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UREA AND COPPER SULPHATE IN DIFFERENT COMBINATIONS ALTER HAEMATOLOGY AND SOME SERUM PROTEINS IN BROILERS

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A study was carried out to document the use of urea and copper sulphate in different combinations in broilers as both the compounds are invariably present/used in poultry feed in Pakistan. The total erythrocyte count (TEC), packed cell volume (PCV) and haemoglobin (Hb) showed a decrease, while lymphocytes increase in birds fed higher levels of urea and copper after the second week. The monocytes increased in birds fed lower levels of urea and copper alone after withdrawal period. The serum total proteins and globulins decreased ($P<0.05$) in birds fed lower levels of copper alone and when higher levels of both the compounds were used together and these remained lower after the end of the withdrawal period. While albumin decreased in birds fed higher levels of both the compounds after the second week of treatment but returned to non-significant levels during the withdrawal period. The fibrinogen concentration increased in birds fed lower levels of urea and copper together, while decreased in birds fed high levels of both the compounds together after the first week of treatment. At the end of the second week, the fibrinogen decreased in birds fed lower levels of urea and copper alone and in those fed higher levels of both the compounds together. However, at the end of withdrawal period fibrinogen showed a decrease in all treatment groups. It was concluded that the hematological parameters especially those related to RBCs and the serum proteins were affected by the combined use of both the compounds at higher levels (2% urea+1000 mg copper sulphate).
Keywords: copper, urea, poultry, haematology, serum proteins

INTRODUCTION

Poultry meat production is 987 tones and contributes to 28% of the total meat production in Pakistan (Economic Survey of Pakistan, 2013-14). The sector has 1.3% share in the GDP and demonstrated an annual growth rate of 8-10% (Economic Survey of Pakistan, 2013-14). The poultry has shown a considerable increase to supply the poultry meat in local market but it's growth is hampered every now and then with the emergence of diseases. Apart from infectious diseases, toxicants in the feed are responsible for the relatively lower performance of poultry. Poultry farmers use different types of chemicals and drugs haphazardly to achieve more weight from their birds. Due to the indiscriminate use of these substances, toxic effects are discovered in poultry flocks. Urea has been used in poultry feed as an adulterant and its higher levels in the feed are causing toxicity in birds (Shahzad *et al*., 2012; Rasool *et al*., 2012). The toxicity from urea occurs due to the local and generalized effects of sufficient quantities of ammonia released. Higher levels of urea in the feed of broilers have shown untoward effects on the health and growth of broilers and layers (Shahzad *et al*., 2012) and on hematological values including increased total erythrocyte count, haemoglobin (Hb), packed cell volume (PCV) and erythrocyte sedimentation rate (Chandra *et al*., 1984), while otherwise reports have also been published (Pervaz*et al.*, 1996).

Copper sulphate ($CuSO_4$) is commonly used as feed premix to control fungal, bacterial and parasitic diseases (Newberne and Buck, 1957). It has also been employed for the disinfection and to control fungal infections (Biester and Schawarte, 1965). Copper (Cu) is an essential micro-element and is often added to the poultry diets at prophylactic concentrations for its growth promoting effects (Brainer *et al*., 2003). However, an overload of Cu *in vivo* can induce toxicological activities (Shahzad *et al*., 2012). It has been reported that the use of copper as oxide or sulphate above 300 mg per kg of feed causes growth depression (Ahmad *et al*., 2004) and at 400 mg per kg result in lower values of serum total proteins, albumin and globulins (Javed *et al*., 2003).

The use of both of these substances at higher levels causes serious complications to the poultry industry as studied independently and errors in computation and improper mixing of feed can easily result in toxic levels. The data on the combined effects of both these compounds were lacking. Therefore, the present work was planned to investigate the effects of urea and copper sulphate in different combinations through a feed on hematological parameters and serum proteins of broiler chicken.

