Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Effects of Urea and Copper Sulphate on Some Serum Biochemical and Meat Parameters in Broiler Chicken

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ARTICLE HISTORY

Received: January 16, 2012
Revised: March 06, 2012
Accepted: May 12, 2012

Key words:
Broilers
Copper
Enzymes
Meat
Urea

ABSTRACT

In this study we analysed some of the serum enzymes, urea and creatinine to understand the pathological changes occurring in different organs of broilers due to urea and copper. The feeding for 15 days at or higher than 2% urea + 1gm copper sulphate caused significant rise in serum ALT, AST, AKP and creatinine. With further increase in time of 15 days, the levels of urea and LDH also increased significantly, this was seen even in birds fed 1% urea + 250 mg copper sulphate. We found increase in serum urea even in 1% urea fed birds and in all other groups where combination was used, however, serum creatinine increased significantly ($P<0.05$) only in birds fed 2% urea+1 gm copper sulphate or higher than these levels. The combined use of urea and copper sulphate resulted in changes in moisture, ash, crude protein and potassium in thigh and breast meat of broilers. The results of the present study suggest damaging effects of higher levels of urea and copper, alone or together and change in meat quality with lower protein contents and higher salt levels in meat of broilers. Thus the use of urea and copper sulphate together is not recommended in broilers, especially at 1% urea and 250mg copper sulphate or higher. The results of the study can be helpful to poultry farmers, pathologists and nutritionists who are involved in augmentation the meat quality and also to general public with special reference to people having hypertension as the meat salt levels may be higher with use of the these compounds in the broiler ration.

To Cite This Article: Rasool A, MT Javed, M Akhtar, SS Bhatti, MN Shahzad and R Hussain, 2013. Effects of urea and copper sulphate on some biochemical and meat parameters in broiler chicken. Pak Vet J, 33(1): 27-31.

INTRODUCTION

The people of Pakistan are getting relatively cheaper source of animal protein in the form of broiler meat as broiler farming has become one of the major industries in the country and has a lot more potential than what it is contributing at the moment (Javaid *et al*., 2012). There are few reasons that why it is not progressing fast. Two of the major reasons are the infectious diseases and toxicities of various kinds, this industry is facing. Farmers still make haphazard use of various drugs and different chemicals to control certain diseases and to enhance meat and egg production. In addition, feed toxicants in various forms are responsible for relatively lower performance of poultry.

Poultry farmers more often use copper sulphate ($CuSO_4$) at different levels in feed. Copper is needed within mitochondria to supply secreted cupro-proteins and in the cytoplasm to supply the superoxide dismutase 1 (Robinson and Winge, 2010). Copper acts as a catalytic and structural cofactor for various enzymes involved in energy generation, iron procurement, oxygen transport, cellular metabolism, hormone (peptide) maturation, blood clotting, signal transduction and in many other processes (Kim *et al.*, 2008). Levels of copper between 100 to 300 mg/kg in broilers feed can promote growth (Pesti and Bakalli, 1996). However, when it is used at 14.5 mg/kg of feed, it results in growth depression (Song *et al*., 2011). At levels, on or beyond 500 mg/kg causes damage to the duodenal villi (Chiou *et al*., 1999) and thus influence the absorption of nutrients from the GIT with resultant effect of poor growth and can induce toxicity with wide range of effects including anemia, osteoporosis and neutropenia (Barceloux, 1999; Bjorn *et al.,* 2003), increase in the level of different enzymes and other serum parameters owing to its effects on liver and kidney as well (Chen *et al.,* 1996).

The use of copper at intentional higher levels to control fungus problem or due to errors in computation and improper mixing in feed can cause toxicity.

Urea is occasionally present in higher levels in the poultry feed owing to adulteration of fish meal being used in poultry feed formulation in Pakistan. Urea is a rich source of non-essential nitrogen in the form of ammonia released by urease enzyme. Urea has been used as a cheap protein substitute in the poultry feed (Pervaz *et al*., 1993). The use of urea can result in an increase in live weight of chicks (Pervaz *et al*., 1993). However, toxicity can occur when chicks are kept on urea treated feed. Chicks show mild to severe pathological changes including poor growth rate and altered blood picture. The diet containing urea was found responsible for low egg production in layers with symptoms of diarrhoea, weak legs and paralysis (Guo, 1983). Use of urea at 1% level did not show significant effect on serum AST levels (Pervaz, 1994); however, at 5% levels it can cause nephritis in broiler chicks (Chandra *et al*., 1984). The toxicity is related with the amount of ammonia released.

