

Research Article



Influence of Dietary Supplementation of Mannanligosaccharide (Bio-Mos®) Prebiotic on the Genotoxic and Antioxidant Status in Japanese Quail

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ABSTRACT

The goal of this research was to determine the effect of different doses of mannanligosaccharide (Bio-Mos®) prebiotic feed additives as a growth promoter on the antioxidant enzyme activities, level of lipid peroxidation and extent of DNA damage in Japanese quail chicks. Sixty Japanese quail chicks were randomly divided into four groups with 15 chicks in each group. The first group was considered as control group and fed the basal diet only while the prebiotic Bio-Mos® was added to combined feed of the 2nd, 3rd and 4th group of chicks at a rate of 0.5, 1, 2 g/kg basal diet, respectively. At the end of the experimental period, that is, after 56 days chicks were killed. The results presented in the current study revealed that Bio-Mos® is a beneficial dietary supplement for improving antioxidant activities and decreasing lipid peroxidation level. Also, Bio-Mos® has a positive impact on the extent of DNA damage in blood cells estimating by comet assay and RAPD-PCR with decrement of fragmented DNA level in hepatocytes of quail chicks. The study suggested that Bio-Mos® can be used in practice as a dietary additive for Japanese quail, stimulating the mechanisms of the birds' antioxidation defense and preventing DNA damage.

Keywords: Bio-Mos®, prebiotic, comet assay, RAPD-PCR, antioxidant, lipid peroxidation.

INTRODUCTION

Quail considered as economically important avian species and used as alternative to the more commonly used chicken, they require less space and have good export potential.¹ Quail belong to the family Phasianidae of order Galliformes of class Aves of the animal kingdom, species or subspecies of the genus *Coturnix* are native to all continents except the Americas.² Japanese quail (*coturnix coturnix japonica*) is one of the smallest birds used for its egg and meat production³ and it has also assumed worldwide importance as laboratory animals.⁴

In recent years, breeding of quail has taken an important place in alternative poultry production. Many factors such as genetics, age, fertility rate, egg quality, nutritional status and live weight of breeders affect quail's performance.⁵ To increase the performance of farm animals and lower the feeding cost, antibiotic growth promoters have been included in animal feeds since 1950.⁶

However, in recent years great concern has arisen about the use of antibiotics as supplement at subtherapeutic level in poultry feed due to emergence of multiple drug resistant bacteria,⁷ and the adverse effects of their residuals that can be found in animal products.^{8, 9} The dietary inclusion of non-digestible ingredients has become important to develop alternatives for antibiotics and enhance microbial growth of beneficial microorganisms.¹⁰

Alternative to the use of antibiotics, natural origin additives such as probiotics, prebiotics,

immunostimulants and medicinal plants are used as growth promoters.¹¹ Prebiotics are nondigestible carbohydrates that stimulating the growth and/or activity of a limited number of bacteria and beneficially affect host health (e.g., *bifidobacteria* and *lactobacilli*).¹² They are consisting of non digestible oligosaccharides such as galactooligosaccharide, transgalactooligosaccharide and mannanligosaccharide (MOS).¹³

The mannanligosaccharide (MOS) can be found in plant and animal cells such as the yeast *Saccharomyces cerevisiae*.¹⁴ The glucan, the manna's and chitin are the principal components of yeast cell wall.¹³ MOS increased beneficial organisms in gastrointestinal tract¹⁵ such as *Lactobacillus* and *Bifidobacterium* which are of particular interest and several studies have reported their antioxidant activities.^{16,17}

Bio-Mos® activity is commonly attributed to mannanligosaccharide but it is not pure MOS, it contains yeast cell fragments¹⁵ and can be considered as glucomannoprotein complex.¹⁸ It has been shown to enhance immune protection in different animal species and as a result of this enhance overall animal performance.¹⁹ In general, prebiotic supplementation have protective effects against a broad range of events such as induction of DNA damage in the colonic mucosa of rats and reducing the levels of enzymes involved in carcinogen formation.²⁰

There are several researches referring the positive effect of Bio-Mos® on performance and health of animals but few data relating genetic and biochemical effects to this dietary prebiotic. Therefore, this study was performed to investigate the effect of prebiotic mannanligosaccharide



(Bio-Mos®) as dietary supplementation on molecular genetics, antioxidant enzyme activities and lipid peroxidation in Japanese quail chicks.