MATERIALS AND METHODS

A total of 100 one-day old broilers were reared until 47 days of age in a semi-open housing system on the floor. All the birds were kept under identical conditions of feeding and management. The chicks were fed commercial Feed No. 15 (Ani Feeds Private Limited) with 16.9% crude Protein. All the birds were vaccinated against ND, IBD, IB and HPS following the local recommendations. After day 7, the chicks were distributed in a completely randomized experimental design with 6 treatments (Group A = no treatment, Group B = $CuSO_4$ 250 mg Kg^{-1}, Group C = urea 1%, Group D = 1% urea+250 mg $CuSO_4$ Kg^{-1}, Group E = 2% urea+1000 mg $CuSO_4$ Kg^{-1}, Group F = 4% urea+2000 mg $CuSO_4$ Kg^{-1}). The birds were kept in six treatment groups with 15 birds in the control group, 16 in groups B and C, 17 in group D, while 18 in groups E and F. The extra birds kept in groups B to F were to compensate for an expected mortality. The treated feed was fed to the birds from days 8-37. After days 37, the treatment was stopped and plain feed was fed to the birds of all the groups for 8 days (up to 45 days) to document the withdrawal effect of these treatments. Five birds from each group were sacrificed on days 22, 37 and 45 by cutting the jugular vein and blood samples were collected with and without EDTA for blood and serum studies. The studies on blood included haematology, while on serum included estimation of total proteins and fractions. The haematological studies including total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin (Hb), packed cell volume (PCV) and differential leukocyte count (DLC) were carried out. The TEC and TLC was carried out by haemocytometer method, PCV by micro-haematocrit method, haemoglobin by cyanmethaemoglobin method and DLC by Geimsa staining method of blood smears (Benjamin, 1978). Serum total proteins were determined by the Biuret method as described by Oser (1976), serum Albumin was determined by Bromocresol green dye binding method as described by Varley *et al.* (1980), Globulins in the serum were estimated by subtracting the concentration of albumin from the total proteins. Plasma was separated after centrifugation of blood containing anticoagulant for fibrinogen estimation by heat precipitation technique (Schalm method) as described in Benjamin (1978). Data obtained were analysed by the General Linear Model procedure and means were compared with a DMR test by using SAS statistical software (SAS, 2004).

RESULTS

***Haematological studies*:** Among hematological parameters, total erythrocyte count (TEC; in all treatment groups) and haemoglobin (in all treatment groups except one) showed a significant ($P<0.05$) decrease after one week of treatment which was similar for Hb in second week also and for Hb and TEC during the withdrawal period (Table 1). The PCV decreased significantly ($P<0.05$) in birds given higher levels of urea + higher levels of copper sulphate throughout the treatment and withdrawal period. Nonetheless, the TLC, heterophil, eosinophil and basophil counts did not show significant difference between treatment groups and control (Table 1, 2). However, lymphocyte count was significantly ($P<0.05$) higher in birds fed higher levels of both the compounds together after the second week of treatment but not during the withdrawal period. While the monocyte count was significantly higher in birds fed lower levels of urea and copper sulphate alone after the withdrawal period (Table 2).

***Biochemical studies*:** The serum total proteins and globulins showed similar pattern during the treatment and withdrawal periods and these were lower in birds fed lower levels of copper alone and in those fed higher levels of both the compounds together after the second week of treatment (Table 3). The serum proteins and globulins remained lower after the end of the withdrawal period in birds fed both the

Table 2. Effect of urea and copper sulphate alone and in combination on hematological parameter including heterophils, lymphocytes, monocytes, eosinophils and basophils in broiler chicks.