Research reports on combined effects of copper sulphate and urea in broilers are lacking. Therefore, we investigated the untoward effects of urea and copper in different combinations on various parameters including serum enzymes (indicative of liver and other tissue mal-function/damage), serum urea and creatinine (indicative of kidney mal-function/damage) and on crude protein, fat, ash, moisture, sodium, and potassium in meat of broilers.

MATERIALS AND METHODS

A total of 90, day-old broiler chicks were obtained from a local market (Sadiq Brothers Chicks, Pvt. Ltd). All the chicks were kept under similar management conditions with average maximum temperature around 37°C and relative humidity of 49% and were vaccinated against ND, IBD, IB and HPS following the local recommendations.

After day 7, the chicks were randomly divided into six groups (A-F) and were assigned different treatments as shown in Table 1. The feed and fresh water was available *ad libitum.* A commercial feed for broilers with 15% protein was used as basal feed (Punjab Feeds Ltd). Urea available in the market as nitrogenous fertilizer with 46% nitrogen was used in this study. It was incorporated in the experimental feed in ground form. Copper sulphate (anhydrous) was purchased from the local market and was used in the basal feed. The treated feed was fed to the birds from days 8 to 37. At day 38, all the experimental groups were switched on to basal feed, for 7 days (up to 42 days). Five birds from each group were sacrificed on days 22, 37 and 45 by cutting the jugular vein and blood samples (minimum of 5 ml) were collected without EDTA for collection of serum. Immediately after killing, meat samples (breast and thigh area) were collected in clean polythene bags. All the samples (blood and meat) were properly labelled and stored for further studies at -20°C.

Serum samples were used for the determination of biochemical parameters including alanine aminotransferase (Liquick Cor-ALAT 120, PZ Cormay S.A. Poland), aspartate aminotransferase (Fluitest GOT AST H7003, Analyticon Biotechnologies AG Germany), alkaline phosphatase (Liquick Cor-ALP 60, PZ Cormay S.A. Poland), lactic dehydrogenase (Liquick Cor-LDH 30, PZ Cormay S.A. Poland), urea (Liquick Cor-UREA 120, PZ Cormay S.A. Poland) and creatinine (CREATININE JAFFE BR2810, AMP Medizintechnik GmbH, Austria).

Study on meat samples included estimation of moisture percentage (hot air oven method), ash (crucible - muffle furnace method), crude fat (petroleum ether - Soxhlet apparatus method), crude protein (Kjeldadh`s method), sodium (flame photometric method) and potassium (flame photometric method) as described in AOAC (1996).

Data obtained were analysed by General Linear Model procedure and means were compared by Dunnett's test by using SAS statistical software (SAS, 2004).

Table 1: No. of birds in each group, and the proposed treatments

Group	No. of birds	Treatment
A	15	Control (basal feed)
B	15	250 mg Kg-1 Copper sulphate
C	15	1% urea
D	15	1% Urea+250 mg kg^{-1} Copper sulphate
E	15	2% Urea+1 g kg^{-1} Copper sulphate
F	15	4% Urea+2 g kg^{-1} Copper sulphate

RESULTS

There was no mortality recorded in any group during the experiment which has previously been indicated in another paper along with clinical signs, gross and histopathological changes (Shahzad *et al*., 2012). Results of four serum enzymes, urea and creatinine in broilers of treatment and control group are presented in Table 2. After 15 days of treatment trial, the values of ALT, AST, AKP and creatinine were significantly ($P<0.05$) higher in birds fed 2% urea+1gm copper sulphate and 4% urea+2gm copper sulphate than values in control group (Table 2). In addition to these treatment groups, urea and LDH were significantly higher in birds fed 1% urea + 250mg copper sulphate, however, LDH was also higher in birds fed 1% urea alone than the values in control group (Table 2). After 30 days of treatment, the values of AKP and creatinine remained higher in groups as after 15 days. However, values of ALT, LDH and urea were significantly higher in birds fed 1% urea, 1% urea+250 mg copper sulphate, 2% urea+1gm copper sulphate and 4% urea+2gm copper sulphate than control group. The values of AST were also higher in the same groups except those given 1% urea. During withdrawal period, values of all these parameters came down but still remained significantly ($P<0.05$) higher in most groups than control group.