MATERIALS AND METHODS

Prebiotic

The prebiotic used in this study; mannanoligosaccharide (Bio-Mos®) is derived from the cell wall of certain strain of *Saccharomyces cerevisia*. It was obtained from Alltech, Inc., USA.

Experimental design and animal management

Sixty Japanese quail chicks were housed in breeding battery with 21x20x27cm individual cages in a room maintained at 37°C until 21°C at the end²¹, with 16-hr light: 8hr darkness throughout the 56 days experimental period. Birds were allowed *ad libitum* access to feed and fresh water. The study design consisted of four dietary treatments, each containing fifteen chicks. The first group was considered as control group and fed the basal diet only (Table 1), while the prebiotic Bio-Mos® was added to combined feed of the 2nd, 3rd and 4th group of chicks at a rate of 0.5, 1 and 2 g/kg basal diet, respectively. At the end of experiment liver and blood samples were collected from the wing vein of the chicks, placed in vials with EDTA as an anticoagulant, and transported in coolers to the laboratory on the day of collection for biochemical and genetic analysis.

Table 1: Dietary formulations (%) and proximate composition

Ingredients	%
Corn	52.20
Soya Bean Meal 44%	36.30
Corn gluten meal	7.00
Di calcium phosphate	1.40
Lime stone	1.35
Vegetable oil	0.90
Sodium chloride	0.35
Vit & min premix	0.30
L_Lysine	0.13
DL-Methionine	0.08

Biochemical analysis

a) Estimation of lipid peroxidation

The level of malondialdehyde (MDA) in liver tissue homogenates was measured using colorimetric assay kit according to the method of Ohkawa et al.²² (Biodiagnostic, Egypt, CAT.No.25 29). Thiobarbituric acid reacts with MDA in acidic medium at temperature of 95°C for 30 min to form a pink colored product. The absorbance of this product at 534 nm is directly proportional to MDA concentration in the sample.

b) Estimation of Superoxide dismutase (SOD)

Superoxide dismutase activity was determined by a kinetic assay at 37°C using a test reagent kit

(Biodiagnostic, Giza, Egypt, CAT No.25 21) according to the method described by Nishikimi et al.²³ The absorbance was measured at 560 nm and the results were expressed as U/gm tissue.

Genetic analysis

a) DNA extraction and Random amplification of polymorphic DNA (RAPD-PCR) analysis

Genomic DNA was isolated from blood sample of Japanese quail chicks by phenol/chloroform method described by John et al.²⁴ Six commercially available decamer random primers (Operon, Alameda, CA, USA) were designed and chosen arbitrarily to generate RAPD profiles from quail DNA including: A01 (5'-CAGGCCCTTC-3'), A02 (5'-TGCCGAGCTG-3'), A05 (5'-AGGGGTCTTG-3'), A08 (5'-GTGACGTAGG-3'), A13 (5'-CAGCACCCAC-3') and C13(5'-CAGCACCCAC-3'). The PCR protocol for RAPD analysis was followed as described by Williams et al.²⁵ Briefly, the amplification reactions were performed in volume of (15µl) consisted of 1.5 µl (50ng genomic DNA), 1.5 µl of 10X PCR reaction buffer, 1.5 µl DNTPs (200µM), 1.5 µl primer (1pmol), 1U Taq DNA polymerase. The final reaction mixture was placed in a DNA thermal cycler (ependorff). The PCR programme included an initial denaturation step at 94°C for 4 mins followed by 45 cycles with 94°C for 1 min for DNA denaturation, 1 min at 36°C, at 72°C for 2min and final extension at 72°C for 5 min were carried out. Approximately 3 µl of the amplified DNA product plus 2 µl of 1X loading dye were loaded on 2% agarose gel and then subjected to electrophoresis in 1X TBE buffer and stained with ethidium bromide (0.5µg/ml) for verification. A BIO-RAD XR⁺ Molecular Imager apparatus was used to visualize the PCR products. The marker used is DNA ladder 100pb (Fermentas).

b) Comet assay (single cell gel electrophoresis, SCGE)

