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Research Article

Efficacy of Tribasic Copper Chloride (TBCC) to Reduce the Harmful Effects of Aflatoxin in Broilers*

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Abstract: This study was conducted to evaluate the effects of copper as tribasic copper chloride on serum biochemical values and growth performance of broilers intoxicated with aflatoxins. Aflatoxins significantly decreased the level of albumin ($P < 0.001$), total protein ($P < 0.001$) and total cholesterol ($P < 0.01$), and increased the activities of alanine amino transferase, L-lactic dehydrogenase and alkaline phosphatase ($P \leq 0.001$) in serum. These AF-induced changes were significantly improved by adding tribasic copper chloride to the diet. The decrease in serum copper concentration induced by aflatoxin was reversed by tribasic copper chloride. Serum zinc concentrations were not affected in tribasic copper chloride and tribasic copper chloride plus aflatoxin groups. Serum iron levels decreased in all the treatment groups significantly ($P < 0.05$). Aflatoxins caused significant decrease in the body weight gain and significant increase in the feed conversion ratio. Adverse effects of aflatoxins on feed conversion ratio were reversed by tribasic copper chloride supplementation to the diet. It is suggested that tribasic copper chloride might be used for reducing the adverse effects of aflatoxins in broiler production.

Key Words: TBCC, biochemical values, performance, broiler, aflatoxin

Broyler Yetiştiriciliğinde Aflatoksinlerin Zararlı Etkilerinin Önlenmesinde Üç Bazlı Bakır Kloridin (TBCC) Faydaları

Özet: Bu çalışma, yemleriyle aflatoksin alan broylerlerin yemlerine üç bazlı bakır klorid halinde ilave edilen bakırın, serum biyokimyasal değerleri ile büyüme performansı üzerine etkilerinin incelenmesi amacıyla yapıldı. Aflatoksin serum albumin ($P < 0,001$), toplam protein ($P < 0,001$) ve toplam kolesterol ($P < 0,01$) düzeylerini önemli oranda azaltırken; serum alanin aminotransferaz, L-laktik dehidrogenaz ve alkalen fosfataz aktivitelerini önemli oranlarda ($P \leq 0,001$) artırdı. Bu değişiklikler üç bazlı bakır klorid ilavesi ile düzeltildi. Aflatoksine bağlı olarak serum bakır konsantrasyonunda meydana gelen azalma üç bazlı bakır klorid ile düzeltildi. Serum Zn konsantrasyonları üç bazlı bakır klorid ve aflatoksin+üç bazlı bakır klorid gruplarında etkilenmedi. Tüm uygulama gruplarında serum demir konsantrasyonlarında önemli düzeyde ($P < 0,05$) azalma oldu. Aflatoksin grubunda canlı ağırlık artışında önemli bir azalma ve yemden yararlanma oranında ise önemli bir artış belirlendi. Aflatoksininin yemden yararlanma oranında meydana getirdiği olumsuz etki üç bazlı bakır klorid ilavesi ile tersine döndürüldü. Sonuçta, broyler yetiştiriciliğinde aflatoksinlerin zararlı etkilerinin önlenmesinde yeme üç bazlı bakır klorid ilavesinin yararlı olabileceği kanısına varıldı.

Anahtar Sözcükler: TBCC, biyokimyasal değerler, performans, broyler, aflatoksin

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Introduction

Aflatoxins (AFs) cause a variety of undesired effects in poultry, including poor performance, liver pathology, immunosuppression, increased susceptibility to environmental and microbial stresses, and increased mortality (1). AF toxicosis decreases serum concentrations of total protein, cholesterol, triglyceride and glucose (1), inorganic phosphorus and calcium (2) in poultry. Several approaches have been used for removing AFs from contaminated feed and feedstuffs, e.g., use of mold inhibitors, fermentation, microbial inactivation, physical separation, thermal inactivation, irradiation, ammoniation (3) and the use of adsorbents (4). These methods for detoxifying AFs have been employed with limited success. Another approach to the problem has been to use divalent cations, such as Cu^{2+}, Mg^{2+} and Ca^{2+} (5,6). Copper from various compounds has often been added to poultry diets as an antimicrobial agent at concentrations far in excess of the 8 mg kg^{-1} requirement established by National Research Council (NRC) (7). Tribasic copper chloride (TBCC®) occurs naturally as the mineral, atacamite, and was the first discovered in the Atacama Desert. This form of Cu is a secondary mineral that is formed by the oxidation of other Cu-containing deposits. In the laboratory, TBCC can also be produced as crystals by heating Cu_2O with a solution of $FeCl_3$ (8).

