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EFFECTS OF MOLDSTOP® ON AFLATOXICOSIS IN QUAILS

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Abstract

This study was conducted to evaluate the toxic effects of aflatoxin (AF) on the growth performance of quails, and to determine the preventive efficacy of MOLDSTOP® (calcium formate, calcium propionate, citric acid, sorbic acid, acetic acid, and carrier). A total of 60 one-day-old quails (*Coturnix coturnix japonica*) of both sexes were equally divided into four experimental groups each comprising of five replicates of three birds. The supplementation of diet with AF decreased significantly ($P<0.001$) the feed consumption. The 0.5% addition of MOLDSTOP® to the AF diet did not significantly prevent or reduce negative effect of AF on feed consumption at any time period. Light microscopic examination demonstrated that the addition of MOLDSTOP® did not decrease fat deposition caused by the toxin, and besides, an electron microscopic examination indicated the reorganisation in the endoplasmic reticulum and increase in the number of ribosomes and polisomes compared to the AF plus MOLDSTOP® group. The data indicated that the addition of MOLDSTOP® to diets containing AF did not prevent the negative effects of AF observed in the quail.

Key words: quail, aflatoxicosis, MOLDSTOP®, growth performance.

Aflatoxins (AF) cause severe economic losses in the poultry and livestock industries and cause a reduction in feed consumption, body weight gain, and feed efficiency, as well as increased susceptibility to environmental and microbial stress and increased mortality (10). The most suitable method to prevent AF undesired effects in the digestive tract is to add adsorbents to animals' feeds at certain rates, which hinder their absorption and alleviate their adverse effects (7). The administration of MOLDSTOP® is a new approach to prevent the deleterious effects of AF.

Material and Methods

Animals and rearing conditions. Sixty one-day-old quails of both sexes were individually weighed and randomly divided into four experimental groups. There were 5 replicates of 3 birds per dietary treatment. The quails were housed in environmentally controlled cages with paper on wire floors and fed a commercial quail chick starter. Group feeding was applied in all replications. The birds were weighed per group once a week. The feed was weighed weekly to evaluate the feed intake and feed conversion ratio per cage. The birds were maintained on a 24 h constant light schedule and allowed to consume feed and water *ad libitum*.

Diets and experimental design. The diets of experimental groups are presented in Table 1. The experimental design consisted of four dietary treatments: 1) control with no AF and MOLDSTOP (calcium formate, calcium propionate, citric acid, sorbic acid, acetic acid and carrier) supplementation; 2) 0.5% addition of MOLDSTOP®; 3) 2.5 mg of AF/kg of diet; 4) 2.5 mg of AF/kg of diet plus 0.5% addition of MOLDSTOP®. The quails were maintained with these treatments from one day to three weeks of age.

At the end of the experiment, blood samples (2 ml) were collected from 15 randomly selected birds (three birds per group). The analyses of serum glucose, total protein, and cholesterol were performed with the photometer (Merck Microlab 200, Netherlands), using the commercial kits (Randox, United Kingdom). Following blood sampling, chicks were necropsied and the liver, spleen, bursa of Fabricius, and kidney were collected; and organ weights and relative organ weights were determined.

Table 1
Chemical composition of experimental diets

Ingredients (g/kg diet)	Control	MOLDSTOP®
Corn	494.9	494.9
Soybean meal	254.2	254.2
Full-fat soybean	179.5	179.5
Rice *	33.8	33.8
$CaCO_3$	9.6	9.6
Dicalcium phosphate	19.1	19.1
Salt	2.5	2.5
Methionine	2.9	2.9
Vitamin premix[1]	2.5	2.5
Mineral premix[2]	1.0	1.0
MOLDSTOP®	-	5.0
Silica **	5.0	-
Values analysed (g/kg diet)		
Metabolisable energy, MJ/kg	13.17	13.17
Crude protein	219.5	219.5
Crude fibre	31.0	31.0
Ether extract	63.0	63.0
Ash	67.8	67.8
Dry matter	920.0	920.0

* Rice in dietary treatments fed AF included AF at level of 73.96 mg/kg.
** Groups without toxin binder included silica instead of MOLDSTOP.
[1] Each 1 kg of vitamin premix contained: 15,000,000 IU vit. A, 2,500,000 IU vit. D_3, 80,000 mg vit. E, 5,000 mg K3, 300 mg B_1, 600 mg B_2, 5,000 mg B_6, 30 mg B_{12}, 50,000 mg vit.C, nicotin amid 50,000 mg, 12,000 mg Panthotenic acid, 1,500 mg Folic acid, 100 mg D-biotin.
[2] Each 0.5 kg mineral premix contained: 80,000 mg Mn, 60,000 mg Fe, 60,000 mg Zn, 5,000 mg Cu, 200 mg Co, 1,000 mg I, 150 mg Se.

