased the blood glucose level in broiler chickens at the end of experiment period ($P<0.05$).

The blood triglyceride, cholesterol and LDL levels after the end of the experiment were significantly higher in birds fed high level TVN compared to control group ($P<0.05$). Whereas, the blood cholesterol level was significantly lower in 15.02 mg per 100 g of TVN in the diet compared to control group and other groups ($P<0.05$). The results of this experiment showed that by increasing the TVN in the diet the blood triglyceride, cholesterol and LDL level linearly increased ($P<0.05$).

The results indicated that by increasing the TVN levels in the diet, the blood uric acid and urea levels linearly increased ($P<0.05$). So that, higher levels of TVN than 24.66 mg per 100 g increased blood urea and uric acid levels compared to other groups ($P<0.05$). In addition, the blood phosphorus level was found significantly different between treatments and control group ($P<0.05$). Therefore, the blood phosphors level was significantly increased by the use of high level of TVN ($P<0.05$). Also, the higher blood phosphorus level was shown in 26.25 mg per 100 g of TVN group than other groups and control group ($P<0.05$). On the other hand, TVN level in diet did not affect the blood calcium level.

The result showed that TVN levels higher than 26.25 mg per 100 g in the diet was significantly increased the blood total protein and globulins ($P<0.05$). Also, the utilization of different level of TVN in the diet not affect the blood HDL and albumin levels in broilers ($P>0.05$). Finally, the gender had a significant effect on blood cholesterol and LDL levels ($P<0.05$).

DISCUSSION

In the present study, the result showed that the increasing TVN more than 15.02 mg per 100 g in the diet was decreased the blood glucose level in broilers. In similar cases, Abdou *et al*. (2006) and Den (1986) observed that the supplementation of high level urea in diet significantly reduced blood glucose level in broiler chicken.

In this study, the reduction of blood glucose level by high level TVN in diet can be related to intestinal malabsorption or kidney malreabsorption of glucose (Abdou *et al*. 2006). On the other hand, the hypoglycemia was ascribed carbon skeletal for syntheses of non-essential amino acids in diets supplemented with NPN in diet. So, intestinal and renal histological changed shown in broiler chickens can substantiate this suggestion (Abdou *et al*. 2006).

In contrast with our result, Nagalakashmi *et al.* (1999) showed that the use of low level urea in diet had lower blood cholesterol level compared to control group, which this may relate to low level urea in diet can utilization of cholesterol as an energy source for various items such as amino acid metabolism. Therefore, the low level urea in diet can be reduction of cholesterol synthesis and cholesterol esterase activities in the liver had related to low cholesterol level in blood.

Also, the high concentration of blood triglyceride, cholesterol and LDL in this study could be related to the high uric acid level in body because the high uric acid have toxic effects in broilers. Javed *et al*. (1995) reported that the higher level urea in diet showed deleterious effects on blood parameters of broilers. Therefore, the high uric acid showed negative effects on liver and kidney damage. Therefore, the increased of blood triglyceride, cholesterol, LDL levels can be due to the increased of uric acid concentration (Javed *et al*. 1995). On the other hand, Fischbach (2004) indicated that fatty liver and kidney had directly effects on blood lipid concentration and also reported that Liver and kidney damage had deleterious effects in blood cholesterol and triglyceride level. Cambell and Coles (1986), Den (1986) indicated that using of high level urea in diets increased blood cholesterol, triglyceride and LDL level due to liver damage.

Supplementation of high level urea in diets had linearly effect on some blood enzyme, in similar cases, Coles (1986) and Thomson (1984) indicated that the use of high level urea in diet had increased blood ALP and LDH. The high blood level of these enzymes indicated organs damage due to the high level of uric acid in body. The blood urea and uric acid level were obviously higher in this study. Our result was agreement with finding of Abdoo *et al*. (2006), Rahman and Ankari (2006) and Woerpel and Rosskopf (1984) who observed high blood uric acid level in high level of urea in diet. Kidney and liver damaged, demonstrated here was known to be dependent on increased blood levels of urea and uric acid (Chaudhari and Kaul 1996). In similar cases, Chendra *et al*. (1984a), Karasawa *et al*. (1994) showed high blood urea level as a result of using high level of urea in diet.

The recorded difference in the increase of uric acid (hyperuricaemia) was probably the result of increased biosynthesis or decreased renal excretion of uric acid as suggested by (Chandra *et al.* 1984c).

In the presence of increased blood urea concentration, unusual catabolic activities can appear (Sykes 1971). Also the high level of uric acid in this study can be related to the increase of urea level in diet.

