The effect of different levels of diet total volatile nitrogen on hematological parameters in broiler chickens

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Abstract

This study was conducted to determine the effects of different levels of diet total volatile nitrogen on hematological parameters in broiler chickens. A total of 400 one day old, male and female, from the Ross 308 broiler chicks, were randomly allocated to one of the 25 floor pens in a completely randomized design with five treatments and five replicate groups and sixteen chicks in each group (eight males and eight females). The aim of using different levels of urea in this study due to created various levels of total volatile nitrogen in the diet were formulated by adding different amounts of urea to the basal diet different levels of total volatile nitrogen in the diet. Dietary treatments consisted of zero (control), 0.5, 1, 1.5 and 2 percentages of urea in the diets. Before starting the experiment, the TVN levels in all diets were measured after adding different levels of urea and TVN levels were 13.30, 14.95, 17.26, 23.26 and 27.47 mg per 100 g and 16.66, 15.02, 17.81, 24.66 and 26.25 TVN mg per 100 g in starter and grower diets, respectively. At 42 days of age, two males and two females from each replicate were selected and the blood samples were collected from taking wing vein. Total leukocyte counts, total erythrocyte count, packed cell volume (PCV), hemoglobin, monocytes, lymphocytes, eosinophils and heterophils were measured. In addition, increasing TVN in the broiler diets affected on leukocyte counts, erythrocytes count, PCV, hemoglobin, lymphocytes and heterophils count ($P<0.05$). So that increasing urea in diet higher than 0.5% (15 mg per 100 g TVN in diet) had linearly reduced, leucocytes and erythrocytes count and PCV at the end of experiment period ($P<0.05$). Also the regression charts shown that the linearly correlation was between TVN and hematological parameters in this study. In addition, gender was significantly effective on PCV percentage ($P<0.05$). Therefore, from the results this research concluded that, increasing urea in diet with more than 0.5% (15 mg per 100 g TVN in diet) has destructive effects on hematological parameters in broiler chickens.

Keywords: Total volatile nitrogen, urea, hematological parameter, broiler chickens

Introduction

In the poultry nutrition, emphasis has been mostly focused on the quality of diet. Total volatile nitrogen (TVN) is a good parameter for studying to the use of none protein nitrogen as fish meal, urea and meat meal in diets (Ruiter, 1995). The most essential principle in the poultry industry is offering a suitable and balanced diet. Among these proteins, plant or animal, considering that the effect is important for poultry performance is concerned. Nowadays the measurements of protein in the bird diets are common, but the nutrient requirements based on ideal amino acid pattern of the essential amino acids have been determined. The TVN is a suitable criterion for determining the quality of products, especially protein components (Ariyawansa, 2000). The TVN compounds containing nitrogen volatile rate indicator was shown in the food. In addition, the TVN is an index used to indicate the compound rate having volatile nitrogen in food stuffs. TVN count of fish powder is at range 20-100 mg per kilogram of in raw sample (Kerr et al., 2002). Replacing of the non-protein nitrogen (NPN) in the diet leads to food cost reduce. However, the use of NPN and unnecessary nitrogen sources has undesirable effects on the birds' health (Pervez et al., 1996). In non-ruminants diets, some researchers have suggested that urea cannot be utilized and that it has no nutritional advantage for poultry (Kobayashi et al., 1981). The toxicity of urea is related to the release of the sufficient amount of ammonia (Pervez et al., 1996; Javed et al., 1995; Javed et al., 2002). Fallah et al. (2016) suggested that increasing diet TVN to more than 15 mg 100 g^1 reduces performance traits of broiler chickens. As well as Ghasemi-Sadabadi et al. (2015) showed that use of high level TVN in diet had serious effects on some blood biochemical parameters. In addition, Pervaz (1994) studied the hematological and enzymological changes produced by urea feeding and he showed that serum enzymes were significantly higher in chicks fed with one percent urea. The hematological studies revealed normocytic and normochromic anemia characterized by an increase in hemoglobin (Hb) concentration, packed cell volume (PCV), white blood cell (WBC) and red blood cell (RBC) sedimentation rate. The histological findings were described with reference to uric acid granulation and calcinosis. Decreasing WBCs, Hb, PCV in this experiment contradicted with their findings. Also, Nagalakshmi et al. (1999) reported that the urea concentrations increased in blood when the concentration of urea increased in the diet. Javed et al. (2002) showed that the concentrations of serum total proteins, albumin and fibrinogen exhibited an insignificant variation between treatment and the control groups. The use of urea at higher levels has shown deleterious effects on broilers including poor growth rate, altered blood profile and untoward changes different tissues of the body (Javed et al., 1995). The toxicity of high level of NPN occurs due to the effects of ammonia released in body (Jabber, 1994). Hence, blood hematological changes appear in the form of increased RBC, Hb, and PCV and RBCs sedimentation rate. In addition, the use of high level of urea showed lower level of WBC count (Chandra et al., 1984a; Chandra et al., 1984b). However, toxicity can occur when chicks are kept on urea treated feed. Others suggested that urea may replace some non-essential amino acids in diet of non-ruminants (Suciu et al., 1990). The use of urea in diet has effect on blood biochemical parameter. Therefore, Pervaz indicated significantly increase in serum enzymes with the use of high level in diet (Pervaz, 1994). Due to this fact, nowadays, fish meal, meat meal, PBPM and NPN sources such as urea are one of important factor to reduce the cost of feed used, so this experiment was carried out to propose study the effects of different level of diet TVN on blood hematological parameters in broiler chickens.