No study has been conducted to determine the preventive effects of Cu as TBCC in poultry exposed to AFs. The objectives of this research were to determine the effects of AFs on serum biochemical values and growth performance of broiler chickens and the preventive efficacy of TBCC from undesired effects of AFs.

Materials and Methods

Aflatoxin: Aflatoxins were produced by fermentation of converted rice under constant stirring and controlled temperature. The NRLL 2999 strain of *Aspergillus parasiticus* (USDA, Agricultural Research Service, Peoria, Illinois, USA) was used for production of the AFs according to the method described by Shotwell et al. (9) and improved by West et al. (10). The total concentration of AFs in rice powder was analyzed by fluorescence spectrophotometry in Ankara Control Laboratory of the Ministry of Agriculture. The AFs of the rice powder consisted of 42% AFB_1, 15% AFB_2, 29% AFG_1 and 14% AFG_2 based on total AF (detection limit: 1 mg AF kg^{-1} rice powder; recovery of the extraction method: 92%). The rice powder was added to the diet to provide the desired amount of 1 mg AF kg^{-1} diet.

Chickens and diet: Ninety-six 1-day-old Ross broiler chicks of both sexes were divided randomly into four groups, in which there were 3 replicates of 8 broiler chicks. The chicks were housed under fluorescent lighting and fed a starter diet from 1 to 21 day of age then grower diet for the period of 22 to 42 days. Experimental diets were formulated as recommended by the NRC (7) (Table 1). The chicks were allowed ad libitum access to feed and water. The animal care and use protocol was reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine, Mustafa Kemal University.

Table 1. Compositions of the starter and grower diets.[1]

Starter Diet[2]		Grower Diet[3]	
Ingredients	%	Ingredients	%
Corn	32.00	Corn	48.00
Full Fat Soybean	28.00	Full Fat Soybean	22.00
Wheat	16.00	Wheat	11.00
Sunflower Meal	10.00	Sunflower Meal	10.00
Fish Meal	6.00	Fish Meal	4.00
Wheat Bran	6.00	Wheat Bran	3.00
Limestone	0.55	Limestone	0.55
Dicalcium phosphate	0.50	Dicalcium phosphate	0.50
Salt	0.35	Salt	0.35
DL-Methionine	0.20	DL-Methionine	0.20
Vitamin Premix[4]	0.25	Vitamin Premix[4]	0.25
Mineral Premix[5]	0.10	Mineral Premix[5]	0.10

[1]: Batches of diet were prepared weekly and stored in a dry and cool place.
[2]: Crude Protein 23.00%; ether extract 5%; Ash 9.00%; ME (calculated) 3100 kcal/kg; Ca (calculated) 3.00%; P (calculated) 0.60%.
[3]: Crude Protein 20.00%; ether extract 6.00%; Ash 9.00%; ME (calculated) 3200 kcal/kg; Ca (calculated) 3.00%; P (calculated) 0.60%.
[4]: 15,000,000 IU Vit A; 3,000,000 IU Vit D_3; 70,000 mg Vit E; 5000 mg Vit K_3; 3000 mg Vit B_1; 6000 mg Vit B_2; 25,000 mg niacin; 5000 mg Vit B_6; 30,000 mg Vit B_{12}; 75,000 mg biotin; 1000 mg folic acid; 50,000 mg Vit C; 200,000 mg choline chloride; 12,000 mg Ca-D- pantothenate /2 kg premix.
[5]: 80,000 mg Mn; 60,000 mg Fe; 60,000 mg Zn; 5000 mg Cu; 200 mg Co; 1000 mg I; 150 mg Se; 300,000 mg choline chloride /1 kg premix.

Experimental design: The experimental design consisted of four dietary treatments. (1) Control: basal diet, (2) AF: 1 mg AF kg^{-1} diet, (3) TBCC: 200 mg Cu as TBCC kg^{-1} basal diet, and (4) AF + TBCC: 1 mg AF + 200 mg Cu as TBCC kg^{-1} diet. TBCC™ ($Cu_2[OH]_3Cl$) was kindly provided by Micronutrients, Indianapolis, IN 46231, USA.