Groups	Days		
	22	37	45
Heterophils (%)			
A(Control)	33.6±5.12	34.8±2.86	33.0±4.00
B ($CuSO_4$ 250 mg Kg^{-1})	37.0±2.00	35.6±3.20	32.5±2.08
C (Urea 1%)	34.0±3.67	32.6±3.64	32.7±1.25
D (1% urea+250 mg $CuSO_4$ Kg^{-1})	36.6±4.03	35.0±3.39	33.0±5.47
E (2% urea+1000 mg $CuSO_4$ Kg^{-1})	33.2±2.77	32.2±2.48	31.3±1.52
F (4% urea+2000 mg $CuSO_4$ Kg^{-1})	34.8±4.81	33.2±3.19	36.0±2.82
Lymphocytes (%)			
A(Control)	51.0±3.31	50.4±1.94	51.6±1.52
B ($CuSO_4$ 250 mg Kg^{-1})	47.4±3.50	46.8±2.58	49.0±2.44
C (Urea 1%)	47.6±1.67	48.8±3.76	48.2±1.89
D (1% urea+250 mg $CuSO_4$ Kg^{-1})	47.6±3.97	48.4±4.03	51.7±7.54
E (2% urea+1000 mg $CuSO_4$ Kg^{-1})	53.2±2.94	54.8±3.19*	56.0±1.00
F (4% urea+2000 mg $CuSO_4$ Kg^{-1})	54.4±2.51	55.2±2.94*	51.0±4.24
Monocytes (%)			
A(Control)	8.4±1.67	8.0±1.87	8.3±2.08
B ($CuSO_4$ 250 mg Kg^{-1})	9.8±1.30	10.4±2.30	12.0±0.81*
C (Urea 1%)	10.4±1.51	12.0±1.41*	12.2±1.25*
D (1% urea+250 mg $CuSO_4$ Kg^{-1})	8.8±1.64	10.4±2.30	9.5±3.00
E (2% urea+1000 mg $CuSO_4$ Kg^{-1})	7.8±1.64	7.6±2.70	8.0±1.00
F (4% urea+2000 mg $CuSO_4$ Kg^{-1})	6.4±1.14	7.2±0.44	7.5±2.12
Eosinophils (%)			
A(Control)	6.2±2.28	6.0±1.00	6.3±1.52
B ($CuSO_4$ 250 mg Kg^{-1})	6.6±3.04	6.2±2.58	5.5±2.51
C (Urea 1%)	7.2±1.30	5.8±1.92	6.0±1.63
D (1% urea+250 mg $CuSO_4$ Kg^{-1})	6.6±2.07	4.8±1.78	5.0±0.81
E (2% urea+1000 mg $CuSO_4$ Kg^{-1})	5.4±1.34	4.2±1.92	4.3±2.08
(4% urea+2000 mg $CuSO_4$ Kg^{-1})	4.0±1.41	3.4±1.14	5.0±4.24
Basophils (%)			
A(Control)	0.80±0.83	0.80±1.09	0.66±0.57
B ($CuSO_4$ 250 mg Kg^{-1})	1.20±0.44	1.00±1.22	1.00±0.81
C (Urea 1%)	0.80±0.83	0.80±0.83	0.75±0.95
D (1% urea+250 mg $CuSO_4$ Kg^{-1})	0.40±0.54	1.40±1.51	0.75±0.50
E (2% urea+1000 mg $CuSO_4$ Kg^{-1})	0.40±0.54	1.20±0.83	0.33±0.57
F (4% urea+2000 mg $CuSO_4$ Kg^{-1})	0.40±0.54	1.00±0.70	0.50±0.70

Figures (Mean±SD) bearing asterisk in a column differ significantly ($P<0.05$) than control group.

Table 3. Effect of urea and copper sulphate alone and in combination on serum/plasma biochemical parameter including serum total proteins, serum albumin, serum globulins and plasma fibrinogen concentrations in broiler chicks.

