Research Notes P-100

Arm & Hammer Animal and Food Production

BG-MAX reduced effects of mycotoxin contaminated feed fed to broilers.

BG-MAX™ combines the benefits of Refined Functional Carbohydrates™ (RFCs™) with a bentonite specifically processed to reduce poor-quality feed challenges.

STUDY OVERVIEW

A study[1] was conducted to test the effect of BG-MAX supplementation on growth performance, serum chemistry, gut integrity and morphology, immunity function, and aflatoxin metabolism and residue in broilers fed diets naturally contaminated with aflatoxin B1 (AFB1), zearalenone (ZEN) and deoxynivalenol (DON).

Trial design and treatments

A total of 480 (1-day-old) Arbor Acre male broilers received one of six different treatments made up of two diets and three inclusion rates of BG-MAX arranged in a 2 x 3 factorial design. The trial was conducted in eight replicate cages per treatment with 10 birds per cage for 42 days. The BG-MAX inclusion rates were 0 kg/MT, 1 kg/MT (BG-MAX low) and 2 kg/MT (BG-MAX high). The control diet was formulated with clean corn (AFB <20 ppb, DON=1.3ppm, ZEN=269ppb) and the mycotoxin diet was formulated with moldy corn (AFB1 130 ppb, DON=1.5ppm, ZEN=496ppb).

Growth performance parameters

Body weight and feed intake were measured so that average daily gain, average daily feed intake and feed conversion ratio (FCR) could be calculated and analyzed throughout the study.

Physiological and biochemical parameters

At day 42, 8 birds (1 bird per pen) in each treatment were selected. Serum was collected to determine antioxidant parameters and vaccine titers. Birds were sacrificed and samples were collected from the liver to measure expression of aflatoxin B1 metabolizing enzymes using RT-qPCR and reported as fold-change compared to control. Jejunal samples were collected, and RNA extracted, to measure mRNA expression of proteins involved in oxidative stress, intestinal barrier function proteins and Toll-like receptors (TLR) 2 and 4 and reported as fold-change. Jejunal samples were analyzed for morphological indices. Liver samples were analyzed to determine AFB1 residues.

Statistical analysis

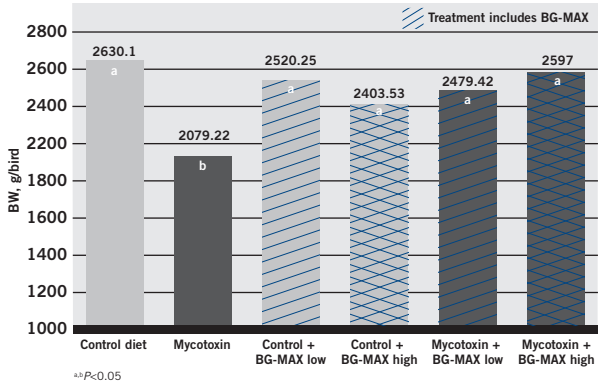
The data were analyzed by two-way analysis of variance using the general linear model (GLM) procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA) as a 2×3 factorial arrangement with dietary mycotoxin and BG-MAX inclusion rate as main effects, as well as their interaction. When interactions were significant *(P*<0.05*)*, differences between means were determined using Tukey's procedure.

RESULTS

Growth performance

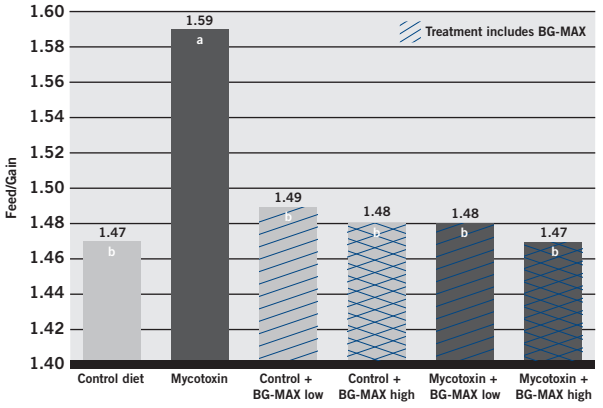
- The body weight of the broilers fed the mycotoxin contaminated diet was 551 g lower and FCR was 12 points higher than broilers fed the control diet, displaying a negative effect when moderate levels of mycotoxins were present in the feed. (Fig. 1).
- Broilers fed the mycotoxin contaminated diet supplemented with either inclusion rate of BG-MAX™ had a higher body weight and lower FCR compared to broilers fed the mycotoxin contaminated diet without BG-MAX supplementation (Fig. 1).
- Broilers fed the control diet or the mycotoxin contaminated diet supplemented with BG-MAX had similar body weights and FCR compared to broilers fed the control diet. (Figs. 1 and 2).
- No significant difference in growth performance was observed in broilers fed BG-MAX at either inclusion rate.

