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RESEARCH ARTICLE

Biointeraction of Chelated and Inorganic Copper with Aflatoxin on Growth Performance of Broiler Chicken

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ABSTRACT

A biological experiment was conducted to evaluate the interactive effects of organic and inorganic copper (Cu) supplementation with aflatoxin (AF) on growth performance of broilers. Day old broiler chicks were randomly divided into nine treatments of three replicates each containing ten birds per replicate. A 3×3 factorial design involving 9 treatments groups was formulated with 3 levels of aflatoxin B_1 (AFB_1) i.e. 0.0, 0.5 and 1.0 ppm and 3 Cu supplemented group, i.e. 0 ppm, 40 ppm organic Cu (as propionate), 40 ppm inorganic Cu (as sulphate). The study was made for 0-42 days and performance of broiler i.e. body weight gain, feed intake, feed conversion ratio and livability were analyzed. The result revealed that the body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were significantly depressed in a dose dependent fashion due to dietary AF levels. The mean BWG showed a significant difference due to Cu supplementation and were highest in Cu chelate group followed by control and it was lowest in Cu inorganic group. The FI and FCR did not differ significantly due to different Cu supplementations. The interaction effect showed that BWG , FI and FCR were highest in all Cu groups at basal AF level and lowest in Cu inorganic group at 1 ppm AF level, while the mean BWG of other groups were found to be intermediary. The livability was highest in basal level followed by 0.5 ppm AF level and it was lowest in 1 ppm AF level. The livability did not differ significantly due to different Cu supplementation. Similarly, the livability percentage among treatments did not differ significantly due to interaction of Cu supplementation with AF levels. It is concluded that there was a difference between organic and inorganic copper in counteracting the effect of aflatoxicosis in broiler chicken.

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INTRODUCTION

Poultry farming has metamorphosed into an organized industry in recent past and registered a rapid growth over last three decades in India. Many problems are continuously challenging against successful poultry farming operations and the common one is threat of mycotoxicosis in poultry. Aflatoxins (AF) have been demonstrated to be carcinogenic, mutagenic, teratogenic and toxic. Surveillance of AFB_1 content of poultry feed stuffs in U.P., India by Johri *et al.* (1986) revealed that 71 per cent of groundnut cake, 47 per cent of maize, 25 per cent of fish meal and 16.7 per cent of rice bran samples were contaminated by AFB_1, and the authors also found AF in starter feed mixture at 0-2 ppm and in grower feed mixture at 0-1 ppm levels. Surveillance in Southern part of India also showed that more than 40 per cent of the poultry and animal feed samples contained moderate to high level of AFB_1, ranged from 0.01 to 12 ppm (Selvasubramanian *et al*., 1987). Dietary manipulations have received considerable attention and increased dietary concentration of protein (Beura, *et al.*, 1993), α-tocopherol, ascorbic acid (Hoehler and Marquardt, 1996), choline, folic acid, pyridoxine, riboflavin were extensively studied for counteracting aflatoxicosis. However, studies on effect of trace minerals supplementation and its interaction with AF *in vivo* in poultry are very few. Increased dietary concentration of copper (Johri *et al.*, 1986), have been tried to counteract AF with moderate to significant response and in all the studies the trace elements were supplemented as inorganic form, not as organic chelates.

It is now recognized that the organic chelates of minerals are different from its inorganic counterpart in that the trace minerals are held with metal binding agents (ligand) by high energy bond known as co-ordinate covalent bond (Leeson and Summers, 2002). Several studies reported that the chelated minerals are more bioavailable to animals and poultry (Wedekind *et al.*, 1992) than its inorganic counterpart and hence one can expect more counteracting effect of this form of minerals against AF than inorganic form. But, studies regarding the effect of chelated minerals on AF and their interaction *in vivo* in poultry received very little attention. Hence, a study was designed to evaluate the interaction of chelated and inorganic copper on growth performances i.e. body weight gain, feed intake, feed conversion ratio, livability of broiler chicken.