The results of meat parameters studied in treatment groups and control are presented in Tables 3 and 4. Among meat parameters, significant ($P<0.05$) differences were observed in moisture, ash, crude protein percentages and potassium levels. Moisture contents in thigh meat were lower in birds fed 2% urea+1gm copper sulphate, 4% urea+2gm copper sulphate after 15 days, while in addition to these groups moisture contents were significantly lower in birds fed 1% urea+250mg copper sulphate after 30 days of treatment as compared to the values by the chicks kept on basal diet. Ash percentage in thigh meat was significantly higher after 15 days of treatment in birds fed 4% urea+2gm copper sulphate. Crude protein percentage in thigh meat was significantly ($P<0.05$) lower in birds fed 2% urea+1gm copper sulphate after 15 days of treatment, while in addition, crude protein was lower in birds fed 4% urea+2gm copper sulphate after 30 days of treatment. Potassium in thigh meat was

Table 2: Effects of urea and copper sulphate on different serum biochemical parameters in broilers

Groups	Day 22	Day 37	Day 45
Alanine transaminase (U/L)			
A	16.02±3.48	18.86±1.36	19.40±1.17
B	17.97±1.05	24.84±4.91	21.41±1.86
C	18.52±1.77	33.68±4.55*	21.08±1.31*
D	22.86±1.48	35.84±4.49*	39.69±8.38*
E	38.49± 6.39*	49.04±3.63*	39.89±6.48*
F	64.79± 17.92*	65.99±13.45*	35.25±5.17*
Aspartate transaminase (U/l)			
A	93.07±18.06	145.92±87.03	163.34±45.79
B	93.26±13.81	109.06±72.97	113.48±15.93
C	98.84±16.98	133.55±60.86	101.81±12.71
D	117.96±33.67	793.49±69.78*	194.98±12.04
E	507.63±61.66*	1393.80±117.3*	932.28±108.11*
F	1459.53±153.79*	1644.92±115.08*	1331.60±96.55*
Lactate dehydrogenase (U/L)			
A	85.02±22.26	108.90±18.69	79.62±11.07
B	122.33±35.58	123.98±36.90	73.79±26.94
C	500.41±95.50*	562.99±95.71*	337.15±68.22*
D	506.09±74.32*	572.99 ± 64.23*	417.21±69.12*
E	601.59±150.46*	887.03±175.06*	691.52±157.06*
F	1036.91±122.17*	1162.79±211.14*	994.76±138.25*
Alkaline phosphatase(U/l)			
A	85.45±7.10	91.96±16.03	76.70±18.2
B	89.55±11.14	92.06±14.35	102.06±11.36
C	106.97±43.96	106.59±8.23	102.38±12.71
D	109.85±41.185	114.53±17.19	72.43±42.79
E	127.07±14.97*	123.07±19.57*	110.70±30.90*
F	159.68±26.36*	160.15±20.37*	151.65±57.94*
Urea (mg/dl)			
A	10.30±2.23	12.11±1.56	21.80±5.24
B	11.87±2.44	18.01±13.58	21.21±12.72
C	42.40±8.94	175.80±42.40*	164.96±48.30*
D	132.00±46.05*	196.94±21.49*	154.75±46.03*
E	140.75±37.71*	257.37±43.01*	265.08±121.29*
F	251.39±52.23*	448.50±81.98*	254.13±83.75*
Creatinine (mg/dl)			
A	0.460±0.043	0.418±0.039	0.503±0.179
B	0.464±0.040	0.438±0.047	0.455±0.044
C	0.508±0.014	0.532±0.125	0.415±0.025
D	0.506±0.030	0.484±0.069	0.435±0.055
E	0.626±0.037*	0.630±0.138*	0.626±0.040*
F	0.636±0.089*	0.758±0.127*	0.715±0.205*

A (control); B (250mg$Cuso_4$/kg feed); C (1% Urea); D (1% Urea+250mg$Cuso_4$/kg feed); E (2%Urea+ 1gm$Cuso_4$/kg feed); F (4% Urea+2gm$Cuso_4$/kg feed); Means with * are significantly different from the control.

