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RESEARCH ARTICLE

Immunomodulatory Activity of β-glucan and Mannan-Oligosaccharides from *Saccharomyces cerevisiae* on Broiler Chickens Challenged with Feed-Borne *Aspergillus fumigatus*

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A B S T R A C T

Aspergillus fumigatus (*A. fumigatus*), a common feed contaminant particularly ubiquitous in conserved feeds, poses a potential health risk of intestinal health in broilers. The aim of this work was to assess the effects of dietary supplementation with β-glucan (BG) and mannan-oligosaccharides (MOS) from *Saccharomyces cerevisiae* (*S. cerevisiae*) on broiler chickens' production and health, especially under the challenge of naturally feed-borne *A. fumigatus*. The study includes one control group fed with regular feed and two supplement-treated groups fed with BG and MOS. The results showed broilers in treatment groups had lower mortality rate and higher antibody titers of Newcastle disease virus (NDV), compared with control group. The immunological analysis found that, when broiler chickens were challenged with *A. fumigatus*, spleen and thymus indices were markedly improved, the cytokine concentrations in serum were increased, and the activities of heterophils and lymphocytes were up-regulated when the feed was supplemented with BG and MOS. These data suggest this dietary supplement not only overcomes the negative effect of *A. fumigatus*, but also improves the production and heath of broiler chickens by up-regulating the immune function in broiler chickens.

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INTRODUCTION

Aspergillus fumigatus, one of the most ubiquitous of the airborne saprophytic fungi, causes severe and usually fatal invasive infections in immune compromised animals and has a negative effect on growth (Geissler *et al.*, 2013). Immunosuppression is common in avian species and may account, in part, for the high incidence of disease seen in broiler chickens (Wideman and Pevzner, 2012; Kumar *et al*., 2015; Valtchev *et al*., 2015). Environmental stressors such as crowding in production facilities may further contribute to immune suppression and susceptibility to pathogenic infection (Liu *et al.*, 2009; Bedanova *et al.*, 2010). Especially, in the first two weeks of life, the development of adaptive immune systems in broiler chickens is delayed. Mycotoxins, pathogens or other stressors can also contribute to immune suppression during this critical growth stage (Sirajudeen *et al.*, 2011). There has been an increased interest in the use of immunomodulators in poultry production. BG and MOS have become ideal candidates because of their positive effects on the avian immune system without adversely affecting poultry performance (Jacob and Pescatore, 2014). BG and MOS, as major source of *Saccharomyces cerevisiae* cell-wall extract, has been well studied in regulating the immune markers of broiler chickens (Li *et al.*, 2012; Zhang *et al.*, 2012). Yeast-derived BG and MOS have been studied to control necrotic enteritis in chickens by promoting commensal bacteria while controlling pathogen invasion (Bagheri *et al.*, 2009). Supplemental MOS has also been shown to increase the production of IgA in laying hens (Koo *et al.*, 2010). The BG and MOS induced DNA synthesis in lymphoid and myeloid cells and played an important role in the regulation of various immune responses, such as cell proliferation/differentiation and adhesion, and immune-regulatory cytokine production. Yeast-derived BG (Y-BG) has also been reported to have antiviral efficiencies in animal and human by reducing viral replication rate, and increasing production of IFN-γ and nitric oxide. In

the study, we hypothesized that BG and MOS have stronger immunological response to the pathogenic contaminated feed because pathogens and BG and MOS shared the pattern recognized by TLR on the surface of immune cells (Wang *et al.*, 2010). Correlating with our findings, especially when chicks were challenged with feed-borne *A. fumigatus*, immune function markers in signal transduction could be utilized to reflect the chicken's health condition, and further to be used as indexes to optimize the dosage to improve their both immune function and performance.

MATERIALS AND METHODS

Supplement: BG and MOS were obtained from Mirigen (Wuxi, China) and mixed (1:1) into feed for treatment groups with 0.5 and 1% (w/w).

