

# Effect of Manna and B-Glucan as Feed Additives on Genotoxicity and Immunity on Broilers

El-Seehy, M.A.,<sup>1</sup>El-Moghazy, G.M.<sup>2</sup>, El-Okazy, A.M.<sup>2</sup>, and Suleyman, M.M.<sup>2</sup>

## ABSTRACT

The present study aims to experiment the Effects of Mannan and  $\beta$  - Glucan as Immuno-stimulants, Prebiotics and Antimycotoxigenic when they are used as a feed additives on Broilers in premix, and then the economical recommendation of the affection of Mannan and  $\beta$  - Glucan in premix. In order to achieve such a purpose, six different types of premix with different concentration of Mannan and  $\beta$ - Glucan, to compare the results two groups of broiler been experiments has been done as negative control and positive control.

Four bioassays were employed to achieve the purpose of the work.

- 1) The molecular cytogenetics parameter.
- 2) The histological parameters.
- 3) The blood parameters.
- 4) The physiological parameters
- 5) The results shows that group (5) and group (6) were the most results which are meets the negative control results in general, that means that the best ratio for Mannan in the premix in between 17% and 25% and the best ratio for B-Glucan in the premix is 25%, also the results indicated that Mannan and B-Glucan have immuno-stimulants, prebiotics and antimycotoxigenic when use as a feed additives on Broilers in premix.

## INTRODUCTION

The use of negative and positive control group is recommended in all mutagenicity tests. According to Preston et al. (1987), positive controls are included to establish the ability of the analyzers to correctly determine aberrations and to ascertain the expected test-to-test and animal-to-animal variations, and to establish the sensitivity of a particular test.

Prebiotics are a category of functional food, defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health. Gibson and Roberfroid (1995) typically, prebiotics are carbohydrates (such as oligosaccharides), but the definition does not preclude non-carbohydrates. The most prevalent forms of prebiotics are nutritionally classed as soluble fibre. To some extent, many forms of

dietary fibre exhibit some level of prebiotic effect. Traditional dietary sources of prebiotics include soybeans, inulin sources (such as Jerusalem artichoke, jicama, and chicory root), raw oats, unrefined wheat, unrefined barley and yacon. Some of the oligosaccharides that naturally occur in breast milk are believed to play an important role in the development of a healthy immune system in infants, but these are not considered prebiotics, as they do not act through the intestinal microflora. Prebiotic oligosaccharides are increasingly added to foods for their health benefits. Some oligosaccharides that are used in this manner are fructooligosaccharides (FOS), xylooligosaccharides (XOS), polydextrose and galactooligosaccharides (GOS). Some monosaccharides such as tagatose are also used sometimes as prebiotics. In petfood also mannoooligosaccharides are being used for prebiotic purposes.

Mannan is a plant polysaccharide that is a polymer of the sugar mannose. Detection of mannan leads to lyses in the mannan-binding lectin pathway. It is generally found in yeast, bacteria and plants. Plant mannans have  $\beta$  (1-4) linkages. It is a form of storage polysaccharide. Ivory nut is composed of mannan.

**$\beta$ -Glucans (beta-glucans)** are polysaccharides of D-glucose monomers linked by  $\beta$ -glycosidic bonds.  $\beta$ -glucans are a diverse group of molecules that can vary with respect to molecular mass, solubility, viscosity, and three-dimensional configuration. They occur most commonly as cellulose in plants, the bran of cereal grains, the cell wall of baker's yeast, certain fungi, mushrooms and bacteria. Some forms of beta glucans are useful in human nutrition as texturing agents and as soluble fiber supplements, but can be problematic in the process of brewing.

Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*, a fungus, the most notable ones being *Aspergillus flavus* and *Aspergillus parasiticus*. Their name is derived from the early work that discovered *Aspergillus* Flavustoxins. Aflatoxins are toxic and among the most carcinogenic substances known. After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide

<sup>1</sup>Department of genetics, faculty of agriculture, Alexandria univervisty, Alexandria.

<sup>2</sup> regional center for food and feed, agricultural research center.

