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Research Article

BIOINTERACTION OF CHELATED AND INORGANIC ZINC WITH AFLATOXIN ON FEED INTAKE AND NUTRIENT RETENTION OF BROILER CHICKEN

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ABSTRACT
A biological experiment was conducted to evaluate the interactive effects of organic and inorganic zinc (Zn) supplementation with aflatoxin (AF) on nutrient retention and carcass quality 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 Zn supplemented group, i.e. 0 ppm, 200 ppm organic Zn (as propionate), 200 ppm inorganic Zn (as sulphate). The study was made for 0-42 days and a nutrient balance trial was conducted during 6^{th} week of age for determination of feed intake and retention of dry matter (DM), crude protein (CP), calcium (Ca) and phosphorus (P). The results revealed that the feed intake and absolute retention of nutrients were significantly reduced due to dietary AFB_1 in a dose dependent fashion with largest feed intake and nutrient retention in 0 ppm AFB_1 groups and lowest in 1 ppm AFB_1 group. Significantly ($P<0.01$) higher feed intake, retention of DM, P, Mn and Zn were noticed in Zn chelate and Zn inorganic groups than Zn unsupplemented group, due to Zn supplementation. The mean feed intake, retention of DM, CP, Ca, P and Cu were significantly ($P<0.01$, except for CP, Ca for which $P<0.05$) highest in all Zn groups at basal AF level and lowest in Zn unsupplemented group at 1 ppm, whereas the other mean retention of other treatment groups were found to be intermediary, due to the interaction between different Zn supplementations and AF levels. But the mean retention of Zn among treatments due to interaction of Zn and AF level was significantly higher ($P<0.01$) in Zn chelate group than Zn unsupplemented group at 1 ppm AF level, while Zn retention of other treatment groups were found to be intermediary. However, the retention of Mn did not differ significantly among treatment groups, due to interaction between different Zn supplementation and AF levels.

Key Words: *Aflatoxin, Zinc, Feed intake, Nutrient retention*

INTRODUCTION
The Aflatoxins (AF) AF are group of closely related secondary metabolites (B_1, B_2, G_1 & G_2,) produced by certain strains of Genus *Aspergillus*. Aflatoxins have been demonstrated to be carcinogenic, mutagenic, teratogenic and toxic (Cole and Cox, 1981) and the threat of aflatoxin is the common problem in poultry. 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. 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 zinc-Zn (Hegazy and Adachi, 2000), 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 reports 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 zinc on feed intake and nutrient retention of broiler chicken.

MATERIALS AND METHODS

The study was carried out in broilers at the Avian Nutrition and Feed Technology (ANFT) Division, Central Avian Research Institute (CARI), Izatnagar, Bareilly, Uttar Pradesh, India. Zinc sulphate was used as source of inorganic mineral supplementation, while zinc propionate with 30% Zn (supplied by M/s. Kemin Nutritional Technologies India Pvt. Ltd., Gummidipundi, Chennai) was used as source of organic mineral supplementation.

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) 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.

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 obtained from Experimental Broiler Farm, CARI belonging to single hatch were used. The birds 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 birds were immunized against New Castle Disease and Marek's Disease at day old by occulonasal route and against Infectious Bursal Disease at 14th day of age. The experimental protocol had the agreement of Animal Ethics Committee.

Preparation of experimental diets

Two standard basal 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.

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

T1 – Basal diet
T2 – Basal + 0.5 ppm AF B_1
T3 – Basal + 1 ppm AF B_1
T4 – Basal diet + 200 ppm Zn from organic source
T5 – Basal + 0.5 ppm AF B_1 + 200 ppm Zn from organic source
T6 – Basal + 1 ppm AF B_1 + 200 ppm Zn from organic source
T7 – Basal diet + 200 ppm Zn from inorganic source
T8 – Basal + 0.5 ppm AF B_1 + 200 ppm Zn from inorganic source
T9 – Basal + 1 ppm AF B_1 + 200 ppm Zn from inorganic source

Zinc sulphate and zinc propionate were used as the source of inorganic and organic zinc, respectively.