Serum biochemical analysis: At the end of the study (day 42), 12 broilers were randomly selected (4 chicks/each 3 replicates) for each treatment group. Blood samples were collected from the v. tibialis caudalis. Serum concentrations of albumin, total protein, total cholesterol, glucose, creatinine, Ca and P, and the activities of aspartate amino transferase (AST), alanine amino transferase (ALT), lactic dehydrogenase (LDH), alkaline phosphatase (ALP), g-glutamyl transferase (GGT) and creatine kinase (CK) were measured on an autoanalyzer (AMS, Roma) using diagnostic kits (Teco Diagnostics, CA, USA).

Element analysis in serum: Serum samples (2 ml) were treated with 8 ml of 1 N nitric acid and then briefly centrifuged. The supernatant was used for element analysis (11). Serum trace elements (Cu, Zn and Fe) were determined in an inductively coupled plasma-atomic emission spectrometry (ICP-AES, Liberty Series-II Varian, USA). All specimens were analyzed 3 times. The wavelength used was 324.754 nm for Cu, 213.856 nm for Zn and 259.837 nm for Fe. Calibration standard series were prepared by appropriate dilutions from 1000 mg L^{-1} single-element standard solutions and the acidity of standards were matched to that of the sample solutions. The concentrations of elements were reported as mg L^{-1} for serum assays.

Performance parameters: The chickens were weighted to determine the body weight and body weight gain (BWG) at 1, 7, 14, 21, 28, 35 and 42 d of ages and mortality was recorded as it occurred. Feed consumption (FC) was also measured and feed conversion ratio (FCR) was calculated as feed-to-gain ratio in the same days and over days 1 to 42 of the experiment.

Statistical analysis and quality assurance: Recovery tests were done during the analysis of elements. Indium was used as an internal standard at concentrations of 0.1 and 10.0 mg l^{-1}, and the results of recovery tests ranged from 98.5% to 101.8%. The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Results are given as mean ± standard errors of means (SEM). For performance, the number of samples (n) used for analysis was 3 and for biochemical analysis and trace element analysis, the number of samples used (n) was 12 (3 replicates of 4 broiler chicks each). All statements of significance are based on the 0.05 level of probability.

Results

Serum biochemical values: At the end of the experiment significant decreases in the levels of albumin, total protein and total cholesterol were detected in AF group (Table 2). In the same group, significant increases were detected in the activities of ALT, LDH and ALP (Table 3). Significant improvements in the levels of albumin ($P < 0.001$), total protein ($P < 0.001$) and total cholesterol ($P < 0.03$), and in the activities of ALT ($P < 0.001$), LDH ($P < 0.002$) and ALP ($P < 0.001$) were

Table 2. Effects of AF (1 mg kg^{-1} diet) and TBCC (200 mg kg^{-1} diet) on serum levels of albumin, total protein, total cholesterol, creatinin, glucose, calcium and phosphorus of broiler chicks at 42 d of age.[1]

Treatment								
AF	TBCC	Albumin (g dL^{-1})	Total protein (g dL^{-1})	Total cholesterol (mg dL^{-1})	Creatinine (mg dL^{-1})	Glucose (mg dL^{-1})	Ca (mg dL^{-1})	P (mg dL^{-1})
-	-	2.15 ± 0.03^a	3.67 ± 0.07^{ac}	147.36 ± 3.39^a	5.15 ± 0.05	254.33 ± 2.67	8.68 ± 0.26	4.00 ± 0.12
+	-	1.50 ± 0.07^b	2.88 ± 0.03^b	133.55 ± 2.64^b	5.15 ± 0.06	258.17 ± 3.17	8.68 ± 0.23	4.31 ± 0.31
-	+	2.21 ± 0.05^a	3.72 ± 0.13^c	146.30 ± 2.91^a	5.01 ± 0.1	259.00 ± 2.29	8.97 ± 0.25	4.50 ± 0.12
+	+	2.06 ± 0.06^a	3.46 ± 0.04^a	145.00 ± 4.58^a	5.00 ± 0.07	257.25 ± 3.07	8.60 ± 0.19	4.15 ± 0.12

[a-c] Means within a column with no common superscript differ significantly ($P < 0.05$), according to Duncan's multiple range tests.
[1]Values represent the mean±SEM of 3 replicates of 4 broiler chicks each.