MATERIALS AND METHODS

Production of aflatoxin

AF was produced from *Aspergillus parasiticus* NRRL 2999 in rice as per method of Shotwell *et al.* (1966) and in Yeast Extract Sucrose (YES) broth as per Tsai *et al.* (1984). The AF B_1 content was measured by preliminary extraction of AF (Pons *et al.*, 1966) and subsequent analysis by TLC method. The AF B_1 in contaminated rice ranged from 25 to 35 ppm and in YES broth the AFB_1 ranged from 250 to 300 ppm.

Experiment

The feeding experiment was conducted in broiler chicken from day one to 42 day of age. Two hundred and seventy day-old synthetic dam line broiler chicks were obtained from Experimental Broiler Farm, CARI and were wing banded, weighed and randomly allotted into nine treatment groups. Each treatment had three replicates and containing ten chicks per replicate.

All the chicks were reared on wire floor, electrically heated, battery brooder with provision of feeder and waterers under uniform and standard management practices. Feed and water were offered *ad libitum.* All the chicks were apparently healthy and were free from Marek's Disease, NewCastle Disease and Infectious Bursal Disease. All birds were immunized against NewCastle Disease and Marek's Disease at day old by occulonasal route and against Infectious Bursal Disease at 14th day of age by occulonasal route. The experimental protocol had the agreement of Institute of Animal Ethics Committee. The feed ingredients of broiler starter and finisher diets were checked for presence of AF before preparation of experimental feed to ensure that the feed was free from natural AF.

Preparation of experimental diets

Two standard control diets were formulated (Table 1) separately for starter (0-21 days) and finisher phase (22-42 days) of growth to meet the requirement of all the essential nutrients for broilers.

Table 1: Ingredient and Chemical Composition of the Control Diets

Ingredients (%)	Starter rations (0-21 d)	Finisher ration (21-42 d)
Maize	55.20	61.73
Broken rice	4.00	4.00
De oiled rice bran	-	2.00
Soya bean meal	31.50	23.00
Sunflower oil cake	6.00	6.00
Dicalcium phosphate	1.40	1.10
Lime stone	1.20	1.50
Trace mineral mixture[1]	0.15	0.15
Vitamin premix[2]	0.10	0.10
Common salt	0.30	0.30
Lysine	-	0.02
Methionine	0.15	0.10
Total	100.00	100.00
Nutrient composition (Analyzed)		
Crude protein (%)	21.98	19.50
Calcium (%)	1.01	1.09
Total phosphorus (%)	0.79	0.78
Copper (ppm)	10.00	12.00
Manganese (ppm)	42.00	38.00
Zinc (ppm)	42.00	39.00
Nutrient composition (Calculated)		
Metab. energy (Kcal/kg)	2849	2901
Lysine (%)	1.20	0.99
Methionine (%)	0.51	0.42

[1] Trace mineral mixture at this added level (mg/kg diet) provides: $CuSO_4.5H_2O$– 20 mg; $FeSO_4.7H_2O$ – 200 mg; KIO_3 – 2 mg; $MnSO_4.H_2O$ – 123 mg; $ZnSO_4.7H_2O$ – 176 mg; [2] The vitamin premix supplied vitamin A, 8250 IU; vitamin D_3, 1200 ICU; vitamin K, 1 mg; vitamin E, 40 IU; vitamin B_1, 2 mg; vitamin B_2, 4 mg; vitamin B_{12}, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg; choline, 500 mg kg^{-1} diet.

The experimental design followed was 3 × 3 factorial and the experiment consisted of nine treatments as follows:

T1 – Control diet

T2 – Control diet + 0.5 ppm AF B_1

T3 – Control diet + 1 ppm AF B_1

T4 – Control diet + 40 ppm Cu from organic source

T5 – Control diet + 0.5 ppm AF B_1 + 40 ppm Cu from organic source

T6 – Control diet + 1 ppm AF B_1 + 40 ppm Cu from organic source

T7 – Control diet + 40 ppm Cu from inorganic source

T8 – Control diet + 0.5 ppm AF B_1 + 40 ppm Cu from inorganic source

T9 – Control diet + 1 ppm AF B_1 + 40 ppm Cu from inorganic source

Copper sulphate and copper propionate with 30 per cent Cu (supplied by M/s. Kemin Nutritional Technologies India Pvt. Ltd., Gummidipundi, Chennai) were used as the source of inorganic and organic copper, respectively.