Table 3: Effects of urea and copper sulphate on different parameters of thigh meat in broilers.

Groups	Day 22	Day 37	Day 45
Moisture percentage			
A	76.75±0.44	76.22±0.44	75.98±1.05
B	76.50±0.49	76.54±0.78	75.96±1.13
C	75.95±0.33	76.34±0.48	75.63±1.94
D	74.85±0.67	74.63±0.40*	75.37±1.51
E	73.85±0.37*	74.06±0.44*	74.71±1.86
F	73.49±0.45*	74.26±0.52*	74.62±1.70
Ash percentage			
A	0.514±0.071	0.510±0.112	0.520±0.112
B	0.518±0.072	0.518±0.108	0.513±0.101
C	0.508±0.070	0.512±0.117	0.512±0.113
D	0.534±0.084	0.526±0.114	0.523±0.110
E	0.536±0.032	0.538±0.132	0.533±0.131
F	0.540±0.027*	0.528±0.122	0.520±0.120
Crude protein percentage			
A	18.98±0.30	19.53±0.56	19.72±1.51
B	19.16±0.20	19.77±1.44	19.58±.40
C	19.01±1.08	19.69±0.77	19.31±1.37
D	19.64±1.17	20.02±0.85	19.71±1.50
E	18.33±1.43	18.68±1.36*	18.75±1.54
F	17.78±0.89*	17.68±1.00*	18.29±1.72
Crude fat percentage			
A	4.30±0.36	4.57±0.53	4.85±0.60
B	4.12±0.21	4.26±0.33	4.61±0.54
C	4.31±0.61	4.31±0.32	4.68±0.33
D	4.53±0.37	4.46±0.74	4.66±0.73
E	4.84±0.58	4.30±0.58	4.57±0.65
F	4.31±0.64	4.69±0.52	5.02±0.66
Sodium (mg/100gm meat)			
A	80.62±5.82	85.27±4.87	98.23±7.70
B	83.20±5.11	86.66±14.23	91.73±9.51
C	83.40±8.36	81.77±9.28	98.86±9.00
D	85.27±5.52	86.10±13.24	87.67±13.48
E	83.93±9.23	79.72±7.24	77.85±16.46
F	78.33±8.39	79.44±3.45	75.63±13.50
Potassium (mg/100gm meat)			
A	111.86±8.21	116.87±13.00	152.19±51.77
B	134.27±27.15	161.55±55.57	136.57±30.93
C	103.83±17.13	165.82±57.67	169.88±25.68
D	133.17±17.65	179.07±55.02	230.40±88.24
E	134.47±25.64	232.83±35.08*	199.07±64.01
F	136.28±14.12	259.33±70.24*	207.56±94.63

A (control); B (250mg$Cuso_4$/kg feed); C (1% Urea); D (1% Urea+250mg$Cuso_4$/kg feed); E (2%Urea+ 1gm$Cuso_4$/kg feed); F (4% Urea+2gm$Cuso_4$/kg feed); Means with * are significantly different from the control.

significantly higher in birds fed 2% urea+1gm copper sulphate, 4% urea+2gm copper sulphate after 30 days of treatment. The results were almost similar for these parameters in breast meat with few exceptions. In addition to results in thigh meat, moisture was significantly ($P<0.05$) lower in birds fed 1% urea+250mg copper sulphate after 15 days of treatment, while it remained lower in these three groups after one week of withdrawal period. Ash percentage did not reveal significant differences between groups during treatment period. In addition to results in thigh meat, the crude protein in breast meat was significantly lower in birds fed 2% urea+1gm copper sulphate after 15 days of treatment and in birds fed 1% urea+250 mg copper sulphate after 30 days of treatment. Sodium showed significantly lower values in breast meat after 30 days of treatment in birds fed highest levels of urea and copper sulphate. In addition to results in thigh meat, potassium levels were significantly ($P<0.05$) higher in birds fed highest levels of urea and copper sulphate after 15 days of treatment, while these were also higher in breast meat in birds fed 1% urea and those fed 1% urea+250 mg copper sulphate after 30 days of treatment. Further, it remained higher in birds fed highest levels of both the compounds after withdrawal period in breast meat.