Housing and vaccination: Nine thousand 1-d-old Ross broiler chickens were randomly assigned to control and two treatment groups (3000/group) and housed at 26 to 28°C. Lighting: 23h/d for days 1 to 4, 20h/d for days 5 to 10 and 18h/d thereafter. Feed and water were provided ad libitum. Vaccination: Marek's disease on day 1, Newcastle disease on days 7, 14 and 27; Infectious bronchitis on days 1, 12 and 21.

Performance: Morbidity and mortality records were kept daily, and body weight (BW) on days 1, 14, 28 and 45. On day 45, thirty birds from each group were sacrificed by cervical dislocation. Spleen and thymus were immediately dissected and weighed for the calculation of their indices: thymus index=thymus weight (mg)/body weight (g) × 10; spleen index=spleen weight (mg)/body weight (g) × 10.

***A. fumigatus* quantification:** *A. fumigatus* concentration of feed (collected weekly) was analyzed using quantitative real-time PCR. 25mg DNA was extracted using GeneJet Genomic DNA kit (Fermentas, Pittsburgh, PA). CHROMA SPIN Column (BD Bioscience, Palo Alto, CA) were used to purify DNA. Forward primer (5'-CTTTGTCACCTGCTCTGTAG-3') and reverse primer (5'-TCCTCCGCTTATTGATATGC-3') were synthesized. Bio-Rad CFX96™ system was used for PCR with annealing temperature set for *A. fumigatus* specific primer pair as 60°C.

Heterophil purification and gene expression: Blood samples were collected from thirty birds from each group on days 1 (prior to treatment diets), 14, 28 and 45, using 4.5mL vaccutainers® (BD Bioscience, Franklin Lakes, NJ) containing sodium citrate. Heterophils were purified using the method by Weber (Weber *et al.*, 2001). Two-third of purified hetereophils was used immediately for phagocytic activity and ROS production as described below. The rest were preserved in 1mL TRIzol for gene expression analysis.

Heterophil RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA was dissolved in RNase-free water and stored at -80°C until analysis.

Quantitative RT-PCR (Q-RT-PCR) was performed in a 96-well PikoREAL qPCR system (Thermo Fisher, PA). Individual RNAs were digested by DNAase (Invitrogen, Carlsbad, CA) to remove DNA contaminations. Then Q-RT-PCR was performed using the SYBR Green PCR Core Reagents (P E Applied Biosystems, Warrington, UK). Three gene-specific primer pairs were designed using Beacon Design (Premier Biosoft International, Palo Alto, CA) as shown in Table 1. The ΔΔCT method was used to analyze relative changes in gene expression according to the method of Livak and Schmittgen (2001) using β-actin as the endogenous control. The target genes were normalized to the average C_t value of β-actin, and the mean of $2^{-\Delta\Delta Ct}$ was used to determine the fold changes. Fold change was presented as the arithmetic mean of the replicates.

Determination of phagocytic activity and ROS production of heterophils: Hereophils were counted and incubated with *Escherichia coli* at 1: 30 ratio for 2 hours, then live bacteria were quantified by reading the absorbance at 570nm of MTT dye solution (Wiggins *et al.*, 2011). ROS generation was carried out using OxiSelect™ ROS Assay Kit (Cell Biolabs, CA).

Chicken serum $CD4^+$, $CD8^+$, IFN-γ, TNF-α, MIP-1β ELISA and NDV titration: Chicken $CD4^+$, $CD8^+$, IFN-γ, TNF-α, MIP-1β ELISA and NDV titration kit (Cusarbio Biotech Co., Shanghai, China) was used to determine their concentrations in serum samples.

Statistical analyses: Data analysis was performed by comparing treatments to control using two-side t-test with pooled standard errors with S-PLUS software. The data were analyzed as a completely randomized design to examine the overall effect of treatments. All statements of differences were based on significance at $P<0.01$ or $P<0.05$.