Data collection

The data on weekly body weigh gain of broiler chicks during 0-6 weeks of age were collected and analyzed for starting (0-21days), finishing (22-42 days) and overall (0-42 days) experimental period. The feed intake of the experimental birds was measured at weekly intervals up to 6 weeks of age. The weekly and cumulative feed conversion ratio (FCR) was calculated by dividing the amount of feed consumed with corresponding per unit gain in body weight by the chicks. The mortality as and when occurred under each experimental treatment was duly recorded and accordingly calculated.

Statistical analysis

The experimental design followed was 3 × 3 factorial design. The data obtained from the above experiments were subjected to statistical analysis as per standard procedure of Snedecor and Cochran, (1989) and Duncan's multiple range test (Duncan, 1955) for verifying significance of treatment means.

RESULTS AND DISCUSSION

The result revealed that the BWG was significantly ($P<0.01$) depressed in a dose dependent fashion due to dietary AF levels and it was lowest in 1 ppm AF level and highest in basal AF level during 0-3 weeks, 4-6 weeks and also during 0-6 weeks, due to the effect of AF levels (Table 2). The mean BWG showed a significant difference due to Cu supplementation and it was higher in Cu chelate and Cu supplemented group than Cu inorganic group during 0-3 weeks. The mean BWG during 4-6 weeks and 0-6 weeks were highest in Cu chelate group followed by control and it was lowest in Cu inorganic group. Similar findings was made by several authors (Gopi, 2006; Vasan *et al.*, 1998; Verma, 1990**;** Doerr *et al.*, 1983) who reported reduced weight gain, feed intake and feed efficiency in a dose dependent fashion due to dietary AF in poultry. However, these authors reported reduced performance of broiler at various dietary concentration of AF, ranging from 0.5 ppm AF to 2 ppm AF level. In contrary to this finding, Sinha and Arora (1987) could not observe any adverse effect on the performance of broilers up to 1 ppm level of dietary AF. The reduced BWG might be due to reduced palatability of feed leading to reduced feed intake and feed utilization of nutrients.

The BWG during 0-3 weeks did not differ significantly due to interaction between Cu supplementation and AF levels whereas, the mean BWG during 4-6 weeks and 0-6 weeks differed significantly ($P<0.01$) due to the interaction. The BWG during 4-6 weeks was highest in all Cu groups at basal AF level and lowest in Cu inorganic group at 1 ppm AF level, while the mean BWG of other groups were found to be intermediary. The Cu chelate increased the BWG at 0.5 ppm AF level, although it was not significant. But the Cu chelate significantly increased the BWG at 1ppm AF. In agreement with the result, Llewellyn *et al.* (1981) indicated that Cu acetate caused higher body weight in Syrian hamster. However, Seffner *et al.* (1997) found more severe liver injury in guinea pigs administered AFB1 and Cu as $CuSO_4$ than animals with AFB_1 alone.

The result on feed intake revealed a significant ($P<0.01$) difference due to AF levels during 0-3 weeks, 4-6 weeks and 0-6 weeks, and it was significantly lower at 1 ppm AF level, followed by 0.5 ppm AF than basal AF, due to the effect of AF levels. The feed intake during 4-6 weeks of age differed significantly ($P<0.05$) due to different Cu supplementation. Significantly higher feed intake was observed in Cu unsupplemented group and Cu chelate group than Cu inorganic group. However, the Cu unsupplemented group did not differ significantly with either Cu chelate group or Cu inorganic group. The feed intake during 0-3 and 0-6 weeks of age did not differ significantly due to different Cu supplementations. The feed intake during 0-3 weeks did not differ significantly

Table 2: Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broiler chickens as influenced by different copper supplementation and aflatoxin Levels