Groups	Days		
	2	37	45
Total Proteins (g/dl)			
A(Control)	3.89±0.33	4.77±0.32	5.04±0.08
B ($CuSO_4$ 250 mg Kg^{-1})	4.02±0.33	3.79±0.42*	4.29±0.62
C (Urea 1%)	3.93±0.31	4.87±0.44	4.88±0.87
D (1% urea+250 mg $CuSO_4$ Kg^{-1})	4.17±0.19	5.37±1.10	4.80±0.47
E (2% urea+1000 mg $CuSO_4$ Kg^{-1})	3.72±0.09	3.35±0.43*	3.68±0.11*
F (4% urea+2000 mg $CuSO_4$ Kg^{-1})	3.57±0.08	3.33±0.16*	3.56±0.09*
Albumin (g/dl)			
A(Control)	1.16±0.03	1.50±0.15	1.53±0.10
B ($CuSO_4$ 250 mg Kg^{-1})	1.29±0.08	1.30±0.12	1.60±0.21
C (Urea 1%)	1.31±0.07	1.52±0.15	1.56±0.22
D (1% urea+250 mg $CuSO_4$ Kg^{-1})	1.32±0.22*	1.44±0.16	1.55±0.15
E (2% urea+1000 mg $CuSO_4$ Kg^{-1})	1.19±0.04	0.95±0.20*	1.38±0.09
F (4% urea+2000 mg $CuSO_4$ Kg^{-1})	1.20±0.05	0.98±0.20*	1.27±0.01
Globulins (g/dl)			
A(Control)	2.73±0.31	3.27±0.31	3.50±0.03
B ($CuSO_4$ 250 mg Kg^{-1})	2.72±0.39	2.49±0.47*	2.69±0.69
C (Urea 1%)	2.62±0.28	3.35±0.54	3.31±0.67
D (1% urea+250 mg $CuSO_4$ Kg^{-1})	2.84±0.22	3.92±1.01	3.24±0.62
E (2% urea+1000 mg $CuSO_4$ Kg^{-1})	2.52±0.08	2.39±0.31*	2.30±0.12*
F (4% urea+2000 mg $CuSO_4$ Kg^{-1})	2.37±0.08	2.35±0.21*	2.28±0.08*
Fibrinogen (g/dl)			
A(Control)	1.88±0.109	1.60±0.140	2.20±0.200
B ($CuSO_4$ 250 mg Kg^{-1})	1.92±0.178	1.36±0.167*	1.50±0.115*
C (Urea 1%)	2.00±0.141	1.28±0.109*	1.25±0.251*
D (1% urea+250 mg $CuSO_4$ Kg^{-1})	2.12±0.170*	1.68±0.109	1.75±0.190*
E (2% urea+1000 mg $CuSO_4$ Kg^{-1})	1.68±0.109	1.04±0.167*	0.93±0.115*
F (4% urea+2000 mg $CuSO_4$ Kg^{-1})	1.52±0.220*	0.84±0.089*	1.00±0.000*

Figures (Mean±SD) bearing asterisk in a column differ significantly ($P<0.05$) than control group.

compounds together at higher levels (Table 3). While albumin levels were significantly ($P<0.05$) lower in birds fed both the compounds together at higher levels after the end of second week of treatment but returned to non-significant levels during the withdrawal period. The fibrinogen concentration in plasma increased significantly ($P<0.05$) in birds fed lower levels of urea + copper together, while decreased significantly ($P<0.05$) in birds fed very high levels of both the compounds together after one week of treatment. At the end of the second week, the fibrinogen concentration decreased significantly ($P<0.05$) in birds fed lower levels of both urea and copper alone and in those fed higher levels of both the compounds together. At the end of withdrawal period, the fibrinogen concentration was significantly ($P<0.05$) lower in all treatment groups than control (Table 3).

DISCUSSION

There is inevitable presence or use of urea and copper sulphate in the broiler feed in different proportions owing to fish meal adulteration with urea and copper sulphate, being used by broiler farmers in Pakistan to prevent fungal infections and as a growth promoter (Rasool *et al*., 2012). However, the research results were lacking in the use of both these compounds together and their effects on broiler health. Recently, we reported the untoward effects of both the compounds on feed consumption, weight gain, body organs and on some serum biochemical parameters (Shahzad *et al*., 2012; Rasool *et al*., 2012). The effects of both of these compounds were studied in broilers on haematological parameters and serum proteins during present study following the previous combinations used by Shahzad *et al*. (2012). We found decrease in TEC, PCV and Hb in treated birds, especially when higher levels of both the compounds were used together. However, we were unable to record significant differences in TLC, heterophil, eosinophil and basophil counts between treatment and control groups. The