RESULTS

Flock performance: Comparison on overall feed intake, feed conversion and mortality between the control and treatment groups was made. The results showed that no significant difference was found in feed intake. However, there was significant difference in feed conversion ratio (FCR) between control (1.86) and two feed supplement treated groups (1.71 and 1.78, respectively) ($P<0.05$). Survival rate between control and treatment groups was significantly different ($P<0.01$) with 92.3% in control, 96.1% and 97.9% in two supplement treated groups, respectively.

Effects of supplement on immune organ indices: Results of the immune organ indices are shown in Fig. 1. Consistent with the chicken performance, treatments with β-glucan and mannan-oligosaccharide could significantly boost the spleen and thymus indices compared with control ($P<0.01$).

***A. fumigatus* concentration in feed:** The level of *A. fumigatus* of feed samples on days 1, 14, 28, 45 were 2233, 2522, 3894, 890 spores/g, respectively. However, there was no detectable *A. fumigatus* in blood of broiler chickens in treatment groups, compared with control groups, which had very low but detectable concentration of *A. fumigatus* in blood (<0.1Gu/mL).

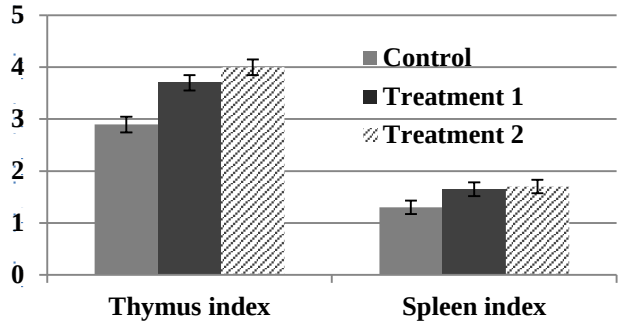

Fig. 1: Effects of BG and MOS on organ indices in broiler chickens.

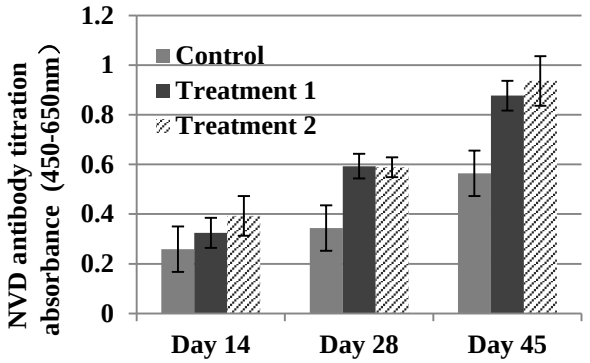

Fig. 2: Effects of BG and MOS on NDV titer of broiler chickens on days 14, 28 and 45.

Table 1: Real-time PCR primers for genes: CD62L, IL-8R and β-actin.

Gene	Sequence (5'-3')
CD62L	Forward: GACACTTCCCTTCAGCCGTAC
	Reverse: AGTTCTTTGCTTCTTCAGTGAGAG
IL8R	Forward: AGACCTTTGGCTTCCTCCTG
	Reverse: GACGAGCACGACAGCAAAG
β-actin	Forward: CGACAATGGATGATGATATTG
	Reverse: AAGTCCGGCCTTGCACAA

Table 2: Concentrations of cytokines and $CD4^+/CD8^+$ ratio in broiler chickens (n=30)

Group	IFN-γ (ng/mL)	TNF-α (ng/mL)	MIP-1β (pg/mL)	$CD4^+/CD8^+$
Control	351.12±9.42	376.41±15.38	103.72±3.52	1.88±0.06
Treatment 1	379.16±11.27**	408.64±15.49**	132.07±8.75**	2.11±0.11*
Treatment 2	398.75±6.75**	417.351±11.20**	139.50±6.16**	2.15±0.19*

Values (mean±SE) in a column bearing *P<0.05 or **P<0.01 differ significantly as compared to control group.

Table 3: Effects of BG & MOS on heterophil phagocytosis and ROS production in broiler chickens (n=30)

Group	Heterophil killing ability of *E. coli*	ROS production (mM)
Control	1.99±0.91	5.51±1.05
Treatment 1	3.16±1.19*	6.21±1.19*
Treatment 2	3.37±1.61*	6.29±1.43*

Values (mean±SE) in a column bearing *P<0.05 or **P<0.01 differ significantly as compared to control group.