Materials and methods

Animals and management and nutrient

This study was carried out at the Poultry Research Farm of Islamic Azad University-Shabestar Branch in Iran to determine the effects of diet TVN level on blood hematological parameters in broiler chickens at 2016. A total of 400 one day old, male and female, from the Ross 308 strain broiler chicken, were randomly allocated to 25 floor pens in a completely randomized design with five treatments, five replicates and sixteen chicks in each replicate (eight male and eight female). The chicks were fed without adding urea during the first week of the experiment. All chicks in the treatment groups were fed on a starter diet (1-21 days) and grower diet from (22-42 days) throughout the experimental period which is lasted for 42 days. The chicks were divided into 25 groups. The broiler chickens were breeding within floor pens and the dimension of each pens were 1×2 m^2. All the chicks were kept under similar management conditions according to Ross 308 strain catalogue. 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. The feed and water were available *ad libitum*. The basal diets were corn and soybean meal so different percentages of urea were added on dry weight of the basal diet at zero (control), 0.5, 1, 1.5 and 2%. After adding urea into diets, the TVN level was analyzed and the diets were similarly formulated in all treatments according to Nutrients Recommendations Council (NRC, 1994). The experimental diets were shown in table 1 and 2. Urea available in the market as nitrogenous fertilizer with 46% nitrogen was used in this study.

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

Ingredients (%)	Treatments				
	Control	urea 0.5%	urea 1%	urea 1.5%	urea 2%
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
Dicalcium 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
Cation-anion balance	237.35	237.35	237.35	237.35	237.35
Diet TVN Analysis (mg/100 g)	13.3	14.95	17.26	23.26	24.47

[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; pentothenic 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.

Diet total volatile nitrogen

The TVN analysis was conducted at the food analysis laboratory of Islamic Azad University-Shabestar Branch in Iran. For the TVN in diets analyses, the diets distributed on a laboratory table then the samples were collected from five different parts of each diet as well as the samples obtained from diets were drained into laboratory bags and properly labeled and transferred to the food analysis laboratory.

The diet was examined by Kjeldahl method by methods for measurement of TVN in the diet. Ten grams of the 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/100g of diet (AOAC, 1992). The amounts of TVN in starter diets were 13.30, 14.95, 17.26, 23.26 and 24.47 mg per 100 grams for control group and other treatments, respectively. Also, the amounts of TVN in grower diets were 14.66, 15.02, 17.81, 24.66 and 26.25 mg per 100 grams for control group and other treatments, respectively. The TVN in diets were shown in table 1 and 2.

Hematological parameters

Four chicks (two male and two female) from each group were sacrificed on days 42 by collecting blood samples from taking wing vein. Blood samples obtained from all birds were drained into tubes with anticoagulant (EDTA 1 mg/mL blood). RBC and WBC counts were determined by using a hemocytometer according to Natt et al. (1952) PCV was determined using hematocrit tubes. Cyanmethaemoglobin method as described by Benjamin (1978) was used to estimate Hb.

Statistical Analysis

Significant differences between mean values were separated by the GLM procedure of SAS software (SAS, 2003) and significant differences between treatments were separated using the Tukey range test at (P<0.05). In addition, the linear regression was compared with the results of TVN and hematological parameters in this study.