The procedure used for measuring the degree of DNA damage was the comet assay which was performed as described by Singh et al.²⁶ Briefly, for the whole blood, we diluted blood with pbs and add equal volumes of diluted blood and 1% LMPA (low melting point agarose) to the coated slide. Place coverslip and put the slide on a slide tray resting on ice packs until the agarose layer hardens (~5 to 10 min). Gently slide off coverslip and add a third agarose layer (80 µL LMPA) to the slide. Replace coverslip and return to the slide tray until the agarose layer hardens (~5 to 10 min). The slides were made to allow DNA unwinding and expression of alkali-labile sites and electrophoresis was conducted by applying 300 volts and 30 mA in the dark for 40 min. Each slide was stained with ethidium bromide. A total of 100 randomly captured comets from each slide were examined at 400 x using an automated fluorescence microscope with an excitation filter of 520-590 nm and the images were captured on a computer equipped with Comet Score software (Komet IV). To quantify the DNA damage; tail length (TL) and tail moment (TM) were evaluated. Tail length (length of DNA migration) is related directly to the DNA fragment size



and presented in micrometres. It was calculated from the centre of the cell. Tail moment was calculated as the product of the tail length and the fraction of DNA in the comet tail.

c) Quantitative analysis of DNA fragmentation

Quantization of DNA fragmentation was determined in liver according to the method described by Ray et al.²⁷ The DNA fragmentation was determined in the pellets and supernatants of the samples. The proportion of fragmented DNA is expressed as a percentage of total DNA appearing in the supernatant fractions to the total amount of DNA in the pellet and supernatant.

Statistical analysis

Statistical significance of the results was evaluated by using One Way ANOVA (analysis of variance) test followed by Duncan's multiple comparisons test to judge the difference between various groups. The results were expressed as mean \pm standard error and values of ($p < 0.05$) were considered statistically significant.²⁸

RESULTS

Biochemical analysis

Molecular biomarkers, such as lipid peroxidation products, antioxidant enzymes are considered as measurable internal indicators of changes in organisms at the molecular or cellular level. Superoxide dismutase (SOD) is one of the main antioxidant enzymes that constitute the first line of antioxidant enzymatic defenses. In the present study the activity of SOD enzyme was significantly increased ($p < 0.05$) among the groups fed with 1 and 2 g of the dietary Bio-Mos[®] as compared with the control group. Meanwhile, no significant differences were detected between the control group and the group fed 0.5 g of the dietary Bio-Mos[®] (Fig. 1).

The concentration of malondialdehyde (MDA) in tissues used as a biomarker for lipid peroxidation. In the current study the mean MDA levels were significantly ($p < 0.05$) decreased in the group fed with 2 g/kg Bio-Mos[®] than the control. Although the MDA levels were decreased among the quail that receiving the additive Bio-Mos[®] (0.5 and 1 g), no significant difference were recorded between these groups and the control group as shown in (Fig. 1).

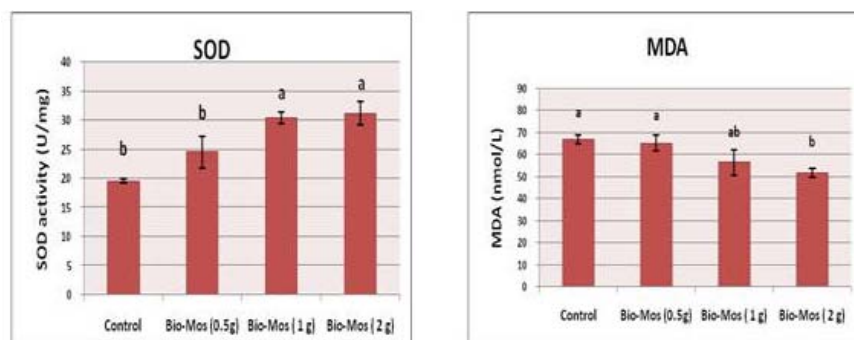


Figure 1: Level of biochemical indices in hepatocytes of Japanese quail on a diet supplemented with Bio-Mos[®]. Data are shown as mean \pm SEM for separate groups of quail. ^{a, b} significant at ($p < 0.05$).