Nutrient balance study

A balance trial of 3 days duration at 6th week of age was conducted for determination of dry matter, protein, Ca, P, Cu, Mn and Zn retention.

The study was preceded with three hour starvation at the beginning and the birds were given weighed experimental diets daily for 3 days at a fixed hour in the morning. The polythene sheets were spread on

faecal tray for collection of excreta. Next day at the same, excreta were collected and weighed. The representative samples of faecal material after mixing it well were collected in polybags for dry matter, nitrogen and trace mineral estimations. The same procedure for faecal material was repeated for two more days and the samples of excreta collected for 3 days were pooled for analysis. On the last day of balance trail period, the feed troughs were removed at an appropriate hours and faecal material from each cage was removed only after a lapse of three hours. The weight of residual feed in each feeder was recorded and feed intake by a group of birds over the 3 days period was worked out. The pooled samples of excreta were subsequently dried in hot air oven at 70^0 C. to constant weights, and then their final weight recorded. The dried samples were then pulverized and stored in air tight plastic container until further analysis.

For dry matter estimation, the total dry matter voided in 3 days was calculated. Samples of feed and excreta were analyzed for crude protein (AOAC, 1990), Ca (Talapatra *et al.*, 1940), total P (AOAC, 1990) Cu, Mn and Zn using Atomic Absorption Spectrophotometer.

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 retention of dry matter, crude protein (CP), Ca and P were expressed as g/ bird/ day, whereas retention of trace minerals was expressed as mg/ bird/ day.

$$\text{Nutrient retention} = \frac{\text{Nutrient intake} - \text{Nutrient output}}{\text{Nutrient intake}}$$

Table 2: Nutrient retention in broiler chickens as influenced by different zinc supplementation and aflatoxin levels

Treatment			Nutrient Retention						
Zn Supplementation	**Aflatoxin level**	**Feed Intake (g/b/d)**	**Dry Matter (g/b/d)**	**Crude Protein (g/b/d)**	**Calcium (g/b/d)**	**Phosphorus (g/b/d)**	**Copper (mg/b/d)**	**Manganese (mg/b/d)**	**Zinc (mg/b/d)**
Interaction effect (Zn supplementation × Aflatoxin level)									
Unsupplemented	basal ppm	116.70^{d}	72.24^{d}	10.63^{d}	0.691^{d}	0.502^{c}	0.373^{d}	1.452	1.290^{b}
	0.5 ppm	94.58^{bc}	56.12^{bc}	7.12^{c}	0.438^{bc}	0.348^{b}	0.185^{bc}	0.907	0.901^{ab}
	1.0 ppm	67.85^{a}	38.83^{a}	3.37^{a}	0.272^{a}	0.194^{a}	0.108^{a}	0.496	0.490^{a}
Zn Chelate (200 ppm)	basal ppm	117.94^{d}	72.40^{d}	10.67^{d}	0.667^{d}	0.495^{c}	0.361^{d}	1.445	6.043^{g}
	0.5 ppm	93.22^{bc}	55.75^{bc}	6.86^{bc}	0.498^{c}	0.358^{b}	0.191^{bc}	0.936	3.519^{de}
	1.0 ppm	89.33^{b}	53.12^{b}	5.45^{b}	0.393^{b}	0.325^{b}	0.159^{b}	0.729	2.277^{c}
Zn Inorganic (200 ppm)	basal ppm	119.93^{d}	76.70^{d}	10.51^{d}	0.716^{d}	0.519^{c}	0.351^{d}	1.515	5.156^{f}
	0.5 ppm	101.67^{c}	60.12^{c}	7.29^{c}	0.520^{c}	0.357^{b}	0.210^{c}	1.055	4.155^{e}
	1.0 ppm	90.00^{b}	54.78^{bc}	6.38^{bc}	0.488^{c}	0.361^{b}	0.177^{bc}	0.747	3.069^{d}
Pooled SEM		3.234	2.276	0.488	0.029	0.020	0.019	0.069	0.361
Main effect - Zn supplementation									
	Unsupple.	93.04^{m}	55.73^{m}	7.04^{m}	0.467^{m}	0.348^{m}	0.222^{m}	0.952^{m}	0.894^{m}
	Zn Chelate	100.16^{n}	60.42^{n}	7.66^{mn}	0.519^{n}	0.393^{n}	0.237^{mn}	1.036^{n}	3.947^{n}
	Zn inorganic	103.87^{n}	63.87^{n}	8.06^{n}	0.575^{o}	0.412^{n}	0.246^{n}	1.106^{n}	4.127^{n}
Main effect - Aflatoxin level									
	basal ppm	118.19^{x}	73.78^{x}	10.60^{x}	0.691^{x}	0.505^{x}	0.362^{x}	1.471^{x}	4.163^{x}
	0.5 ppm	96.49^{y}	57.33^{y}	7.09^{y}	0.485^{y}	0.354^{y}	0.195^{y}	0.966^{y}	2.858^{y}
	1.0 ppm	82.40^{z}	48.91^{z}	5.07^{z}	0.384^{z}	0.293^{z}	0.148^{z}	0.657^{z}	1.945^{z}
Probabilities									
	Interaction	P<0.01	P<0.01	P<0.05	P<0.05	P<0.01	P<0.01	NS	P<0.01
	Zn Supp.	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01
	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