Blood parameters: Immunological function was evaluated using serum samples from broiler chickens, including serum MIP-1β, TNF-α, IFN-γ, $CD4^+$, $CD8^+$ concentrations, and NDV titers. The results demonstrated that there was significantly higher MIP-1β expression level in treatments than in control (P<0.01) (Table 2), representing that there were higher innate immune responses in the feed supplement groups. There were significantly higher TNF-α and IFN-γ in treatment groups (P<0.01) than control (Table 2). Meanwhile, there was significantly higher $CD4^+/CD8^+$ ratio in treatment groups (P<0.05) than control group (Table 2).

NDV vaccination is an effective method to protect the broilers from Newcastle virus disease, the most prevalent avian disease. Fig. 2 shows that supplement resulted in a numerical increase in titer of NDV antibody on Days 28 and 45, and there were significantly higher titration and longer lasting NDV antibodies in two treated groups than control (P<0.01).

Heterophil phagocytic activity and ROS production: On day 45, there was significantly higher *E.coli* killing ability in phagocytosis in treatment groups (P<0.05) than control (Table 3), and ROS production was numerically higher in dietary supplement groups than control (P<0.05) as shown in Table 3, indicating heterophils from treatment groups have stronger killing ability than that from control.

The results of the experiment demonstrated that blood neutrophils from broiler chickens fed with supplement were primed and more readily available for bacterial killing by phagocytosis and ROS producing.

Gene expression of heterophil CD62L and IL-8R: The relative expression level of CD62L was determined using quantitative real-time RT-PCR with relative to β-actin. There were no statistical differences between treatment groups (P>0.05, Table 4) on day 14, but both treatment groups had higher CD62L expression level than control group. There were significantly higher CD62L between treatment and control groups on days 28 and 45 (P<0.05, Table 4). Higher concentration of CD62L mRNA was a sign of enhanced immune response to a pathogen challenge.

There was higher IL-8R expression in two treatment groups than control, but no statistical difference within the treatment groups on day 14 (P>0.05, Table 4). IL-8R expression level difference between treatment groups and the control became more significant on day 28 and 45 (P<0.01, Table 4). The higher relative concentration of IL-8R in the supplement treatment groups could be interpreted as enhanced immune response to a pathogen challenge.

DISCUSSION

β-glucan and mannan-oligosaccharides may help to control or reduce the growth of harmful fungi such as *A. fumigatus* - especially important to the poultry industry because it is a primary cause of necrotic enteritis - which has been estimated to cost the worldwide poultry industry $2 billion each year. This experiment demonstrated a positive advantage to bird performance in both average daily weight gain and average live weight due to the addition of feed supplement at 0.5% and 1% of the diet. BG and MOS has been used in animal feeding since the last decades (Rosen, 2007). Their inclusion in broiler diets has significantly improved animal productivity, which was attributed to the physiological effects on intestinal digestive mucosa (Baurhoo *et al*., 2007; Gao *et al*., 2008). The results showed there was no significant different mortality and body weight (BW) gain between those two treatment groups, however, we noticed that the BW on day 45 was higher, and FCR was lower in the low dose (0.5% w/w) than the higher does (1% w/w), indicating that the high dose of supplement might have caused more energy consumption for immune response, and this innate immune response diverts energy from growth and performance.

Table 4: CD62L and IL-8R mRNA relative concentration (n=30)

Group	CD62L/β-actin mRNA expression level			IL-8R/β-actin mRNA expression level		
	Day 14	Day 28	Day 45	Day 14	Day 28	Day 45
Control	1.00±0.33	1.16±0.39	0.94±0.24	1.00±0.29	1.01±0.56	1.14±0.29
Treatment 1	1.74±0.96	2.88±0.52*	3.06±0.77*	0.81±0.48	1.84±0.33**	2.44±0.68**
Treatment 2	1.67±0.94	3.07±0.84*	3.45±0.96*	0.94±0.37	1.46±0.37**	2.14±0.42**

Values (mean±SE) in a column bearing *P<0.05 or **P<0.01 differ significantly as compared to control group.