The blood total protein and globulins level by using the high level TVN in the diet were increased. In similar case, Javed *et al*. (2002) indicated that the blood globulin was higher with supplementation of urea in diet. In contrast with this study, Chandra *et al*. (1984a) observed no significant difference in blood total protein level by using of differences levels of urea in diets. In this study, total protein showed significantly higher by using high level of TVN in the diet. Nworgu *et al*. (2007) reported that supplemented processed fish waste was adequate enough to meet the normal protein requirements of broiler chicken. This increase may be related to making more amino acid and consequently protein due to increased density of amino caused by consumed urea dissolution (Nagalakashmi *et al*. 1999). In the present study, the high blood globulin level was recorded in urea-exposed birds. It seems that increased the blood globulin level in this study can be related to liver damage due to high uric acid in body (Burtis and Ashwood 1994). But the result showed that TVN in diet had not significantly affect to blood albumin level in broiler chickens. In this experiment, the blood phosphorus level increased with the addition of urea in the diet. The different cases, Singh and Ray (1982) observed that the blood phosphorus level was higher than control group. Rahman and ankari (2006) reported that blood phosphorus level was higher in 2.5 percent urea groups compared to low level urea groups. In addition, the results of blood phosphorus showed significantly lower in birds fed 17.80 mg per 100 g of TVN in the diet. Lierz (2003) concluded that, the use of high level urea in diet causes kidney damage and damage of kidney causes changes in the calcium and phosphorus metabolism in the body and thereby decreases the bone ash. Gou (1983) reported that, use of high level urea in the diet caused leg weakness in laying hens. Moreover, kidney disease which can lead to reduced clearance of phosphate, caused to increase phosphate in blood.

It seems that, the reduction of kidney glomerular filtration can be seen or considered as a reason for increasing of plasma phosphors level. Increased blood phosphors level of this experiment can be related to the increase of uric acid (Den 1986).

CONCLUSION

The results obtained from present study indicated that using high level total volatile nitrogen in diet had serious effect on some blood biochemical parameters.

ACKNOWLEDGEMENTS

This article is a part of M.Sc. thesis in Poultry Production and Management, Islamic Azad University, Shabestar Branch (thesis supervisors: Dr. Y. Ebrahimnezhad). The authors are grateful to Amin Ghasemi-Sadabadi for his valuable assistance. Also we would like to acknowledge Mohammadreza khodai and Hasan Mohammadian for their helps.

REFERENCES

Abdou KA, Mubarak M, and Sharkawy AA (2006). Toxo-pathological effects induced by urea in broiler chicks. Bs Veterinary Medicine Journal, 16: 75-84.

Anderson WAD (1971). Mosby international edition of pathology, 6th ed. Vol., pp. 1178-1190.

AOAC (1992). Official methods of analysis (15th Ed.). Association of Official Analytical Chemists, Inc Arlington Virginia USA.

Ariyawansa S (2000). The evaluation of functional properties of fish meal. Final Project. Icelandic Fisheries Laboratories. 25pp.

Banwart GJ (1981). Basic food microbiology. Avi Publishing Company Inc., Westport, CT.

Brukental I, and Nitsan Z (1981). Effect of urea on growth, food utilization and body consumption of chicks. British Poultry Science, 22: 115-121.

Burtis CA and Ashwood ER (1994). Rietz textbook of clinical chemistry. 2nd ed. W. B. Saunders Co. Philadelphia. Pp: 735-888, 1354-1375.

Cambell TW, Coles EH (1986). Avian clinical pathology. In: Veterinary clinical pathology. Edited by E. H. Coles. 4th ed. W. B. Saunders Co. Philadelphia.

Chandra M, Sigh B, Soni GL, and Ahuja SP (1984a). Renal and biochemical Hematological changes in nephritis induced by diets high in protein, high calcium, urea-containing and vitamin A deficient diet. Avian Diseases, 28: 1-11.

Chandra M, Singh S, Gupta PP, and Ahuja SP (1984b). Comparative pathogenesis of nephritis in poultry induced by high protein, high calcium, urea and vitamin A deficient diet. Acta Veterinaria, 34: 113-134.

Chandra M, Singh B, Singh N, and Ahuja SP (1984c). Hematological changes in nephritis induced by diets high in protein, high calcium containing urea or deficient in vitamin A. Poultry Science. 63:710-716.

Chandra M, Singh B, Gupla PP, and Ahuja SP (1983). Hematological changes in nephritis in poultry induced by diet high in protein, high in calcium containing urea or deficient in vitamin A. Poultry Science. 63: 710-716.