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

Ingredient (%)	Treatments				
	Control	Urea 0.5%	Urea 1%	Urea 1.5%	Urea 2%
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
Dicalcium 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
Cation-anion balance	211.71	211.71	211.71	211.71	211.71
Diet TVN Analysis (mg/100 g)	14.66	15.02	17.81	24.66	26.25

[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; pentothenic 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.

[a–c] Averages in a column with different superscript letters are significantly different (P<0.05). ** Heterophils: lymphocyte ratio (H/L ratio)

Results

The effects of different levels of diet TVN on total WBC counts, total RBCs count, PCV, Hb, monocytes, lymphocytes, eosinophils and heterophils in broiler chickens were shown in table 3.

The results indicated that by increasing the TVN levels in the diet, the WBCs and RBCs count linearly decreased in the end of experiment period (P<0.05). So that the use of urea higher than 1% urea (17.81 mg per 100 g of TVN) in the diet total WBCs and RBCs count significantly were decreased (P<0.05). In addition, regression analysis showed a highly correlation between TVN level (y) and RBCs ($y = -0.049x + 3.336$, $R^2= 0.95$) and WBCs (x) ($y = -0.266x + 25.79$, $R^2= 0.94$) in this study (figure 1 and 2).

The results of PCV showed a significant difference between treatment and control group (P<0.05). The PCV was significantly decreased by the use of high level of TVN in diet (P<0.05). The lower PCV was shown in 2% urea (26.25 mg per 100 g of TVN) in the diet than other groups and control group (P<0.05). While, the regression analysis between TVN level (y) and PCV (x) ($y= -0.365x + 33.77$, $R^2= 0.92$) were demonstrated high correlation in this study (figure 3).

The Hb concentration after the end of the experiment was significantly lower in birds fed high level TVN compared with control group (P<0.05). The Hb concentration was lower in birds fed 2% urea (26.25 mg per 100 g of TVN) in the diet than other treatment. Also, the regression analysis showed high correlation between TVN level (y) and Hb concentration (x) (y= -0.145x + 10.64, R^2= 0.92) (figure 4).
In this experiment, the lymphocyte and heterophils count significantly decreased with the addition of TVN in the diet (P<0.05). The use of urea higher than 1% (17.81 mg per 100 g of TVN) in the diet was significantly decreased lymphocyte and heterophils counts on broiler chickens (P<0.05). The utilization of different level of TVN in the diet not showed significant effect on eosinophil, monocyte and heterophils: lymphocyte ratio (H/L ratio) in broiler chickens (P<0.05). But, PCV, RBCs, leucocyte, lymphocyte, eosinophils, monocytes counts and heterophils: lymphocyte ratio (H/L ratio) data regarding that the gender and interaction between TVN level×gender showed no significant difference between previously mentioned treatments during the experiment. Therefore, the gender had a significant effect on PCV and heterophils count in experiment (P<0.05). So that the female chickens had higher heterophils counts compared to male broiler chickens. But the PCV was significantly higher in male chickens than female chickens (P<0.05). In addition, according to the linear regression charts in this experiment showed that the linearly correlation was between TVN and heterophils (y = -53.24x + 7045, R^2= 0.89) and lymphocytes (y = -211.9x + 17516, R^2= 0.90), as described by the equations at figures 5 and 6.

Table 3. The effect of diet total volatile nitrogen (TVN) level on hematological parameters of broiler chickens at 42 day of age