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

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 feed intake, retention of dry matter (DM), Crude Protein (CP), Ca, P, Cu, Mn and Zn differed significantly ($P<0.01$) among treatments due to different AF levels and these nutrient retention were highest in basal AF followed by 0.5 ppm AF and lowest in 1 ppm AF group (Table 2). The aflatoxin might have caused alteration in intestinal physiology and intestinal mucosal damage due to chronic inflammation which might have lead to decreased absorption of nutrients. This is supported by earlier findings of Kelly and Arora (1976) who observed hemorrhage on intestinal organs due to AF and Balachandran and Ramakrishnan (1987b) who observed catarrhal inflammation in intestinal mucosa.

Significantly ($P<0.01$) higher feed intake, retention of DM, P, Mn and Zn were noticed in Zn chelate and Zn inorganic groups than Zn unsupplemented group, due to Zn supplementation. The mean CP and Cu retention were significantly ($P<0.05$) higher in Zn inorganic group than Zn unsupplemented group, while the Zn chelate group did not differ with other two groups. However, the retention of Ca was significantly ($P<0.01$) higher in Zn inorganic group, followed by Zn chelate group than Zn unsupplemented group.

The mean feed intake, retention of DM, CP, Ca, P and Cu were significantly ($P<0.01$, except for CP, Ca for which $P<0.05$) highest in all Zn groups at basal AF level and lowest in Zn unsupplemented group at 1 ppm, whereas the other mean retention of other treatment groups were found to be intermediary, due to the interaction between different Zn supplementations and AF levels. But the mean retention of Zn among treatments due to interaction of Zn and AF level was significantly higher ($P<0.01$) in Zn chelate group than Zn unsupplemented group at 1 ppm AF level, while Zn retention of other treatment groups were found to be intermediary. However, the retention of Mn did not differ significantly among treatment groups, due to interaction between different Zn supplementation and AF levels.

Supplementation of Zn might have reduced the inflammation of intestinal mucosa and prevented change in the intestinal physiology due to AF, subsequently leading to improvement in absorption of nutrients. Zn administration is the standard therapy for acrodermatitis enteropathica as well as non-specific malabsorption syndrome in humans (Neldner and Hambidge, 1975; Cunningham- Rundles *et al.,* 1980). Hedemann *et al.,* (2006) found that the pigs fed diet fortified with 100 ppm Zn had greater activity of amylase and carboxy peptidase-A in small intestinal content, amino peptidase N in Calldal small intestine, and longer Villi in cranial small intestine and concluded that high dietary Zn increased the activity of several enzymes in pancreatic tissue and increased mucin staining area in the large intestine.

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