Chaudhari GR, and Kaul L (1996). Effect of castor meal feeding on serum and uric acid concentration in white Leghorn cockerels. Indian journal environmental toxicology, 1: 45-47.
Coles EH (1986). Veterinary clinical pathology. 4th end, W.B. Saunders Comnany, Philadelphia, USA.
Davis RH, and Martindale H (1973). The performance of laying hens fed on rearing and laying diets containing urea. British Poultry Science, 14: 153-160.
Den FJ (1986) in: clinical avian medicine and surgery. Philadelphia. Pp.: 174-191.
Egan H, Kirk RS, and Sawyer R, 1981. Pearson's chemical analysis of foods, 8th Edn, Churchill Livingstone Edinburgh.
Featherston WR, Bird HR, and AE Harper, (1962). Ability of the chicks to utilize D- and excess L-indispensable amino acid nitrogen in the synthesis of dispensable amino acids. Journal Nutrition, 78: 95-100.
Fischbach F, (2004). A manual of laboratory and diagnostic test. 8ed, Lippincott Williams and Wilkins. Philadelphia. USA.
Gou XD (1983). Preliminary observation on urea poisoning in chickens, Chinese journal of veterinary medicine, 9: 36-37.
Itoh H, Koike H, and Kobayashi S (1979). Effect of dietary urea on blood levels of non protein nitrogenous compounds in chicks. *Japanese journal of zoo technical science*, 49(12): 880-888.
Javed MT, Sarwar MA. Kausar R, and Ahmad I (2002). Effect of feeding different levels of formalin (37% formaldehyde) and urea on broiler health and performance. Veterinary archives, 72: 285-302.
Javed MT, Pervaz S, Sabri MA, Khan HA, Chatha ZA, and Younis M, 1995. Studies on body weight, gross pathology and some serum enzymes of urea induced toxicity in broiler chicks. Pakistan Veterinary Journal, 15: 109-112.
Karasawa Y, Ono T, and Koh K (1994). Inhibitory effect of penicillin on caecal urease activity in chicken fed on a low protein diet plus urea. British Poultry science, 35: 151- 160.
Kazemi R, and Balloun SL (1973). Urea and diammonium citrate for laying hens. Poultry Science, 52: 44-50.
Kobayashi S, Koike H, and Itoh H (1981). Effect of dietary urea on nitrogen excretion in cockerels (in Japanese). Japanese Poultry Science, 18: 78-85.
Lee DJW, and Blaire R, 1972. Effect on chick growth of adding various non-protein nitrogen sources of dried autoclaved poultry manure to diet containing crystalline essential amino acid. British Poultry Science, 13: 243-249.
Lierz M. (2003). Avian renal disease: pathogenesis, diagnosis, and therapy. *Veterinary* Clinics of North America*:* Exotic Animal Practice, 6: 29–55.
Murray RK, Granner DK, Mayes PA, and Rodewell VM (1988). Harpers biochemistry. 21st edition applcton and large. California. Pp.271-272.
National Research Council (1994). Nutrient requirements of poultry. 9th edition National Academy Press Washington, D. C., U.S.A.
Nagalakashmi D, Sastry VR, Katiyar RC, Agrawal DK, and Verman SV (1999). Performance of broiler chicks fed on diets containing urea ammoniated *neam cazadirachta indica* kernel cake. British Poultry Science 40:77-83.
Nolan JV, and Leng RA (1972). Dynamic of ammonia and urea metabolism in sheep. British Journal Nutrition, 27: 177-194.
Nworgu FC, Ogungbenro SA, and Solesi KS (2007). Performance and some blood chemistry indices of broiler chicken served fluted pumpkins (*Telfaria accidentalis*) leaves extract supplement. American-Eurasian Journal of Agricultural & Environmental Sciences, 2: 90-98.
Okumura J, and Kino K (1984). Growth promoting effect of dietary urea and diammonium citrate in the chick. Journal Poultry Science, 21: 49-56.
Pervaz, S., 1994. Hematological and enzymological studies of urea induced toxicity in broiler chicks. M. Sc. (Hons.) thesis, Department Veterinary Pathology, University of Agriculture, Faisalabad.
Pervaz S, Javed MT, Sabri MA, and Pervaiz S, 1996. Hematological and biochemical findings in broilers feed different levels of urea. Pakistan Veterinary Journal, 16: 75-77.
Rahman, AS and Ankari A (2006). Association between serum biochemistry of leghorn chickens and changes in renal tissues induced by high calcium and high urea diets. International Journal of Poultry Science, 5 (10): 992-995.
Sahraei M, Ghanbari A, and Lootfollahian H (2012). Effects of inclusion of poultry slaughterhouse by product meals in diet on performance, serum uric acid and carcass traits of broilers. Global Veterinarian, 8 (3): 270-275.
SAS Institute, (2003). SAS Users guide: Statistics. Version 6.12. SAS Institute Inc., Cary, NC.
Shahzad NM, Javed MT, Shabir S, Irfan M, and Hussain R (2012). Effect of feeding urea and copper sulphate in different combinations on live body weight, carcass weight, percent weight to body weight of different organs and histopathological tissue changes in broilers. Experimental and Toxicological Pathology, 64: 141-147.
Snedecor GW, and Cochran WG (1989). Statistical Methods, 8th edn. Iowa State University Press, Ames, Iowa, USA.
Singh C, and Roy AK (1982). Studies on the utilization of different levels of protein and non-protein nitrogenous substance related to proteins in hens. Indian Veterinary Journal, 59: 349-351.
Sykes AH (1960). The renal clearance of uric acid and p-amino hippurate in the fowl. Research Veterinarian Science, 1: 308-310.
Thomson RG (1984). General Vet. Pathol. W B. Saunders Co., Philadelphia.
Tukey JW (1949). Comparing individual means in the analysis of variance. Biometrics, 5: 99-114.
Woerpel WR, and Rosskopf W (1984). Clinical experiences with avian laboratory diagnostics. Veterinary Clinics of North America: Small Animal Practice, 14(2): 249–86.