DISCUSSION

Changes in serum enzymes along with urea and creatinine are considered as good indicators of health status of chicken. These can be monitored during life by drawing blood from wing vein from selected birds. It is always better to monitor the flock before it is seriously sick and many birds are dead. Therefore, in this study we analysed some of the serum enzymes along with urea and creatinine to understand the pathological changes occurring in different organs of the chicken due to use of higher levels of urea and copper, especially in the liver and kidneys. The effects of these compounds on some meat parameters were also studied. Previously, we reported effects of the same combinations on mortality, organs pathology and on weight of organs/birds (Shahzad *et al*., 2012).

Table 4: Effects of urea and copper sulphate on different parameters of breast meat in broilers.

Groups	Day 22	Day 37	Day 45
Moisture percentage			
A	75.04±0.19	74.92±0.09	74.78±0.41
B	75.05± 0.50	74.19±0.82	74.15±1.55
C	74.87±0.53	73.91±0.46	73.39±1.18
D	72.43±0.42*	71.91±0.58*	72.80±0.48*
E	72.38±0.28*	71.94±0.10*	72.76±0.59*
F	71.87±0.38*	71.46±0.52*	72.65±0.33*
Ash percentage			
A	0.610±0.032	0.610±0.025	0.610±0.020
B	0.612±0.071	0.610±0.025	0.605±0.021
C	0.610±0.031	0.608±0.013	0.632±0.026
D	0.626±0.005	0.622±0.028	0.602±0.009
E	0.636±0.011	0.624±0.009	0.620±0.010
F	0.650±0.034	0.636±0.008	0.630±0.014
Crude Protein percentage			
A	22.98±0.90	22.97±0.91	20.94±0.49
B	23.40±0.58	22.71±0.37	22.60±1.54
C	23.55±0.73	22.96±0.86	22.24±1.66
D	23.40± 0.60	20.85±0.18*	22.34±1.37
E	21.89±0.86*	20.85±0.54*	21.86± 0.82
F	20.34±0.49*	20.26±0.63*	21.12±0.72
Crude fat percentage			
A	1.47±0.13	1.65±0.11	1.58±0.12
B	1.44±0.13	1.56±0.11	1.56±1.05
C	1.43±0.11	1.60±0.16	1.56±1.07
D	1.48±0.12	1.40±0.19	1.68±0.19
E	0.63±0.11	1.68±0.16	1.58±0.19
F	1.46±0.13	1.59±0.18	1.59±0.12
Sodium (mg/100gm meat)			
A	51.47±20.97	71.14±12.60	73.99±19.41
B	76.20±22.23	65.74±9.32	74.01±20.97
C	85.84±52.04	66.55±18.82	75.27±14.53
D	73.94±20.32	59.33±2.59	77.45±11.28
E	69.56±11.85	57.36±13.90	93.33±12.32
F	70.95±27.54	50.89±7.55*	74.26±4.03
Potassium (mg/100gm meat)			
A	113.33±13.38	116.77±14.80	129.64±13.83
B	113.04±15.70	138.34±21.15	148.82±29.19
C	133.89±15.91	179.35±56.52*	207.79±98.39
D	132.92±14.04	189.32±49.08*	153.47±34.89
E	146.04±23.80	241.28±41.62*	143.37±24.90
F	182.99±54.89*	265.43±55.44*	246.29±49.38*

A (control); B (250mg$Cuso_4$/kg feed); C (1% Urea); D (1% Urea+250mg$Cuso_4$/kg feed); E (2%Urea+ 1gm$Cuso_4$/kg feed); F (4% Urea+2gm$Cuso_4$/kg feed); Means with * are significantly different from the control.