Treatment		BWG, FI and FCR								
Cu Supplementation	Aflatoxin level	0-3 weeks			4-6 Week			0-6 week		
		BWG	FI	FCR	BWG	FI	FCR	BWG	FI	FCR
Interaction effect (Cu supplementation × Aflatoxin level)										
Unsupplemented	basal	362.57	661.35	1.82	872.23^{e}	1933.47^{c}	2.22^{a}	1234.83^{e}	2594.82^{c}	2.10
	0.5 ppm	342.53	640.09	1.87	592.20^{d}	1665.13^{b}	2.81^{c}	934.77^{d}	2305.22^{b}	2.47
	1.0 ppm	289.37	565.82	1.95	392.67^{b}	1222.37^{a}	3.11^{c}	682.03^{b}	1788.19^{a}	2.62
Cu Chelate	basal	363.37	665.22	1.83	856.37^{e}	1914.71^{c}	2.24^{a}	1219.73^{e}	2579.93^{c}	2.12
(40 ppm)	0.5 ppm	330.03	624.29	1.89	629.03^{d}	1578.49^{b}	2.51^{ab}	959.07^{d}	2202.78^{b}	2.30
	1.0 ppm	304.97	582.28	1.91	509.80^{c}	1510.30^{b}	2.96^{c}	814.77^{c}	2092.58^{b}	2.57
Cu Inorganic	basal	339.97	652.03	1.92	897.40^{e}	2040.41^{c}	2.28^{a}	1237.37^{e}	2692.44^{c}	2.18
(40 ppm)	0.5 ppm	326.23	646.80	1.98	584.60^{d}	1471.84^{b}	2.52^{ab}	910.83^{d}	2118.65^{b}	2.33
	1.0 ppm	282.63	588.99	2.09	289.90^{a}	1044.82^{a}	3.60^{d}	572.53^{a}	1633.80^{a}	2.86
Pooled SEM		3.036	8.051	0.018	13.603	64.192	0.093	15.327	69.969	0.053
Main effect – Cu supplementation										
	Unsupple	331.49^{m}	622.42	1.88^{m}	619.03^{n}	1606.99^{mn}	2.71	950.54^{n}	2229.41	2.40
	Cu Chelate	332.79^{m}	623.93	1.88^{m}	665.07^{o}	1667.83^{m}	2.57	997.86^{o}	2291.76	2.33
	Cu Inorg.	316.28^{n}	629.27	2.00^{n}	590.63^{m}	1519.02^{n}	2.80	906.91^{m}	2148.30	2.46
Main effect – Aflatoxin level										
	basal	355.30^{x}	659.53^{x}	1.86^{x}	875.33^{x}	1962.86^{x}	2.24^{x}	1230.64^{x}	2622.40^{x}	2.13^{x}
	0.5 ppm	332.93^{y}	637.06^{x}	1.91^{x}	601.94^{y}	1571.82^{y}	2.61^{y}	934.89^{y}	2208.88^{y}	2.36^{y}
	1.0 ppm	292.32^{z}	579.03^{y}	1.98^{y}	397.46^{z}	1259.16^{z}	3.22^{z}	689.78^{z}	1838.19^{z}	2.68^{z}
Probabilities										
	Interaction	NS	NS	NS	$P<0.01$	$P<0.01$	$P<0.05$	$P<0.01$	$P<0.01$	NS
	Cu Supp.	$P<0.05$	NS	$P<0.01$	$P<0.01$	$P<0.05$	NS	$P<0.01$	NS	NS
	AF level	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$

abcde (interaction); mno (Cu supplementation); xyz (AF level); Values bearing different superscripts within a column differ significantly ($P<0.05$), ($P<0.01$); NS- Non Significant

Table 3: Livability in broiler chickens as influenced by different copper supplementation and aflatoxin levels