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intermediate or hydroxylated to become the less harmful aflatoxin M1.

The aim of work was to investigate the effects of Mannan and Glucan on:

1. The Immune System of the Broilers.
2. The possible genotoxic effect effect.

## MATERIALS AND METHODS

### A- The Experimental Animal:-

Broilers are chickens (*Gallus gallus domesticus*) bred and raised specifically for meat production. Chickens are one of the most common and widespread domestic animals, and although the global population has decreased from more than 24 billion in 2003 to 19 billion in 2011, there are more chickens in the world than any other species of bird.

Broilers have a varied diet. They eat insects, worms, fruit, seeds, acorns, grains, slugs, snails, and many other foods. They have a well-developed gizzard (a part of the stomach that contains tiny stones) that grinds up their food.

### B- Experimental procedures:-

A total number of (80) one day old chicken has been collected from a farm near to Alexandria, and been adapted for 6 days at to laboratory conditions inside prepared hatches, and were treated by all the broilers vaccines to ensure the avoidance of diseases hazard or death, and then the treatments starts to proceed from the 7<sup>th</sup> day till the 45<sup>th</sup> day as a commercial conditions of the broilers breeding, and from through all the treatment proceeding the chicken been feed with a typical standard feed been experimented to be free of Aflatoxin and in the same laboratory environmental conditions.

A total number of (6) types of Mannan and  $\beta$  Glucan premixes with different concentrations of those were treated as a treatments and added as a (1.5 gm/kg feed) as a commercial ratio with a permanent concentration of Aflatoxin (1 $\mu$ g/kg feed) to experiment their Immuno-stimulant and Antimycotoxigenic effect as a feed additives on broilers, and been added.

At the 7<sup>th</sup> day the total number of (80) chicken were divided into (8) groups each group contained a total number of (10) chicken and had sorted as a Group(1) Negative control, Group(2) Positive control and 6 groups as a treatments as been shown in the table (1):-

In the 45<sup>th</sup> day the blood was collected from the veins under the wing and the blood was receives in anti-coagulants tubes and normal tubes and then the chicken were slighted and blood was smeared on clean slides, liver and bone marrow tissue were removed and subjected to the bioassay as follows.

### C- Chromosomal aberrations analysis:-

Each animal had received 0.5ml (4 $\mu$ g) colcemid /100 gm for cells weight of chicken. After four hours the animals were killed and bone marrow were removed. Preparations of chromosome complement were carried out according to the method that described by Seehy (2002b).

Bone marrows were removed by an isotonic and received in a mortar containing isotonic solution and filtered through two layer of nylon mesh and then centrifuged for 10 min. at 2300 rpm, and then transferred to a cup containing 20 ml of hypotonic solution (0.075 M KCl), gently homogenized, and left for 45 min. at 37C°. The homogenate was then centrifuged for 10 min, at 2300 rpm. The supernatant was discarded and the pellet was then suspended in methanol and glacial acetic acid (3:1), and centrifuged. (Repeat these 4 times).

Cells in fixative were dropped onto very clean glass slides and air dried. Spread cells were stained with 10% Giemsa (pH 6.8) for 5 min.

Scanning slides for mitotic spreads was conveniently accomplished with a 25X magnification objective, and analysis was with a 100X objective. For control of bias, all prepared slides were coded prior to scoring for chromosomal abnormalities; at least 100 scorable metaphases per treatment was investigated for, Stickiness, Deletion, RCF, and Fragment...etc and recorded.

**Table 1. classification of Treatments in Experiment.**

| Groups  | Mannan %<br>In premix | B Glucan %<br>In premix | Aflatoxin con.<br>( $\mu$ g/kg feed) |
|---------|-----------------------|-------------------------|--------------------------------------|
| 1(N.C.) | 0                     | 0                       | 0                                    |
| 2(P.C.) | 0                     | 0                       | 1                                    |
| 3       | 3                     | 1                       | 1                                    |
| 4       | 10                    | 0                       | 1                                    |
| 5       | 17                    | 25                      | 1                                    |
| 6       | 25                    | 25                      | 1                                    |
| 7       | 25                    | 30                      | 1                                    |
| 8       | 32                    | 38                      | 1                                    |