lymphocyte counts were found higher in birds fed higher levels of both the compounds together at 37 days, while monocyte counts were found higher in birds fed 1% urea alone. A lowered erythrocyte count has been reported at 10 days post treatment with 8% urea (Pervaz, 1994). A non-significant difference was seen in total erythrocyte count value than the control when copper was given at 200-400 mg Kg^{-1} feed (Javed *et al*., 2003). So the decreased TEC observed during present study is probably due to the effects of urea rather than copper sulphate. Chandra *et al*. (1984) also found lowered erythrocyte count in urea fed chicks and related the effect with hypoglycemia and the associated lowered metabolic activity responsible for shortening of life span of RBCs. In a previous study, lowered haemoglobin concentration in birds given 8% urea (Pervaz, 1994) or 1-5% urea in feed (Abdou *et al*., 2006) has been reported. However, Nagalakshmi *et al*. (1999) recorded non-significant differences in haemoglobin concentration in chicks being supplemented with urea ammoniated neem kernel cake. The feeding of $CuSO_4$ to the layers resulted in an increase in haemoglobin concentration (Jackson *et al*., 1979) including the use of copper at concentrations 250, 500, 1000 and 2000 mg/kg (Stevenson *et al*., 1980). Pervaz *et al*. (1996) attributed the decreased red blood cell count and Hb concentration to the lowered erythropoietin factor due to renal and hepatic damage. Thus the lowered TEC and Hb in the present study are likely due to the effect of urea, while copper sulphate has otherwise effects as indicated in other studies. Pervaz (1994) and Abdou *et al*. (2006) also observed significant decrease in packed cell volume when higher levels of urea were used and the results of the present study showed that not only urea but when it is combined with the copper sulphate it caused decrease in PCV in broilers. The report of Javed *et al*. (2003) revealed no significant effect on PCV when copper was fed at 200-400 mg Kg^{-1}. Chandra *et al*., (1984) documented that a diet high in protein (42.28 %) and urea (5 %) resulted in an increase in total leukocyte number, while a significant decrease in WBC's count has also been reported in chicks fed on a urea supplemented diet (Abdou *et al*., 2006). It can be inferred from the results of the present study and the others that urea has a depressing effect on the hematological parameters. The results on heterophil count in the present study were different from those of Saqib (1993) who observed a transient depression in heterophil count at 14 days post treatment with 250, 500 and 1000 ppm dietary copper sulphate. He further stated that a significant increase in relative lymphocyte count induced by copper sulphate at 14 days post-treatment was in line with the relative decrease in heterophils (Saqib, 1993). However, the non-significant effect on differential leukocyte counts with a relative increase in lymphocyte and monocyte count suggest no effect of urea on WBCs and there appears to be no inflammatory reaction occurring in the body. This is evident from the findings on histopathological grounds as reported by Shahzad *et al*. (2012) as they also observed no inflammatory reaction in any body tissues.

Serum total proteins, albumin, globulins and fibrinogen showed decrease in birds given copper sulphate alone or with urea in higher amounts which may be linked to the change in liver as there were vacuolation in the cytoplasm and other degenerative changes seen not only in the liver and kidney (Shahzad *et al*., 2012). The reports of Javed *et al*. (2003) revealed that the concentration of serum proteins showed lower values in copper treated groups (Cu 200-400 mg Kg^{-1}). When urea (4%) was added in the diet then an insignificant effect was there on the concentration of serum total proteins and albumin (Javed *et al*., 2002). The study by Guclu *et al*. (2008) also revealed lowering in albumin concentration in chicks fed at 300 and 450 mg copper Kg^{-1}. Thus the previous studies and findings of the present study suggest lowering of serum proteins in broilers which can be regarded as an untoward effect and thus both the compounds should be used with care. The results also suggest that the prolonged use of both the compounds are contraindicated.

***Conclusions*:** The haematological parameters and the serum proteins are affected by the combined use of both the compounds with a decrease in the values thus these compounds should be used sparingly in the broilers, especially in combination.

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