FIGURE 1: Effect of treatments on 42 d weight (g/bird).



[a,b]$P<0.05$

FIGURE 2: Effect of treatments on FCR (feed/gain).



[a,b]$P<0.05$

Intestinal gene expression and morphology

Aflatoxin B1 exposure has been reported to cause impaired protein activity which could cause increased translation activity. This study measured expression of two key translation initiation factors, 4EBP1 and S6K1, of the mTOR pathway which is responsible for cell growth, survival and proliferation. Broilers fed the mycotoxin contaminated diet had a significantly higher expression of 4EBP1 compared to broilers fed all other treatment diets, including broilers fed the mycotoxin contaminated diet supplemented with BG-MAX (Table 1). No treatment effects were observed on expression of S6K1.

Mycotoxin exposure causes cellular oxidative stress which can lead to membrane phospholipid degradation and a compromised intestinal barrier. In this study, expression of cellular oxidative stress indicators HMOX, HIF-1α and XOR was measured. Broilers fed the mycotoxin contaminated diet had significantly higher HMOX and XOR expression compared to broilers fed all other treatment diets, including broilers fed the mycotoxin contaminated diet supplemented with BG-MAX (Table 1).

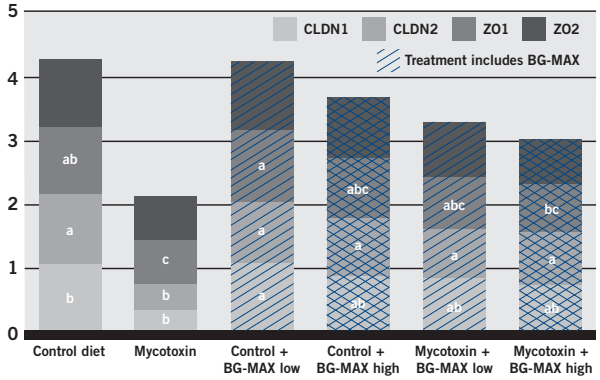
TABLE 1 Effect of treatments on fold-change in mRNA expression of translation factors and markers of cellular oxidative stress.

Treatment	4EBP1	S6K1	HMOX	HIF-1a	XOR
Control	1.06±0.13[b]	1.04±0.12	1.05±0.11[b]	1.10±0.16	1.05±0.12[b]
Mycotoxin	2.08±0.45[a]	1.27±0.09	2.07±0.34[a]	1.45±0.13	1.65±0.28[a]
Control + BG-MAX low	1.03±0.10[b]	0.75±0.15	1.10±0.13[b]	0.90±0.14	0.94±0.12[b]
Control + BG-MAX high	0.88±0.16[b]	0.66±0.08	1.13±0.12[b]	1.14±0.10	0.86±0.10[b]
Mycotoxin + BG-MAX low	1.17±0.17[b]	1.08±0.31	1.24±0.21[b]	1.33±0.16	1.13±0.22[b]
Mycotoxin + BG-MAX high	0.94±0.16[b]	0.81±0.15	1.10±0.26[b]	1.28±0.09	0.95±0.16[b]

[abc] Different superscripts within columns indicate significant differences between treatments ($P \leq 0.05$)

Intestinal barrier function proteins Claudin 1 (CLDN1), Claudin 2 (CLDN2), Zonaoccludin 1 (ZO1) and Zonaoccludin 2 (ZO2) play a key role in maintaining gut integrity and permeability. A decrease in their expression could indicate compromised gut integrity. No treatment effects were noted for ZO2. Expression of CLDN1, CLDN2 and ZO1 was reduced in broilers fed the mycotoxin contaminated diet compared to control fed broilers. Broilers fed diets supplemented with BG-MAX™ had similar CLDN2 expression as broilers fed the control diet, while expression of CLDN1 and ZO1 was intermediate (Fig. 3). A compromised gut integrity can increase translocation of toxic compounds, as well as allow an increase in passage of bacteria.