Preparation of toxin, addition of toxin and MOLDSTOP® to diet. Aflatoxin was produced, with minor modifications (3), by the method described by Shotwell *et al.,* (18) by growing *Aspergillus parasiticus* NRLL 2999 (National Center for Agricultural Utilization Research, Peoria, USDA) on rice. The rice powder was incorporated into the basal diet in order to provide the desired amount of 2.5 mg of AF/kg of feed.

Light microscopic examination. Sub-fractions of the samples taken from various parts of the liver were fixed in 10% formol-calcium solution. Tissue blocks were frozen at -20°C, and sectioned in a cryostat. To demonstrate the presence of fats in the liver, oil red O staining (2) was carried out. The other sub-fractions of the tissue samples were fixed in a formol-alcohol solution (1), and paraplast sections were prepared. The periodic acid Schiff (PAS) reaction was used to identify glycogen (4), while the diastase/PAS reaction was used for enzymatic confirmation of glycogen.

Electron microscopic examination. The liver specimens taken for electron microscopy were kept for 24 h in glutaraldehyde-paraformaldehyde for pre-fixing (pH 7.4) (8), rinsed for 3 h in a cacodylate buffer and then fixed for a second time in a 1% osmic acid solution for 2 h. After storing in a 0.5% uranyl acetate solution for 2 h, the samples were passed through graded alcohols and propylene oxide and embedded in araldite M. Sections of 300–400 Å in thickness were prepared and contrasted according to the method of Venable and Coggeshall (20) and then examined in a Carl Zeiss EM 9S-2 model transmission electron microscope.

Statistical analysis. The data for all response variables were subjected to ANOVA (19) using SPSS programme. Significant differences among treatment means were determined using Duncan's multiple range test (5) with a 5% level of probability.

Results and Discussion

All performance results are given in Table 2 (initial body weight, body weight gain, feed consumption and feed conversion ratio).

The supplementation of feed with AF alone or with MOLDSTOP® resulted in a significant depression in feed consumption while body weight gain and feed conversion ratio (FCR) remained unchanged. Our results agree with previous studies as regards body weight gains (11, 12), feed consumption, and FCR (16-17) in poultry. No significant differences were found between chickens fed the control diet and chickens fed the diets containing MOLDSTOP® alone, indicating that the MOLDSTOP® was inert and non-toxic. Our results as regards the body weight gain seem to differ from those observed by Şehu *et al*. (16). These authors found a significant decrease in the body weight gain. In the present study, the body weight gain results obtained from groups fed AF tend to be lower than other treatment groups.

Table 2
Initial body weight (b.w.), body weight gain (b.w. gain), feed consumption, and feed conversion ratio of experimental groups (n=5)

MOLDSTOP® (%)	0	0.5	0	0.5	SEM
AF (mg/kg)	0	0	2.5	2.5	
Initial b.w.	9.8	10.07	10.17	10.46	0.101
B.w. gain (g), overall	97.26	100.37	85.74	84.18	3.037
Feed consumption (g/bird), overall	120.07^{a}	113.40^{a}	70.60^{b}	80.83^{b}	8.323***
Feed conversion ratio (g/g), overall	2.61	2.55	2.22	2.43	0.085

a, b - means on the same row followed by different letters significantly differ; AF – aflatoxin; ***P<0.001

Table 3
The effect of toxin binder on organ weights and some blood parameters (n=15)

MOLDSTOP® (%)	0	0.5	0	0.5	SEM
AF (mg/kg)	0	0	2.5	2.5	
Organ weights (g)					
Liver	2.39	2.74	2.49	2.37	0.064
Spleen	0.04	0.06	0.05	0.09	0.010
Bursa of Fabricius	0.09	0.11	0.07	0.11	0.010
Kidney	0.49^{b}	0.72^{a}	0.58^{b}	0.61^{ab}	0.023**
Relative organ weights (g/100 g b.w.)					
Liver	2.23	2.48	2.72	2.59	0.076
Spleen	0.04	0.05	0.06	0.09	0.009
Bursa of Fabricius	0.07	0.10	0.08	0.12	0.011
Kidney	0.45^{b}	0.65^{a}	0.64^{a}	0.66^{a}	0.022***
Blood parameters					
Glucose (mg/dL)	15.40	20.62	21.55	18.33	1.698
Total protein (g/dL)	2.97	3.05	2.65	2.60	0.088
Cholesterol (mg/dL)	109.00^{a}	130.10^{a}	83.30^{b}	86.55^{b}	4.734***

a-c - means on the same column followed by different letters differ significantly; AF - aflatoxin
** P<0.01, *** P<0.001