Table 3. Effects of AF (1 mg kg^{-1} diet) and TBCC (200 mg kg^{-1} diet) on serum enzymes' activities and trace element levels of broiler chicks at 42 d of age.[1]

Treatment		AST	ALT	LDH	ALP	GGT	CK	Cu	Zn	Fe
AF	TBCC	(IU L^{-1})	(IU L^{-1})	(IU L^{-1})	(IU L^{-1})	(IU L^{-1})	(IU L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})
-	-	113.0 ± 4.0^{ab}	44.0 ± 2.0^{a}	436.0 ± 28.0^{a}	355.0 ± 22.0^{ac}	4.67 ± 0.51	10077.0 ± 325.0	0.450 ± 0.056^{ac} (0.214-2.210)	(0.308-0.739) 0.450 ± 0.056^{ac}	1.487 ± 0.296^{ab} (0.308-0.739)
+	-	128.0 ± 9.0^{ac}	57.0 ± 2.0^{b}	620.0 ± 38.0^{b}	593.0 ± 36.0^{b}	5.09 ± 0.51	10763.0 ± 283.0	0.317 ± 0.007^{b} (1.134-2.181)	(0.283-0.345) 0.317 ± 0.007^{b}	1.562 ± 0.118^{b} (0.283-0.345)
-	+	108.0 ± 6.0^{b}	40.0 ± 1.0^{ac}	391.0 ± 38.0^{a}	371.0 ± 17.0^{c}	5.42 ± 0.34	10380.0 ± 272.0	0.496 ± 0.051^{a} (0.822-1.945)	(0.340-0.392) 0.496 ± 0.051^{a}	1.219 ± 0.144^{ab} (0.340-0.392)
+	+	112.0 ± 7.0^{bc}	40.0 ± 2.0^{c}	443.0 ± 26.0^{a}	436.0 ± 32.0^{c}	4.08 ± 0.60	10947.0 ± 373.0	0.371 ± 0.007^{bc} (0.762-2.210)	(0.340-0.392) 0.371 ± 0.007^{bc}	0.979 ± 0.093^{a} (0.340-0.392)

[a-c] Means within a column with no common superscript differ significantly ($P < 0.05$), according to Duncan's multiple range tests.
[1]Values represent the mean±SEM of 3 replicates of 4 broiler chicks each.

detected by adding TBCC (200 mg Cu kg^{-1} diet) to the AF-containing diet (Tables 2 and 3).

Serum trace element concentrations: At the end of the study, Cu and Fe concentrations in the serum of the AF group were significantly decreased ($P < 0.03$ and $P < 0.001$ respectively). The addition TBCC to the diet caused Cu levels to return to the control level, but decreased Fe levels induced by AF were not affected (Table 3).

Performance parameters: The results are summarized in Table 4. At the end of the experiment, the group containing AFs alone had significantly lower ($P < 0.05$) BWG than the control and TBCC supplemented groups. Addition of TBCC in combination with AFs resulted in a slight but not significant increase in BWG. FCR of the AF treated group was significantly higher ($P < 0.01$) than that of the other three groups, which did not differ significantly from one another. Mortality rates of groups during the experimental period were 2/24 and 1/24 in AF treated group and TBCC treated group, respectively. At the autopsy examination, no abnormal signs of toxicity were observed in dead chickens.

Table 4. Effects of copper (200 mg kg^{-1} diet) as TBCC on body weight gain, feed consumption and feed conversion ratio for broiler chicks fed on a diet containing 1 mg AF kg^{-1} diet between 1 to 42 days of age.