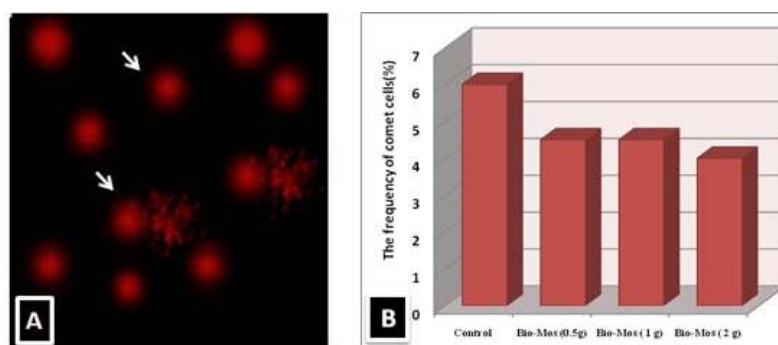


Figure 2: DNA damaged cells as detected by comet assay measured. (A) Fluorescent microscopy of blood cells. The upper cell represents a normal comet image. The lower cell is considered to be an image of a typical comet undergoing DNA damaged. (B) The frequency of comet cells detected by the comet assays in different experimental group.

Genetic analysis

Analysis of DNA damage: The DNA damage expressed as tail length (TL), DNA %, and tail moment (TM) in the blood cells of the Japanese quail birds were determined using comet assay. Analysis of comet cells revealed that

supplementation of the basal diet with different doses of the prebiotic Bio-Mos[®] in Japanese quail decreased the percentage of DNA damaged cells than the control group that fed basal diet (Fig. 2). The results summarized in Table (2) revealed that, there were no statistically significant difference in terms of TL and TM in the blood

cells of the control and group fed with 0.5 g of Bio-Mos®. While the mean values of comet TL and TM were significantly decreased in groups that supplemented with

the dietary (1 and 2 g) Bio-Mos® as compared with the control group.

Table 2: Effect of a diet supplemented with different doses of Bio-Mos® on DNA damage detected by comet assay in blood cells.

Treatment	Tail length (µm)	% DNA	Tail moment
Control	3.02 ± 0.03 ^a	2.83 ± 0.29	8.55 ± 0.96 ^a
Bio-Mos® (0.5 g)	2.90 ± 0.02 ^a	2.61 ± 0.07	7.58 ± 0.67 ^a
Bio-Mos® (1 g)	2.60 ± 0.14 ^b	2.43 ± 0.12	6.36 ± 0.16 ^b
Bio-Mos® (2 g)	2.56 ± 0.01 ^b	2.69 ± 0.03	6.89 ± 0.03 ^b

^{a,b} Means in the same column with different superscripts differ significantly ($p < 0.05$).

Analysis of DNA Fragmentation: DNA fragmentation is a quantitative evaluation of chromatin damage. As shown in the results illustrated in Fig. (3), there were no significant differences among the three groups fed with the dietary Bio-Mos®. Addition of 2 g of Bio-Mos® to the basal diet of the Japanese quail significantly decreased the DNA fragmentation percentage in liver cells as compared to the control group.

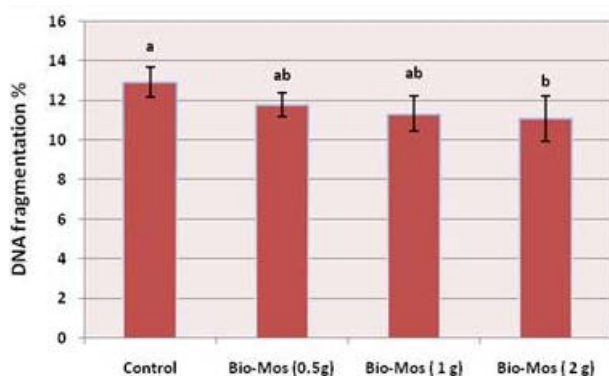


Figure 3: Effect of Bio-Mos® level on DNA fragmentation % in Japanese quail. Data are shown as mean ± SEM for separate groups of quail. ^{a,b} significant at $p < 0.05$.

RAPD fingerprinting pattern: Molecular markers derived from polymerase chain reaction (PCR) amplification of genomic DNA has been created new possibilities for the selection and genetic improvement of poultry species. The molecular genetic variability among the treated quail and their control were evaluated using six oligodecamers (6-mer random primers). All of the primers gave positive and detectable bands (Fig. 4). They amplified a total of 148 different bands with an average of 24.6 bands per primer. They revealed monomorphic bands in the control and the experimental diet samples supplemented with varying levels of Bio-Mos® (0.5, 1 and 2 g/kg). For primers A1, A2 and A8, the bands resulted from samples supplemented with Bio-Mos® (0.5, 1 and 2 g/kg) were monomorphic to those resulted from control. On the other hand the bands resulted from experimental diet samples and created by primers A5, A13, C13 were relatively similar to the scorable bands from control.