Some studies reported the performance of chicken in response to β-glucan and MOS treatment (Huff *et al*., 2006). Considering the lower mortality in higher dose (1% verse 2% death in low dose group) and given a worse environment and animal conditions, it might be a necessary approach to increase the dose to prevent pathogens infection.

Improving immune function through feed supplementation may aid in preventing infections and decreasing treatment and culling costs due to diseases. As broiler heterophils are the first phagocytes to be activated during bacterial infection (Lillehoj *et al*., 2004), their primary function is to phagocytize and kill bacteria. The results of ROS generation and phagocytosis assays showed that heterophil killing ability was markedly improved through feed supplement. The respiratory burst and degranulation processes produce ROS that are instrumental in preventing bacterial growth and colonization (Wang *et al*., 2009).

Cytokine production during the initial stages of infections leads to an influx of heterophils into the sites. During migration, TNF-α and IFN-γ originating from the site of infection activate the neutrophil to become a mature "killer cell". In our study, significant increases in IFN-γ and TNF-α enhanced by supplement stimulation signify a direct relationship between this dietary supplement and the immune response. The importance of IFN-γ in the immune system stems in part from its ability to inhibit viral replication directly, and most importantly from its immunostimulatory and immunomodulatory effects. MIP-1β, expressed by microphages on their cell surfaces, which is instrumental in killing of the invading bacteria. In the study, the enhancing in MIP-1β reflects that the supplement diet was provided in sufficient amounts to result in immune response in animals. This feed supplement contains molecules that interact with the innate immune system to prime leukocyte antimicrobial processes, specifically heterophils (Heine and Ulmer, 2005). Actually, cytokines and intercellular communication networks are self-stabilizing and self-regulating system in health individuals, which should be free from excessive intervention, under illness state, BG and MOS with long-term and high dose intake can play an important role by stimulating immune response as above. In the study, we observed the higher $CD4^+/CD8^+$ ratio in treatment groups than control group. The enhanced $CD4^+/CD8^+$ ratio in the blood was associated with increases in the ability of lymphocytes to respond to T cell mitogen and in the antibody response to a T-independent antigen (Ghosh *et al*., 2012). The ratio of $CD4^+$ cells to $CD8^+$ cells is often an indicator of immune response status (Teixeira *et al*., 2013).

Many studies have shown that BG and MOS potentiate the immune system (Yoon *et al*., 2012), and tested as an effective adjuvant for immunomodulation in avian. Vaccination is an extremely effective strategy for protection of birds against virus diseases, but protection depends on the nature of the vaccine itself and the method of the application, most of all, the response of broiler chickens to vaccination (Swayne, 2006; Peyre *et al*., 2009). Higher antibody titration after routine vaccination indicates a better protection in animals. The results showed that dietary supplement improve the broilers' response to NDV vaccine with higher titration and less variation of titration comparing with control group. NVD is the most prevalent avian diseases, and vaccination is an effective method to protect broiler chicks, however, some chicks had no and weak immune response because of stress or chicks' health condition. In fact, BG and MOS has been used as adjuvant in antigen-specific antibody production in B lymphocytes. BG and MOS for animal every before and after vacation, could be increasingly becoming a vital component of the dietary supplement. Studies (Chae *et al*., 2006; Huff *et al*., 2006) have shown that BG and MOS may be an excellent adjuvant that improves the immune response by modulating the immune system and the effector cells to produce cytokines. In this paper, we present data obtained on immune response experiments with the vaccines and the adjuvant immunomodulatory effect of BG and MOS tested in poultry. The immunomodulatory effect of β-glucan and MOS in general indicates that the β-glucan and MOS as adjuvants may be a good source for a multitude of purposes including the enhancing effect of the immune system in broiler chickens.