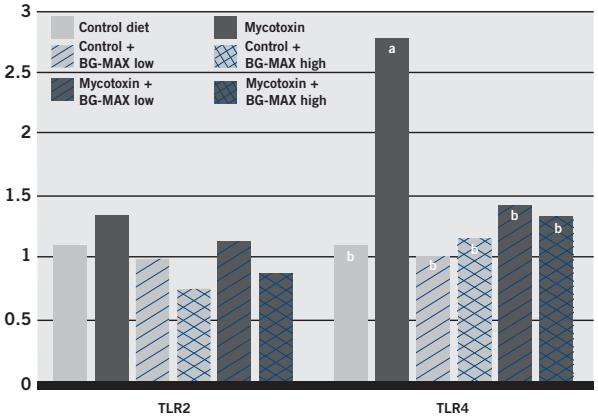
FIGURE 3: Effect of treatments on fold-change in expression of intestinal barrier function proteins.



[abc] Different superscripts within columns indicate significant differences between treatments ($P \leq 0.05$)

A significant increase in jejunal expression of TLR4, but not TLR2, was noted in broilers fed the mycotoxin contaminated diet compared to control fed broilers or broilers fed the mycotoxin contaminated diet supplemented with BG-MAX (Fig. 4). Increase in TLR4 expression is generally associated with the presence of gram negative bacteria and TLR2 with gram positive bacteria.

FIGURE 4: Effect of treatments on fold-change in expression of Toll-like receptors in the jenunum.



[abc] Different superscripts within columns indicate significant differences between treatments ($P \leq 0.05$)

Mixed treatment effects were noted on villi height (VH), crypt depth (CD) and VH/CD ratio. Broilers fed the control diet supplemented with BG-MAX had significantly greater VH and VH/CD compared to broilers fed any other treatment. Presence of mycotoxins in the diet negatively affected VH and VH/CD ratio. Broilers fed diets supplemented with BG-MAX at the higher inclusion rate in the mycotoxin contaminated diet had increased VH and VH/CD similar to control fed broilers (Table 2).

TABLE 2 Effect of BG-MAX on the villus height, crypt depth and villus height/crypt depth of jejunum.

Treatment	VH (µm)	CD (µm)	VH/CD
Control	1312.85±81.83^{ab}	155.71±15.24	9.02±0.85^{abc}
Mycotoxin	1220.46±49.55^{a}	175.41±11.00	7.31±0.59^{a}
Control + BG-MAX low	1431.78±58.66b^{bc}	137.47±7.90	10.76±0.71^{cd}
Control + BG-MAX high	1544.80±58.60^{c}	134.49±8.22	11.99±0.91^{d}
Mycotoxin + BG-MAX low	1194.13±31.25^{a}	152.91±9.48	8.06±0.42^{ab}
Mycotoxin + BG-MAX high	1328.98±61.36^{ab}	145.98±11.51	9.72±0.88^{bc}

[abcd] Different superscripts within columns indicate significant differences between treatments ($P \leq 0.05$)

Serum markers of oxidative stress

Toxic effects of mycotoxins can lead to oxidative stress and generation of free radicals. The increased number of free radicals along with disruption in the host antioxidant management system can damage DNA, lipids and proteins. Different indicators of oxidative stress were measured in this trial. Broilers fed the mycotoxin contaminated diet had increased levels of oxidative stress indicators malondialdehyde

(MDA) and 8-Hydroxydeoxyguanosine (8-OHDG), compared to broilers fed the control diet. Broilers fed BG-MAX™ supplemented, mycotoxin contaminated diets had reduced levels of oxidative stress indicators (Table 3). Total antioxidant capacity (T-AOC), as well as enzymes responsible for breakdown of reactive oxygen species, glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD), helps the host manage oxidative stress. No treatment effects were noted for SOD, but levels of T-AOC and GSH-PX were reduced in broilers fed mycotoxin contaminated diets compared to those fed the control diet or mycotoxin contaminated diet supplemented with BG-MAX (Table 3).

TABLE 3 Effect of treatments on indicators of oxidative stress in broiler serum at 42 days of age.