DISCUSSION

To strengthen the resistance of poultry against pathogenic agents, additives with immunomodulating characteristics, among others prebiotics, are increasingly being used in the animal feed industry. A prominent product in this group is mannan oligosaccharide (Bio-Mos®), which positively influence on defense mechanisms of the digestive system and neutralization of pathogens, they activate digestive enzymes and improve nutrients absorption from the diet.^{29, 30} Broiler chickens are exposed to natural or induced stressors, some of which induce reactive oxygen species (ROS) generation, which may promote morphological and/or physiological malfunctions. Nutrition play an important role in maintaining a balance between free radicals produced by metabolism or derived from environmental sources and the antioxidant system of the body.³¹ The present study revealed that dietary Bio-Mos® supplementation in Japanese quail chicks significantly increased SOD activity and decreased the MDA level that may reflect a significant improvement in health and oxidative status of the birds. A similar study in turkey revealed that Bio-Mos® used as dietary additives stimulate the mechanism of oxidative defense of the birds.³² Consumption of prebiotics, such as oligosaccharides and xylo-oligosaccharides, were capable of increasing intestinal numbers of *lactobacilli* and *bifidobacteria*.³³ Lactic acid bacteria have SOD activity and *in vitro* studies have shown that lactic acid presence and the fermentations of the prebiotic, Fructooligosaccharide, by different strains of *bifidobacteria* lead to the elimination of free radicals.³⁴ Hsia et al.³⁵ suggested that the Fructooligosaccharide may diminish D-galactose induced oxidative molecule damages by contributing antioxidants produced from its fermentation in the colon and by stimulating the proliferation of colonic bacteria that exert antioxidative capacity. Also, it may be that *lactobacilli* resident in gut lyses and release their intracellular antioxidative constituents that in turn help to decrease the MDA.³⁶ Actually, many *lactobacilli* have been reported to have antioxidative capacities, and they have been applied to the alleviation of oxidative stress induced by various dysfunctions.^{37,38} Previous studies have reported that

lactobacilli function as antioxidants by improving the lipid metabolism, increasing antioxidant enzyme activities and inhibiting lipid peroxidation reactions.^{34,39} Others have revealed that the prebiotics had the ability to modify gene expression of antioxidant enzymes. It is reported that the consumption of chicory reduces oxidative stress, restores GSH levels and induces gene expression, which

results in the over expression of the activity of the antioxidant enzyme catalase and in turn, up-regulating the endogenous antioxidant defense system.⁴⁰ It has been suggested that the consumption of the Bio-Mos® supplementation exerts these same systemic antioxidative effects in the colon.