The feeding of urea and copper sulphate together for 15 days at or higher than 2% urea + 1gm copper sulphate caused significant (P<0.05) rise in serum ALT, AST, AKP and creatinine. With further increase in time of 15 days, the levels of urea and LDH also increased significantly not only in these birds but also in those fed 1% urea + 250 mg copper sulphate. Thus it appears from the results that 1% urea+250 mg copper sulphate are also not safe when given for longer times (30 days). However, both increase (Rehman *et al*., 2001) and decrease (Guclu *et al*., 2008) in serum ALT in copper fed birds have previously been reported. Similarly, increase in serum AST (Rehman *et al*., 2001) and AKP (Guclu *et al.,* 2008; Abdou *et al*., 2006) in copper fed birds have been reported. Thus the results of the present study are in agreement with the results of the previous studies and suggest damage to the liver and other tissues of the chicken causing increase in serum enzymes due to leakage of the latter from the damaged cells. We found increase in serum urea even in 1% urea fed birds and in all other groups where combination was used, however, serum creatinine increased significantly only in birds fed 2% urea+1 gm copper sulphate or higher than these levels. An increase in serum urea (Abdou *et al*., 2006) and creatinine (Wei *et al.,* 2009) in copper fed birds/duckling has already been reported. Previously, we found mild to moderate gross changes in liver, kidneys and other organs (Shahzad *et al*., 2010). Histopathological studies revealed degenerative and necrotic changes in liver, kidneys and other tissues of these birds. Therefore, the present results confirm the earlier changes in these birds fed with urea and copper sulphate in different combinations. The mild histopathological changes observed in about 10% cells of liver of birds fed 250 mg copper sulphate did not result into significant changes in serum enzymes. The microscopic changes described earlier for birds fed 1% urea + 250 mg copper sulphate (Shahzad *et al*., 2010) correlated well with the changes in serum enzymes. Thus the results described earlier are strengthened with the findings on serum enzymes along with urea and creatinine levels in these birds. The enzyme LDH can increase under different circumstances and is a good indicator of tissue turnover in the body. The enzyme alkaline phosphatase is a good indicator of liver, kidney and bone tissue integrity apart from some other tissue cells. Looking at the results, one can see that the use of urea alone at 2% has much higher values for various parameters than the values of 250mg copper sulphate fed birds. Thus, when these were fed in combination it is possible that the effect of urea was much pronounced than the effect of copper sulphate. This finding is also supported by the results of liver and kidney weights and pathology noticed in these groups which is published earlier (Shahzad *et al.,* 2012). Now, whether these effects were additive or synergistic is difficult to explain as we did not include the groups where higher levels of urea and copper were fed separately. This can be included in future studies to clarify the facts. However, our previous experience with use of urea and copper sulphate suggest that the effect is cumulative where urea has more pronounced effect at higher levels (Pervaz *et al.,* 1993; Javed *et al.,* 1995; Pervaz *et al.,* 1996). The toxicity of copper and urea is well documented in literature. The copper toxicity occurs through generation of reactive oxygen species such as superoxide, hydrogen peroxide and the hydroxyl radical ultimately causing injury to the cells. The urea toxicity is related with release of ammonia after metabolism and also due to uric acid produced.

Among meat parameters, significant (P<0.05) differences between groups were observed in moisture, ash, crude protein percentages and potassium levels. The combined use of urea and copper sulphate resulted decrease in moisture contents in both breast and thigh meat. The ash percentage was significantly higher in thigh meat only in birds fed the highest levels of both the compounds together but differed non-significantly in breast meat. Crude protein percentage decreased in thigh meat in birds fed 2% urea+1gm copper sulphate; however, in breast meat it was lower even in birds fed 1%urea + 250mg copper sulphate. Among the salts, potassium levels increased in thigh meat in birds fed 2% urea + 1gm copper sulphate or higher than these levels, while in breast meat it increased in birds fed 1% urea alone or in combination with copper sulphate. The changes in serum sodium were mostly non-significant. Previous studies have reported significant effect of copper on meat fat

percentage, especially in breast meat (Javed *et al*., 2010; Skrivan *et al*., 2002). Significant increase in meat protein, while non-significant effects on moisture and ash percentage in copper and chromium fed birds have also been reported (Javed *et al*., 2010). A significant increase in potassium in meat has been reported in chromium fed birds (Javed *et al*., 2010). Thus it appears that the use of copper alone or with urea cause changes in meat parameters including protein, fat, moisture and ash.

The results of the present study suggest damaging effects of higher levels of urea and copper, alone or together thus they should be carefully used in the poultry industry, especially at higher doses.

Conclusion: Results obtained in the present experiment highlighted the significance of both of these substances in broilers. Both urea and copper sulphate at and beyond 1% and 250mg, respectively produced deleterious effects in broilers and these data can be of help to poultry farmers.

Acknowledgement: The help in some of the laboratory procedures extended by the Director and staff, Institute of Animal Feed Science and Technology is highly acknowledged.

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