Urea level (TVN mg/100 g) in diet	Gender	RBCs ($x10^6$ /µl)	WBCs ($x10^3$ /µl)	PCV (%)	Hb (g/dl)	Heterophils ($x10^3$/µl)	Lymphocytes ($x10^3$/µl)	Eosinophils ($x10^3$/µl)	Monocytes ($x10^3$/µl)	H/L ratio
Control (14.66)		2.63^{a}	22.21^{a}	29.06^{a}	8.83^{a}	6233^{a}	14887^{a}	543.63	500.81	0.41
0.5% (15.02)		2.53^{ab}	21.56^{ab}	28.06^{ab}	8.20^{b}	6281^{a}	14114^{b}	498.94	525.25	0.44
1% (17.81)		2.48^{b}	20.83^{b}	26.602^{bc}	7.93^{b}	6067^{ab}	13317^{c}	583.63	572.13	0.45
1.5% (24.66)		2.18^{c}	19.68^{c}	25.37^{cd}	7.15^{c}	5884^{ab}	12609^{c}	490.44	561.88	0.46
2% (26.25)		1.96^{d}	18.47^{d}	23.87^{d}	6.78^{d}	5525^{b}	11800^{d}	484.56	493.31	0.46
SEM		0.03	0.21	0.48	0.07	174.42	185.38	55.24	67.98	0.015
Gender										
	Female	2.35	20.47	26.15^{b}	7.72	6098^{a}	13237	473.33	481.80	0.46
	Male	2.36	20.63	27.05^{a}	7.83	5897^{b}	13454	567.15	579.55	0.43
SEM		0.01	0.13	0.30	0.04	85.39	117.33	34.96	35.81	0.009
Urea (TVN) in diet × Gender										
Control (14.66)	Female	2.61	21.96	28.62	8.73	6207	14720	464.00	517.12	0.42
Control (14.66)	Male	2.65	22.47	29.50	8.93	6258	15054	623.25	484.50	0.39
0.5% (15.02)	Female	2.49	21.31	27.75	8.08	6327	13724	503.00	500.12	0.46
0.5% (15.02)	Male	2.57	21.81	28.37	8.31	6234	14505	494.87	550.37	0.43
1% (17.81)	Female	2.49	20.71	25.50	7.98	6203	13234	487.50	434.87	0.48
1% (17.81)	Male	2.47	20.95	27.75	7.87	5931	13401	679.75	709.37	0.45
1.5% (24.66)	Female	2.19	19.60	25.00	7.11	5997	12497	441.37	513.75	0.48
1.5% (24.66)	Male	16.1	19.76	25.75	7.18	5770	12722	539.50	610.00	0.45
2% (26.25)	Female	2.01	18.80	23.87	6.70	5757	12010	470.75	443.12	0.48
2% (26.25)	Male	1.91	18.15	23.87	6.86	5293	11590	498.37	543.50	0.45
SEM		0.04	0.30	0.68	0.08	247.40	262.95	69.43	64.77	0.02
P Value										
Urea (TVN) in diet		0.0001	0.0001	0.0001	0.0001	0.0001	0.0210	0.6724	0.8958	0.0531
Gender		0.8161	0.4285	0.0404	0.1119	0.0432	0.2016	0.0617	0.1125	0.0535
Urea (TVN) in diet × Gender		0.3302	0.3063	0.5710	0.5284	0.0514	0.8717	0.6717	0.6062	0.9989

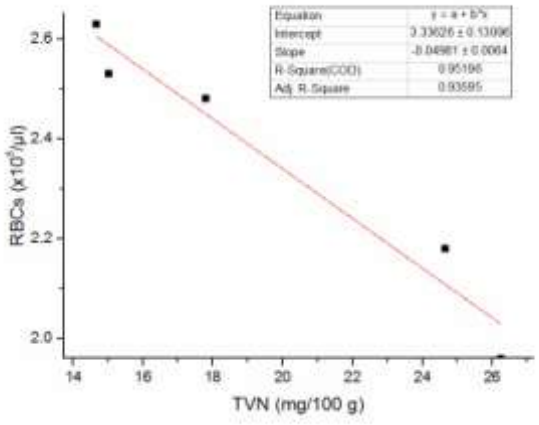

Fig 1: The regression chart of correlation between dietary TVN (mg/100 g) and RBCs (x10^6/µl) in the experiment at 42 days of age.

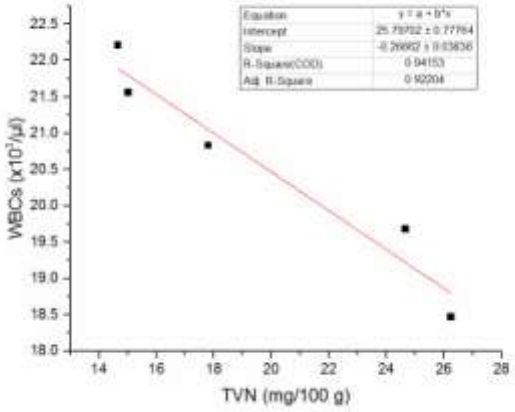

Fig 2: The regression chart of correlation between dietary TVN (mg/100 g) and WBCs (x10^3/µl) in the experiment at 42 days of age.