		Groups			
Period		Control	AF	TBCC	AF+TBCC
	BWG (g)	99.9 ± 2.0	93.3 ± 2.4	101.0 ± 7.1	94.0 ± 2.4
1 to 7*	FC (g)	112.8 ± 2.0	105.3 ± 2.4	111.9 ± 6.4	105.0 ± 1.8
	FCR (g of feed/g of gain)	1.13 ± 0.005	1.13 ± 0.006	1.11 ± 0.01	1.12 ± 0.01
	BWG (g)	219.2 ± 13.0	196.2 ± 10.7	215.8 ± 13.6	197.0 ± 4.7
8 to 14	FC (g)	286.8 ± 13.6	268.8 ± 13.6	281.4 ± 14.1	269.3 ± 4.7
	FCR (g of feed/g of gain)	1.31 ± 0.01	1.37 ± 0.005	1.31 ± 0.03	1.37 ± 0.01
	BWG (g)	333.5 ± 16.1	298.7 ± 10.2	335.3 ± 6.0	333.7 ± 7.6
15 to 14	FC (g)	509.4 ± 16.5	486.4 ± 10.6	501.5 ± 7.9	502.6 ± 11.3
	FCR (g of feed/g of gain)	1.53 ± 0.02^{a}	1.63 ± 0.02^{b}	1.50 ± 0.03^{a}	1.51 ± 0.02^{a}
	BWG (g)	464.9 ± 20.6	426.3 ± 20.6	480.6 ± 19.5	445.5 ± 28.7
22 to 28	FC (g)	824.4 ± 19.0	821.3 ± 26.1	799.7 ± 18.7	815.0 ± 27.3
	FCR (g of feed/g of gain)	1.78 ± 0.07	1.93 ± 0.03	1.67 ± 0.06	1.84 ± 0.11
	BWG (g)	530.7 ± 22.6^{ab}	498.2 ± 7.6^{a}	551.0 ± 12.0^{b}	520.8 ± 11.0^{ab}
29 to 35	FC (g)	1006.3 ± 21.5	1071.0 ± 36.8	1009.3 ± 43.9	961.5 ± 66.09
	FCR (g of feed/g of gain)	1.90 ± 0.05^{a}	2.15 ± 0.07^{b}	1.83 ± 0.04^{a}	1.84 ± 0.08^{a}
	BWG (g)	552.3 ± 11.0^{a}	490.7 ± 8.3^{b}	597.3 ± 12.1^{c}	536.27 ± 16.7^{a}
36 to 42	FC (g)	1087.0 ± 8.6	1107.2 ± 35.6	1154.7 ± 21.8	1128.8 ± 61.7
	FCR (g of feed/g of gain)	1.97 ± 0.05^{a}	2.25 ± 0.03b	1.94 ± 0.04^{a}	2.11 ± 0.09^{ab}
	BWG (g)	2200.5 ± 68.5^{ac}	2003.4 ± 31.8^{b}	2280.9 ± 5.6c	2128.3 ± 46.3^{ab}
1 to 42	FC (g)	3826.7 ± 65.0	3860.3 ± 73.5	3858.5 ± 68.9	3782.2 ± 125.9
	FCR (g of feed/g of gain)	1.74 ± 0.03^{a}	1.93 ± 0.007^{b}	1.69 ± 0.03^{a}	1.78 ± 0.02^{a}

a-c Means within a row with no common superscript differ significantly ($P < 0.05$), according to Duncan's multiple range tests.

*Days of age; BWG: Body weight gain; FC: Feed consumption; FCR: Feed conversion ratio

Discussion

It was reported that a dietary concentration of Cu up to 800 mg kg^{-1} as Cu sulfate has been fed to chickens without any adverse effects on performance (12). Miles et al. (13) reported that relative bioavailability of Cu in TBCC compared to 100% for Cu sulfate ranges from 90% to 106%, implying that TBCC does not appear to be an environmental risk factor. In this study copper was used at a dose of 200 mg as TBCC kg^{-1} basal diet. In broiler feeding, the best economic performance was achieved at levels of 188 mg kg^{-1} copper from TBCC and 250 mg kg^{-1} copper from copper sulfate (14).