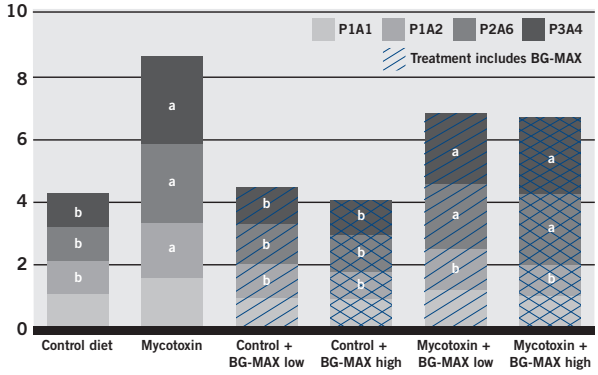
	Indicates ability to manage oxidative stress			Indicators of oxidative stress	
Treatment	**T-AOC (U/mL)**	**SOD (U/mL)**	**GSH-PX (μmol/L)**	**MDA (nmol/mL)**	**8-OHDG (ng/mL)**
Control	9.077±0.27^{ab}	73.07±5.11	1901.85±97.79^{bc}	8.36±0.53^{ab}	20.46±3.53^{b}
Mycotoxin	8.21±0.30^{a}	63.77±2.95	1369.85±149.28^{a}	10.83±0.57^{c}	29.34±1.31^{a}
Control + BG-MAX low	10.43±0.62^{c}	73.33±4.37	2375.69±131.83^{d}	7.07±0.60^{ab}	17.23±2.82^{bc}
Control + BG-MAX high	10.61±0.38^{c}	76.61±3.84	2046.15±156.43^{cd}	6.67±0.71^{a}	12.74±0.65^{c}
Mycotoxin + BG-MAX low	10.09±0.16^{bc}	72.33±3.49	1565.85±124.04^{ab}	9.38±1.33^{bc}	18.42±1.70^{bc}
Mycotoxin + BG-MAX high	10.21±0.24^{c}	78.22±3.90	2012.62±165.15^{cd}	7.55±0.81^{ab}	15.23±1.93^{bc}

^{abcd} Different superscripts within columns indicate significant differences between treatments ($P \leq 0.05$)

Aflatoxin metabolism in the liver

Aflatoxin B1 gets metabolized in the liver by the cytochrome P450 enzyme system to a more toxic Aflatoxin B 8,9 epoxide (AFBO). Four key enzymes involved in this conversion were monitored (Fig. 5). Expression of three of the four enzymes was upregulated when broilers were fed the mycotoxin contaminated diet only. Broilers fed the mycotoxin contaminated diet supplemented with BG-MAX showed significant reduction in expression of P1A2 and numerical reduction in the other enzymes compared to broilers fed mycotoxin contaminated diets without BG-MAX supplementation. This could suggest lower rate of conversion of aflatoxin B1 to more toxic form AFBO in broilers fed mycotoxin contaminated diets supplemented with BG-MAX.

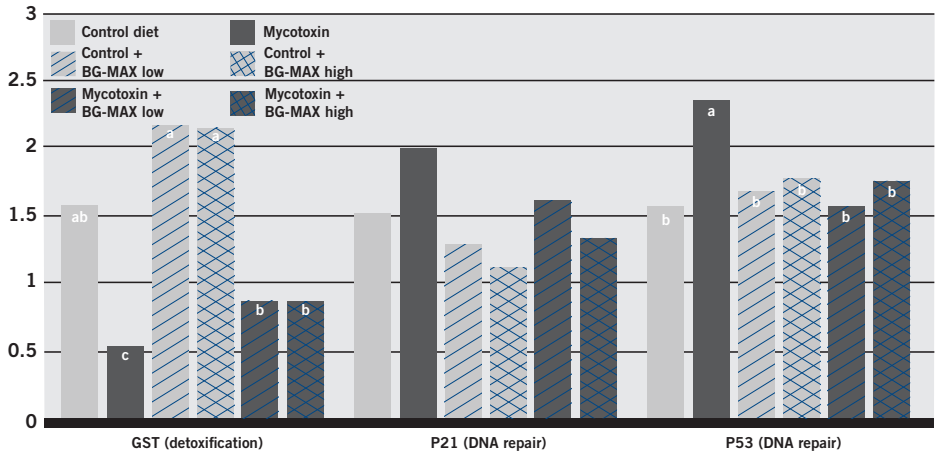
FIGURE 5: Effect of treatments on fold-change in mRNA expression of cytochrome P450 metabolizing isozymes in liver.



^{ab} Different superscripts within columns indicate significant differences between treatments ($P \leq 0.05$)

AFBO binds to DNA to form DNA adducts, resulting in mutations. Glutathione S transferase (GST) can bind to AFBO and detoxify it. Additionally, DNA repair enzymes p53 and p21 can also help reduce DNA damage done by aflatoxin and its toxic forms. In this study, expression of GST was reduced and that of p53 was increased in broilers fed mycotoxin contaminated diets compared to broilers fed the control diet or mycotoxin contaminated diet supplemented with BG-MAX (Fig. 6). This indicated reduced ability to bind toxic AFBO and detoxify it in broilers fed mycotoxin contaminated diets. This may have led to increased DNA damage and an increase in expression of DNA repair enzyme p53 in this same treatment group. Compared to that, broilers fed mycotoxin contaminated diets supplemented with BG-MAX had similar expression of GST and p53 as control fed broilers (Fig. 6). No treatment effects were noted for P21.