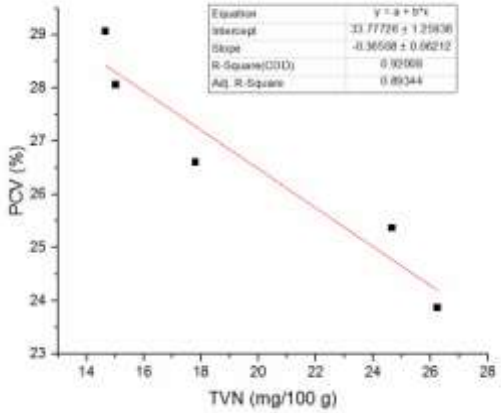

Fig 3: The regression chart of correlation between dietary TVN (mg/100 g) and PCV counts in the experiment at 42 days of age.

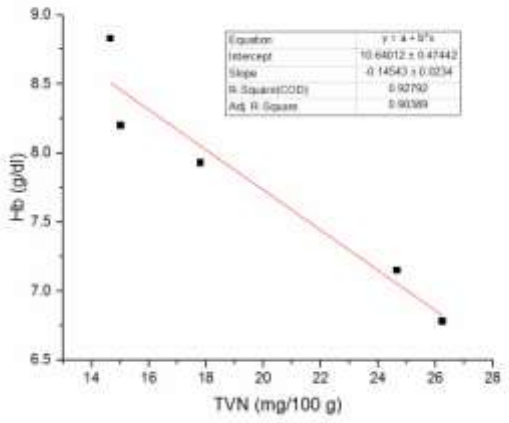

Fig 4: The regression chart of correlation between dietary TVN (mg/100 g) and hemoglobin concentrations (g/dl) in the experiment at 42 days of age.

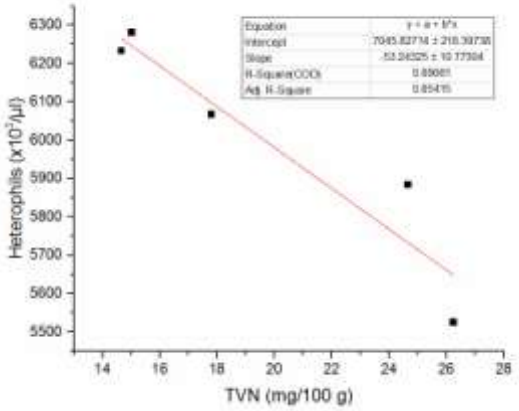

Fig 5: The regression chart of correlation between dietary TVN (mg/100 g) and Heterophils counts in the experiment at 42 days of age.

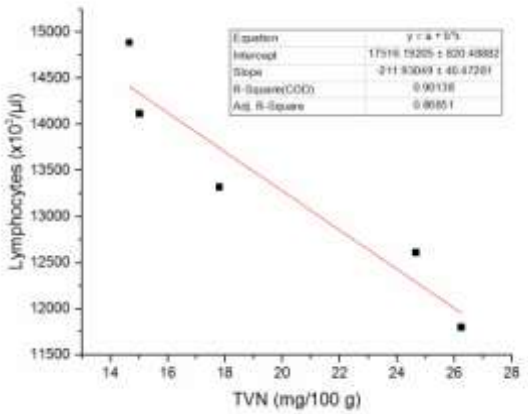

Fig 6: The regression chart of correlation between dietary TVN (mg/100 g) and Lymphocytes counts in the experiment at 42 days of age.