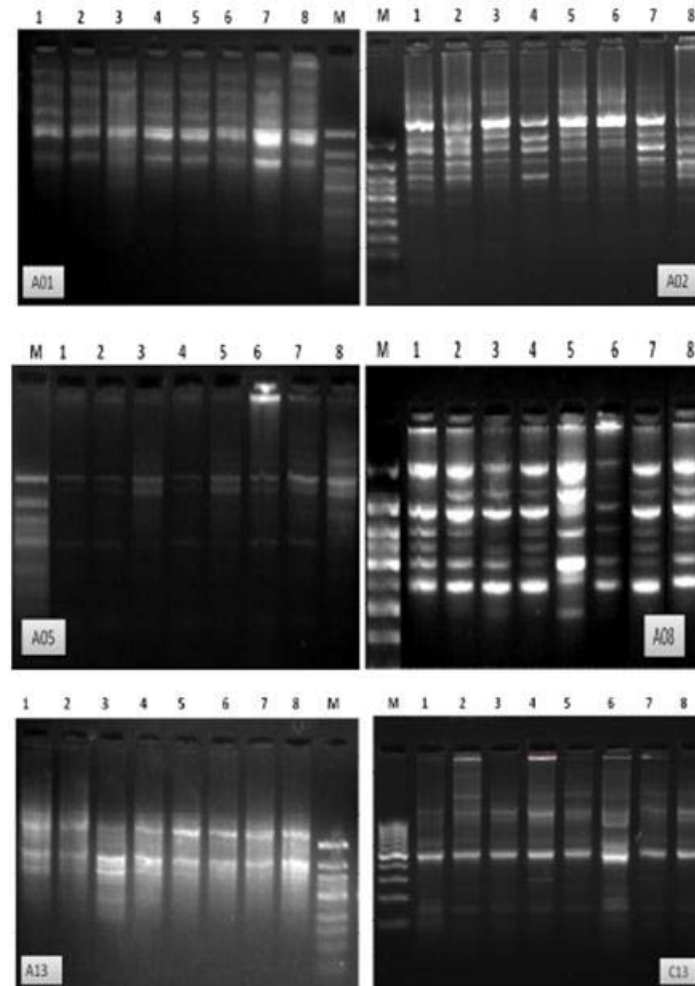


Figure 4: RAPD profiles of genomic DNA from quail blood exposed to varying concentrations of Bio-Mos® (0.5, 1 and 2g/kg diet) for 56 days. M: 100 bp molecular marker. Lanes 1 and 2 represent control group. Lanes 3 and 4 represent quail samples treated with 0.5 g/kg Bio-Mos®. Lanes 5 and 6 represent quail samples treated with 1 g/kg Bio-Mos®. Lanes 7 and 8 represent quail samples treated with 2 g/kg Bio-Mos®.

The increase in *bifidobacteria* and/or *lactobacilli* numbers, associated with the use of prebiotics, has also displayed a direct protective effect on DNA in animal models of colon cancer.⁴¹ Through the Comet assay that enables the detection of the broadest spectrum of DNA damage; double-and single-strand breaks, the extent of DNA damage was decreased by increasing the level of dietary Bio-Mos® supplementation in quail basal diet as compared with control diet. The comet assay has previously been used to evaluate the *in vivo* effect of a prebiotic, lactulose, on DNA damage in the colonic mucosa. Rats that were fed a diet containing 3% lactulose exhibited less DNA damage in colon cells than similarly treated animals fed a sucrose diet.⁴² Recently, Narumi et al.⁴³ concluded that the comet assay of the prebiotic,

galactooligosaccharides in stomach, colon and peripheral blood of rats didn't reveal any DNA damage.

Concerning DNA fragmentation, Bio-Mos® dietary supplementation enhanced the reduction in chromatin damage. Administration of prebiotics leads to decreases in certain bacterial enzymes which involved in synthesis or activation of carcinogens, genotoxins and tumour promoters.²⁰ In calves, Fleige et al.⁴⁴ showed that administration of prebiotic, lactulose decrease the anti-apoptotic rate. In addition, in the present study the supplementation of Bio-Mos® to quail diet reveal no alteration in DNA fingerprint among the control and experimental diet supplemented with varying levels of Bio-Mos®. These findings revealed that the prebiotic Bio-

Mos[®] didn't induce any genotoxic effect on Japanese quail birds which is may be due to its antioxidative properties. Such antioxidative and antimutagenic effect of yeast cell wall mannans were described by Krizkova et al.^{45, 46} In agreement with these results, Hafner et al.⁴⁷ reported that Bio-Mos[®] supplementation in the feed had significant protective effect against genotoxic effect of T-2 toxin in rabbits that is may be by its binding capacity or antioxidative properties. Prebiotics have the ability to scavenge the free radicals of the body and trigger the enhanced SOD activity as well as minimize lipid peroxidation⁴⁸ and overcome the mutagenic and carcinogenic effects of MDA.⁴⁹ Results of Gracia et al.⁵⁰ revealed that in vivo genotoxic studies of fructooligosaccharides (Metlin[®] and Metlos[®]), have shown the absence of mutagenic or genotoxic potential. Moreover, Wollowski et al.⁵¹ observed that LAB (lactic acid bacteria) which increased by supplementation of dietary Bio-Mos[®] have been investigated for their ability to prevent mutations resulted from DNA damage. Also, Zsivkovits et al.⁵² revealed that different *Lactobacillus* strains are highly protective against the genotoxic effects of heterocyclic amine induced in colon and liver of rats.

In conclusion the success of poultry production has been the direct result of improved genetics, nutrition and management as well as maximizing disease control. Recently the prebiotics, mannanoligosaccharide Bio-Mos[®] has been used as a potentially alternative to antibiotics growth promoters in the poultry. The results presented in the current study revealed that Bio-Mos[®] is a beneficial dietary supplement for improving antioxidant activities, decreasing lipid peroxidation level and has a positive impact on the extent of DNA damage which are important in evaluating the efficiency of commercial poultry production system that is influenced primarily by the quality of feed and feed intake.

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