Treatment		Livability percentage
Cu supplementation	Aflatoxin level	
Interaction effect (Cu supplementation × Aflatoxin level)		
Unsupplemented	basal	96.67
	0.5 ppm	90.00
	1.0 ppm	83.33
Cu Chelate(40 ppm)	basal	93.33
	0.5 ppm	90.00
	1.0 ppm	86.67
Cu Inorganic (40 ppm)	basal	100.00
	0.5 ppm	90.00
	1.0 ppm	83.33
Pooled SEM		1.250
Main effect - Cu supplementation		
	Unsupple.	90.00
	Cu Chelate	90.00
	Cu Inorganic	91.11
Main effect - Aflatoxin level		
	basal	96.67^{x}
	0.5 ppm	90.00^{y}
	1.0 ppm	84.44^{z}
Probabilities		
	Interaction	NS
	Cu Supp.	NS
	AF level	P<0.01

xyz (AF level); Values bearing different superscripts within a column differ significantly (P<0.05), (P<0.01); NS- Non Significant

due to interaction between Cu supplementations and AF levels. But the feed intake during 4-6 weeks and 0-6 weeks differed significantly (P<0.01) due to the interaction. The mean feed intake during 4-6 weeks and 0-6 weeks was significantly highest in Cu groups at basal AF level followed by Cu group at 0.5 ppm AF level than Cu chelate or Cu unsupplemented groups and 1 ppm AF, while the feed intake of other treatments were found to be intermediary.

The FCR during 0-3 weeks, 4-6 weeks and 0-6 weeks differed significantly (P<0.01) among treatments due to AF levels. The FCR during 0-3 weeks was lower in 0 and 0.5 ppm AF level than 1 ppm AF level. The FCR during 4-6 weeks and 0-6 weeks was significantly increased in a dose dependent fashion with lowest FCR in basal AF level and highest at 1 ppm AF level. The FCR during 0-3 weeks was significantly (P<0.01) lower in Cu unsupplemented and Cu chelate group than Cu inorganic group, due to the effect of Cu supplementation. Non significant effect was observed on FCR during 4-6 weeks and during 0-6 weeks due to Cu supplementation. The interaction effect of Cu supplementation with AF level on FCR was significant (P<0.05) at 4-6 weeks and non-significant at 0-3 weeks and at 0-6 weeks. The FCR during 4-6 weeks was lower in Cu groups at basal AF level than Cu inorganic group at 1 ppm AF level, whereas the FCR of other groups were found to be intermediary.

The result of the study is also supported by earlier finding of many authors (Sharma *et al.*, 1989; Bartov, 1983) who observed that $CuSO_4$ did not counteract aflatoxicosis in chicken. In contrary, Johri *et al.* (1986) found that the growth depression due to mouldy corn was overcome by addition of 150 ppm of $CuSO_4$.

The result suggests that there is a difference between chelated and inorganic Cu in their effect under AF challenge. The role of propionic acid in Cu-propionate against aflatoxicosis is also doubtful because of the trace amount of propionic acid, whereas the minimum effective level of propionic acid in feed was found to be 0.15 per cent (Johri *et al.*, 1986). Arias and Koutsos (2006), in one experiment found that broilers with supplemented Cu as tribasic copper chloride had higher body weight and not with Cu in the form of $CuSO_4$ and concluded that the broiler performance could be influenced by dietary Cu source and level. However, there is a little study comparing the chelated and inorganic Cu under AF challenge and more detailed study and comprehensive information appears necessary on the form of Cu and its influence during aflatoxicosis.

The present study indicates that the livability percentage ranges from 96.67 to 84.44 with higher livability in basal AF group and lower livability in 1 ppm AF group. This finding are well in agreement with Gopi (2006), Reddy *et al.* (1982) who reported significant reduction in livability at 0.5 to 1.25 ppm level of dietary AF in broilers.

The supplemental Cu in either form at 40 ppm was found to have no effect on the decrease in livability due to dietary AF. On the contrary, Llewellyn *et al.* (1981) observed higher livability in Syrian hamster undergoing AF and Cu treatment, than AF alone.

Conclusion

From the results, it was observed that there was a difference between organic and inorganic copper in their effect in counteracting aflatoxicosis in broilers. The organic form of copper was able to counteract the aflatoxicosis in broiler, while inorganic copper was found to aggrevate the aflatoxicosis.

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