Discussion

In this study leucocyte count was decreased in the use of high level TVN in diets. In similar cases, Abdou et al. (2006) indicated that leucocyte and heterophils count decreased in the birds exposed high level of urea. Also, Pervaz (1994) and Chandra et al. (1983) observed that decrease in total leucocyte count due to lymphopoenia, heterophilia and monocytosis in the birds fed higher level of urea. The WBCs circulate in the blood and carry oxygen throughout the body. They are produced in the bone marrow and then released into the bloodstream as they mature. A relatively stable number of WBCs count is maintained in the circulation by increasing or decreasing the rate of production by the bone marrow. Isikwenu (2012) reported that the use of high level of urea in the diet had decreased leucocyte count in broiler chickens. This lower leucocyte count can relate to depressed haemopoiesis caused by increasing substances such as urea and uric acid (Abdou et al., 2006). Total RBCs count at the end of the experiment was lesser in higher urea than the control group. In addition the use of TVN in the diet with higher than 15.02 mg per 100 g significantly was reduced the total RBCs count. Pervaz (1994) observed decrease in the RBCs count with utilization of one and two percent urea in the diet. Also Pervaz et al. (1996) indicated lowered hematological parameters in urea intoxicated birds and attributed the decreased RBCs count to lowered erythropoietin factor due to renal and hepatic damage. In similar cases, Abdoo et al. (2006) and Rahman and Ankari (2006) observed the significant increase of 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 (Chawdhury et al., 1996). In addition, Ghasemi-Sadabadi et al. (2015) reported that a higher level of TVN in the diet increased blood uric acid and urea levels in broiler chickens. However, finding in this study is agreement with the finding of Chandra et al. (1983). This researcher found a significant increase in total RBCs count in the birds fed high level urea. This difference may be related to different levels of urea in diets in experiment (Chandra et al., 1983). The result in our study showed that the use of high level TVN in diet can decreased Hb concentration and PCV in broiler chickens. It seems that in this experiment, reducing of PCV in relation to loss total RBCs count was due to TVN as a result of the toxic effects of uric acid. The PCV is the proportion of the volume comprised of RBCs in the whole blood (Clark et al., 2009). All hematological parameters measured in the present work, including RBCs count, PCV and Hb concentration was decreased and significant decrements were recorded in high level of TVN. Isikwenu (2012) did not show significant differences among cockerel chicks fed the experimental diets in PCV, Hb content, RBC count, mean corpuscular Hb (MCH) and mean cell Hb concentration (MCHC) values. Pervaz et al. (1996) recorded lowered hematological parameters in urea intoxicated birds and attributed the decreased total RBCs count to lowered erythropoietin factor due to kidney damage. Finding in this study is agreement with the finding of Abdou et al. (2006). This researcher reported that use of high level urea in broiler diet significantly decreased PCV and Hb concentration. The gender was affected the PCV in this experiment. Male chickens were significantly higher in urea-treated broiler than in the female broiler chickens. Pervaz (1994) observed that the use of high level urea decreased the WBC counts, total RBCs, Hb and PCV compared with other group. Isikwenu (2012) reported that the use of urea in broiler diets the similarity in the PCV, Hb, RBCs, MCH and MCHC values shows that urea–treated and fermented BDG diets had no adverse effect on the blood levels, the air exchange or oxygen carrying capacity and caused no anemic conditions in the birds. In addition, Hunsaker et al. (1964) Showed higher PCV to male chicks, this could be due to stimulation of androgen hormones in male chickens. Nirmalan et al. (1972) and Sturkie (1965) reported that changes in RBCs count can have the effect of PCV which is compatible with our study, but the results of interaction between TVN level×gender, did not showed significantly effect to total RBCs count, Hb concentration and PCV in this experiment. The hematological values may vary depending on the reproductive status of the bird. For example a higher PCV was observed in non-reproductive female pigeons (0.54 L/L) compared to those undertaking reproductive activities such courtship, mating, incubation and brooding (0.41-0.45 L/L) (Gayathri and Hedge, 2006). In the present study, the feeding of urea contaminated diets resulted in a decrease in the total heterophils count (per micro liter) compared with the control group. These responses may indicate predictable physiological changes to the bone marrow because RBCs and heterophils in bone marrow are common ancestral source and urea has an inhibitory effect on the cells in the bone marrow (Coles, 1986). The heterophils and lymphocytes counts observed decrease in the present study. This finding is agreement with Coles (1986) results. This researcher indicated that reducing urea levels in diet in line of WBCs in the body. The gender and interaction between TVN level×gender were having a significant effect on heterophils count in experiment. The female broiler chickens had higher heterophils counts in this study. These results are in line with the finding of Nirmalan and Robinson (1972). These researchers showed that the use of estrogens significantly increased the heterophils counts in male chickens. Meyer (1973) indicated that injection of estrogens on birds increased leucocyte count in rooster. By contrast, no differences in the RBCs concentration and Hb concentration were observed between males and females, during this experiment. In addition, mature male common pheasants had a greater PCV than mature females (Dos Santos Schmidt et al., 2007).

Conclusion

The results obtained from present study indicated that using high level TVN in diet had serious effect on hematological parameters in broiler chickens. Consequencely, this study result shows that the use of low level TVN in the diet has no destructive effect on broiler chickens health. Therefore, the results indicated that, increasing urea in diet with more than 0.5% (15 mg per 100 g TVN) has destructive effects on broiler chickens health.

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Conflict of interest

The authors declare no potential conflict of interest.

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