In this study, we use both heterophil CD62L and IL-8R as markers, which were upregulated in treatment groups, indicating heterophils in treatment groups have stronger ability in "killing" pathogens than control group. After the bacteria have been brought into the heterophils in a phagosome they are killed by a variety of mechanisms. These protein molecules include defensins. CD62L (cluster of differentiation number 62 ligand) is a primary adhesion molecule on the heterophil cell surface that allows these cells to migrate from capillaries into the tissue to reach infected areas. An increase in circulating CD62L is indicative of an enhanced immune response (Wang *et al*., 2009). IL-8R on the heterophils binds with IL-8, which mediates neutrophil migration and activation.

A stronger immune response invariably leads to a healthier animal, resulting in less antibiotic use, only novel alternatives will pave the new ways to enhance an animal's immune response. With the increased pressure to reduce antibiotic use in food animal production, producers are looking at boosting immune function as an alternative approach to reducing the detrimental effects of harmful intestinal pathogens. The use of compounds that may have prebiotic effects is a possible way to improve intestinal health and animal performance in the absence of antibiotic growth promoters (Gibson and Roberfroid, 1995). This investigation sheds light on the effects of dietary supplementation on immunity, and demonstrates

the resolve of animal scientists to appeal to the public and offers an alternative management tool to improve animal health without increasing antibiotic use and jeopardizing both consumer well-being and public perception of the agricultural industry. With the accumulating information on the mechanism of BG and MOS, which dietary feed additive wound be widely used to improve poultry health and increase their performance.

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Author's contribution: YL designed the study, LC, TJ, XL, QW, YW conducted the experiment and processed the samples, and QW and YW analyzed the data and graphed them. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES

Bagheri K, Alimoghadam K, Pourfathollah AA, Hassan ZM, Hajati J *et al*., 2009. The efficient generation of immunocompetent dendritic cells from leukemic blasts in acute myeloid leukemia: A local experience. Pathol Oncol Res, 15: 257-267.

Baurhoo B, Phillip L and Ruiz-Feria CA, 2007. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poult Sci, 86: 1070-1078.

Bedanova I, Chloupek J, Chloupek P, Knotkova Z, Voslarova E *et al*., 2010. Responses of peripheral blood leukocytes to chronic intermittent noise exposure in broilers. Berl Munch Tierarztl Wochenschr, 123: 186-191.

Chae BJ, Lohakare JD, Moon WK, Lee SL, Park YH *et al*., 2006. Effects of supplementation of beta-glucan on the growth performance and immunity in broilers. Res Vet Sci, 80: 291-298.

Gao J, Zhang HJ, Yu SH, Wu SG, Yoon I *et al*., 2008. Effects of yeast culture in broiler diets on performance and immunomodulatory functions. Poult Sci, 87: 1377-1384.

Geissler A, Haun F, Frank DO, Wieland K, Simon MM *et al*., 2013. Apoptosis induced by the fungal pathogen gliotoxin requires a triple phosphorylation of bim by jnk. Cell Death Differ, 20: 1317-1329.

Ghosh S, Hoselton SA and Schuh JM, 2012. Characterization of cd19(+)cd23(+)b2 lymphocytes in the allergic airways of balb/c mice in response to the inhalation of aspergillus fumigatus conidia. Open Immunol J, 5: 46-54.

Gibson GR and Roberfroid MB, 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J Nutr, 125: 1401-1412.

Heine H and Ulmer AJ, 2005. Recognition of bacterial products by toll-like receptors. Chem Immunol Allergy, 86: 99-119.

Huff GR, Huff WE, Rath NC and Tellez G, 2006. Limited treatment with beta-1,3/1,6-glucan improves production values of broiler chickens challenged with escherichia coli. Poult Sci, 85: 613-618.

Jacob JP and Pescatore AJ, 2014. Barley beta-glucan in poultry diets. Ann Transl Med, 2: 20.

Koo BC, Kwon MS, Lee H, Kim M, Kim D *et al*., 2010. Tetracycline-dependent expression of the human erythropoietin gene in transgenic chickens. Transgenic Res, 19: 437-447.