In this study AF intoxication resulted in alterations of selected biochemical parameters of broilers. AFs decreased the serum cholesterol level in AF-treated chicks. This result is in agreement with Santurio et al. (15), who reported a decreased total cholesterol level in broilers as a result of aflatoxicosis. Decreases in serum albumin and total protein concentrations have been proposed (16) as indicators of liver function disorders or alterations in protein synthesis in aflatoxicosis. Serum albumin and total protein levels-decreased by AFs were elevated significantly by simultaneous additions of TBCC and AFs to the diets in this study. Increased activities of serum ALT, AST, ALP and LDH are well-known diagnostic indicators of hepatic injury. In the present study, ALT, LDH and ALP activities were increased by AFs. TBCC addition in combination with AFs resulted in a significant reduction in the activities of ALT, LDH and ALP, approaching the control levels. TBCC is totally insoluble in water and yet very easily and quickly soluble in animal gut owing to the factors such as low pH and complexing environment. In the intestine, undissolved TBCC will continue to slowly solubilize due to the action of natural organic complexing agents in digestive fluids. Thus it displays a form of controlled release, giving effectiveness over a long path-length of the intestine (14). This is consistent with the increased liver copper level as reported by Miles et al. (13) that copper concentrations of the liver were detected at 16.6-18.5 mg kg^{-1}, 48.9-155.3 mg kg^{-1} and 799.1-940.4 mg kg^{-1} in broilers treated with 200 mg kg^{-1}, 400 mg kg^{-1} and 600 mg kg^{-1} copper as TBCC, respectively. In addition, it was reported that the low water solubility resulted in reduced reactivity in a food or feed mixture, thus improving the stability of vitamins (14). The stabilization of vitamins by TBCC could be accounted for by antioxidant or less oxidant capacity of TBCC. There is a lack of literature concerning Cu-induced oxidation in poultry diets. Copper has been shown to be a prooxidant or antioxidant (17) in meat systems. It was reported that both Cu sulfate and TBCC are prooxidants and the amount of water in the system might have an effect on observed rates of oxidation. Because TBCC is not water soluble, this might explain why the smaller particles of TBCC did not promote the rate of oxidation observed with large particles of Cu sulfate. Salem et al. (18) reported that ascorbic acid might prevent the aflatoxin-induced oxidative damage. Furthermore there is a report by Yu et al. (19) saying that ascorbic acid also may inhibit the binding of AFB_1 to DNA. In the present study, in AF plus TBCC group, an improvement was observed in the liver enzymes' activities (ALT, ALP and LDH) and albumin and total protein levels, which may be associated with stabilization of vitamins including ascorbic acid, which might prevent liver damage.

In this study, Cu, Zn and Fe concentrations in the serum of the control group were 0.450, 1.487 and 3.439 mg L^{-1} respectively (Table 3). These levels of elements are comparable to the results (0.17, 1.77 and 1.28 mg L^{-1} respectively) reported by Richards and Augustine (20). In the present study, it appears that there is a positive correlation between the copper level and the iron levels in serum. This is similar to the report by Huan et al. (21) in broiler chicks. In contradiction to this, Richards and Augustine (20) have reported that serum iron level was reduced, while copper level was increased in chicks infected with E. tenella. This could be accounted for by the parasitic infection.

Growth inhibition and poor BWG caused by AFs have long been known in broilers (1,22). Similar to our results, Kubena et al. (1) and Giambrone et al. (22) have reported that BWG was reduced by AF addition to the diet. These negative effects of AF on growth may be due to its inhibitory action on protein synthesis and lipogenesis. TBCC seems to mediate the positive effects on performance, improving the stability of vitamins including Vitamin C and E (18,23). Dudley-Cash (23) reported that TBCC appeared to reduce the growth of bacterial colonies on MacConkey and blood agar. This could also account for the improvement of performance by TBCC, inhibiting the secondary bacterial infections that reduce the performance of broilers.

FC values of all the experimental groups did not differ from each other (Table 4). While this result is in agreement with the report by Oguz and Kurtoglu (24), it is not consistent with Parlat et al. (25), who reported that AF caused a significant decrease in FC.

At the end of the experiment, the addition of AFs to the diet clearly depressed the feed efficiency in terms of FCR. The highest FCR value (1.93) was recorded in the AF-feeding alone group ($P < 0.01$). TBCC supplementation to the AF-containing diet significantly ameliorated the negative effect of AF on FCR (Table 4). TBCC improved the feed efficiency decreased by AFs. This finding agrees with previous results (22,25), which showed that AFs have adverse effects on FCR. The deleterious effects of AFs on FCR are due to impaired liver functions and protein/lipid utilization mechanism. One mechanism of action of the AFs is related to the inhibition of protein synthesis. Our research suggests that this mechanism may explain the observed effects, and is in agreement with the results reported by other authors (16).

This study has shown that copper (200 mg kg^{-1} diet) addition as TBCC to the AF-containing diet significantly ameliorated the disturbing effects of AFs on some biochemical values and FCR. The addition of TBCC to diet containing AFs (1 mg kg^{-1} diet) can be beneficial for broiler chickens. Therefore, TBCC might be a valuable chemical agent for reducing the harmful effects of AFs in broiler production.

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