FIGURE 6: Effect of treatments on fold-change in mRNA expression of Glutathione S transferase and DNA repair enzymes in liver.



[ab] Different superscripts indicate significant differences between treatments ($P \leq 0.05$)

Immune function

Mycotoxin exposure can cause immunosuppression. Vaccine antibody titers for NDV and IBD were measured in all treatment groups at different time intervals. Treatment effects were noted at d21 and d28 of age for NDV and at d28 for IBD. Antibody titers were reduced in broilers fed mycotoxin contaminated diets compared to broilers fed control or mycotoxin contaminated diets supplemented with BG-MAX™ (Table 4 & 5).

TABLE 4 Effect of BG-MAX on serum NDV ELISA antibody titer.

Treatment	14-day NDV (ng/mL)	21-day NDV (ng/mL)	28-day NDV (ng/mL)	42-day NDV (ng/mL)
Control	722.06±49.99	808.86±18.39[ab]	926.95±22.89[b]	967.27±36.03
Mycotoxin	693.24±36.56	698.80±48.55[a]	803.60±30.74[a]	912.32±21.33
Control + BG-MAX low	735.37±62.65	912.99±62.78[b]	925.03±13.88[b]	932.97±38.47
Control + BG-MAX high	729.00±27.51	837.08±25.64[b]	923.02±13.65[b]	990.96±18.08
Mycotoxin + BG-MAX low	723.84±46.32	816.83±19.75[b]	921.03±40.91[b]	976.08±23.76
Mycotoxin + BG-MAX high	716.64±67.56	804.17±12.88[ab]	920.07±17.74[b]	1012.09±15.38

[ab] Different superscripts within columns indicate significant differences between treatments ($P \leq 0.05$)

TABLE 5 Effect of BG-MAX on serum IBD ELISA antibody titer.

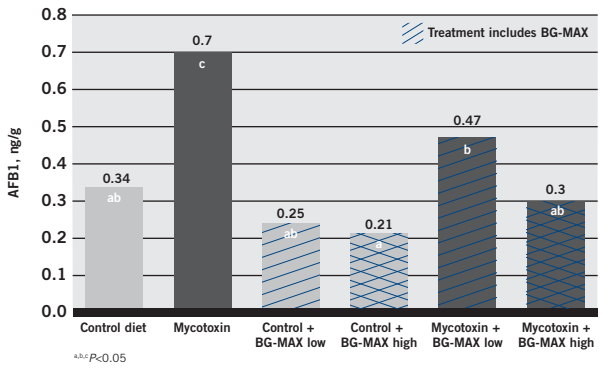
Treatment	14d IBD (ng/mL)	21d IBD (ng/mL)	28d IBD (ng/mL)	42d IBD (ng/mL)
Control	24.27±0.65	26.05±0.30[a]	18.89±0.29	18.28±0.95
Mycotoxin	23.58±1.54	23.68±0.37[b]	17.69±0.33	16.65±0.38
Control + BG-MAX low	24.09±0.83	25.48±0.50[a]	18.07±0.23	17.99±0.30
Control + BG-MAX high	25.05±1.50	25.79±0.96[a]	18.21±0.42	17.92±0.49
Mycotoxin + BG-MAX low	24.23±1.32	26.72±0.50[a]	18.42±0.79	16.95±0.52
Mycotoxin + BG-MAX high	23.97±1.43	25.86±0.51[a]	18.92±0.57	17.34±0.38

[ab] Different superscripts within columns indicate significant differences between treatments ($P \leq 0.05$)

Aflatoxin residue in the liver

A compromised gut barrier allows translocation of toxic compounds, including mycotoxins, from the digesta into the blood stream and to different organs. Broilers fed the mycotoxin contaminated diets had higher AFB1 in the liver compared to broilers fed the control diet. Broilers fed diets supplemented with BG-MAX™ had decreased AFB1 residue in the liver compared to broilers fed the mycotoxin contaminated diet and were not different from broilers fed the control diet (Fig. 7).

FIGURE 7: Effect of treatments on AFB1 residue in liver (ng/g).



$^{a,b,c}P<0.05$

CONCLUSION

Negative effects caused by moderate levels of aflatoxin, zearalenone and deoxynivalenol contamination on broiler performance, immunity, gut health, and tissue residue could be reduced significantly by supplementation with BG-MAX.

To learn more about BG-MAX contact your nutritionist, veterinarian or ARM & HAMMER™ representative or visit AHfoodchain.com.

1 Fang Y, Fu Y, Jalukar S, Ma J, Zhao L. Effect of BG-MAX on broilers exposed to aflatoxin B1, zearalenone and deoxynivalenol. State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University. Data on file. 2021.