Kumar CB, Reddy BSV, Gloridoss RG, Prabhu TM, Suresh BN et al., 2015. Amelioration of aflatoxicosis through a bio-technologically derived aflatoxin degrading commercial product in broilers. Pak Vet J, 35: 217-221.

Li Z, Yang ZB, Yang WR, Wang SJ, Jiang SZ *et al*., 2012. Effects of feed-borne fusarium mycotoxins with or without yeast cell wall adsorbent on organ weight, serum biochemistry, and immunological parameters of broiler chickens. Poult Sci, 91: 2487-2495.

Lillehoj HS, Min W and Dalloul RA, 2004. Recent progress on the cytokine regulation of intestinal immune responses to eimeria. Poult Sci, 83: 611-623.

Liu L, Yuan S, Long Y, Guo Z, Sun Y *et al*., 2009. Immunomodulation of rheum tanguticum polysaccharide (rtp) on the immunosuppressive effects of dexamethasone (dex) on the treatment of colitis in rats induced by 2,4,6-trinitrobenzene sulfonic acid. Int Immunopharmacol, 9: 1568-1577.

Peyre M, Samaha H, Makonnen YJ, Saad A, Abd-Elnabi A *et al*., 2009. Avian influenza vaccination in egypt: Limitations of the current strategy. J Mol Genet Med, 3: 198-204.

Rosen GD, 2007. Holo-analysis of the efficacy of bio-mos in broiler nutrition. Br Poult Sci, 48: 21-26.

Sirajudeen M, Gopi K, Tyagi JS, Moudgal RP, Mohan J *et al*., 2011. Protective effects of melatonin in reduction of oxidative damage and immunosuppression induced by aflatoxin b1-contaminated diets in young chicks. Environ Toxicol, 26: 153-160.

Swayne DE, 2006. Principles for vaccine protection in chickens and domestic waterfowl against avian influenza: Emphasis on asian h5n1 high pathogenicity avian influenza. Ann N Y Acad Sci, 1081: 174-181.

Teixeira D, Longo-Maugeri LM, Duarte YA, Lebrao ML and Bueno V, 2013. Evaluation of renal function and immune system cells in elderly individuals from sao paulo city. Clinics (Sao Paulo), 68: 39-44.

Valtchev I, Koynarski T, Sotirov L, Nikolov Y and Petkov P, 2015. Effect of aflatoxin B1 on Moulard duck's natural immunity. Pak Vet J, 35: 67-70.

Wang M, Krauss JL, Domon H, Hosur KB, Liang S *et al*., 2010. Microbial hijacking of complement-toll-like receptor crosstalk. Sci Signal, 3: ra11.

Wang YQ, Puntenney SB, Burton JL and Forsberg NE, 2009. Use of gene profiling to evaluate the effects of a feed additive on immune function in periparturient dairy cattle. J Anim Physiol Anim Nutr (Berl), 93: 66-75.

Weber PS, Madsen SA, Smith GW, Ireland JJ and Burton JL, 2001. Pre-translational regulation of neutrophil l-selectin in glucocorticoid-challenged cattle. Vet Immunol Immunopathol, 83: 213-240.

Wideman RF, Jr. and Pevzner I, 2012. Dexamethasone triggers lameness associated with necrosis of the proximal tibial head and proximal femoral head in broilers. Poult Sci, 91: 2464-2474.

Wiggins MC, Woolums AR, Hurley DJ, Sanchez S, Ensley DT *et al*., 2011. The effect of various mycoplasma bovis isolates on bovine leukocyte responses. Comp Immunol Microbiol Infect Dis, 34: 49-54.

Yoon H, Song JM, Ryu CJ, Kim YG, Lee EK *et al*., 2012. An efficient strategy for cell-based antibody library selection using an integrated vector system. BMC Biotechnol, 12: 62.

Zhang S, Liao B, Li X, Li L, Ma L *et al*., 2012. Effects of yeast cell walls on performance and immune responses of cyclosporine a-treated, immunosuppressed broiler chickens. Br J Nutr, 107: 858-866.