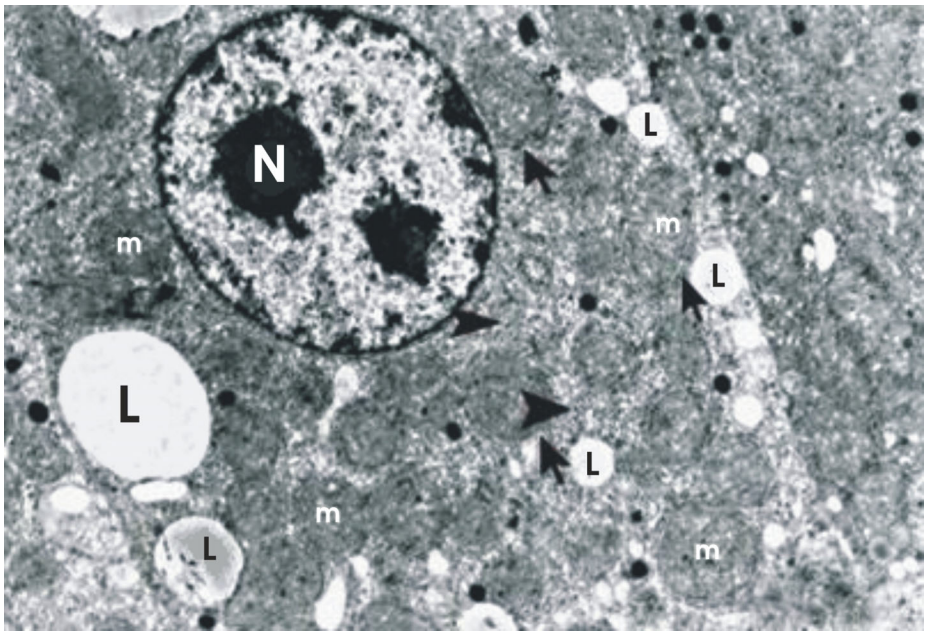


Fig. 1. Electron micrograph of a section of hepatocyte from quail fed ration containing AF plus MOLDSTOP®. Lipid droplets (L), rough endoplasmic reticulum (arrows), ribosomes (arrow heads), mitochondria (m), nucleus (N). x 19,000.

Significant decrease in feed consumption may have resulted from anorexia, listlessness, and the inhibitory effect of AF on protein synthesis and lipogenesis (9, 16). The addition of MOLDSTOP® to the AF diet did not recover the feed consumption-depressing effect of AF. The decreased feed consumption observed in birds fed AF was similar with previous studies (6, 13, 16, 17).

Concerning of organ weight parameters, addition of AF and MOLDSTOP® had no individual or combined effect on organ weights, except kidney weights (Table 3). Data for kidney weight was similar to data reported by Şehu *et al*. (17). Increases in the relative weights of the liver of birds fed AF were reported previously (10, 14). In the present study, AF alone and AF plus MOLDSTOP® did not cause a significant change in the liver, spleen, and bursa of Fabricius weights. A protective effect of MOLDSTOP® against changes in kidney weights was not observed in the current study.

Normally, changes in serum total protein indicate the impaired protein synthesis that was observed previously in quail suffering from aflatoxicosis (12). In the present study, the total protein and glucose levels did not change significantly. The total cholesterol level was found lower in AF groups. Keçeci *et al.* (9), have shown that some serum biochemical changes could be ameliorated by bentonite administration to the diet at doses of 5 mg/kg in broiler chickens given 2.5 mg of AF/kg diet. However, in our case, the biochemical parameters of broilers fed diets containing AF alone and MOLDSTOP® plus AF did not completely return to normal values, showing a decrease in cholesterol levels. These results agree with the findings reported by Rosa *et al.* (15), who observed a decrease in this parameter during the aflatoxicosis in poultry. The findings for blood levels corroborate data reported by Kubena *et al.* (13) and Şehu *et al.* (16).

The typical cytoplasmic vacuolar degeneration observed in the hepatocytes upon the histological examination of the liver complied with the findings reported by other researchers (14, 17). The level of the decrease observed in the fat deposition of the liver in quails that were fed the AF alone and AF plus MOLDSTOP® diet was insignificant. There was no significant difference in the liver glycogen storage between control and experimental groups (PAS staining). However, electron microscopy revealed the reorganisation in the rough endoplasmic reticulum, and an increase in the number of ribosomes and polisomes of hepatocytes was evident in AF plus MOLDSTOP® group (Fig. 1). These results were in agreement with those of Şehu *et al*. (17).

As it was shown in the present study, no beneficial effect of MOLDSTOP® supplementation on quails with aflatoxicosis could be demonstrated. However, this does not exclude the possibility that supplementation of this active substance may be beneficial in other circumstances. We suggest that further *in vivo* experiments comparing these kinds of adsorbents should be conducted.

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