**D- Micronucleus test:-**

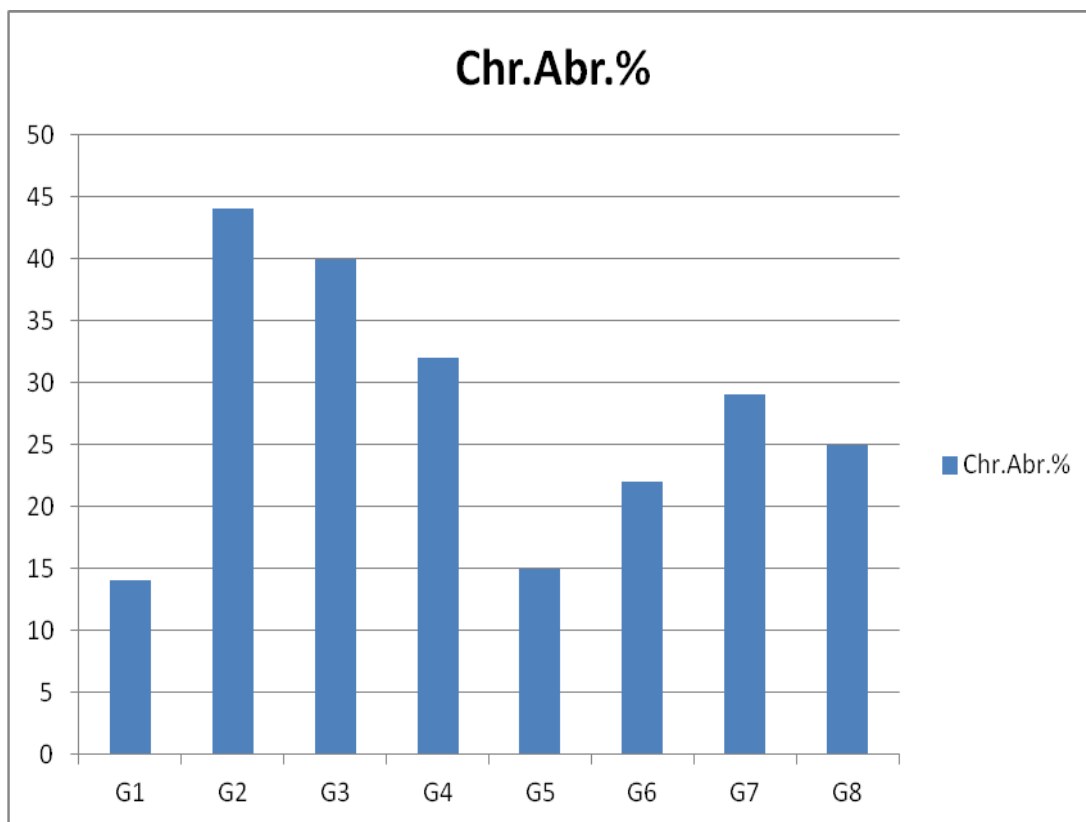
The peripheral blood smears were obtained through the under wings veins and neck vein. The slides were air-dried for 12 hrs and then fixed in methanol for 10 min, followed by 5% Giemsa (w/v) staining. Each chicken had 1000 erythrocytes examined, from both peripheral blood and the kidney. To detect micronuclei in erythrocytes, the slides were analyzed using a 1000X oil-immersion lens. Statistical analysis was performed using Student's t test. This method were carried out through (Grisolia and Starling, 2001).

**E- Immune Globulin Analysis:-**

Bovine polyclonal Immune Globulins G (IgGs) were obtained from Sigma (St. Louis, MO). Blood samples of chicks randomly selected from each pen, was collected for determination of serum IgGs concentrations. The method used LC-MS/MS according to Julien et al., (2002).

**RESULTS AND DISCUSSION****A- Analysis Of Chromosomal Aberrations:-****B- Micronucleus Test:-****Table 2. The total Chromosomal Aberrations of all treatments in the 8 Groups**

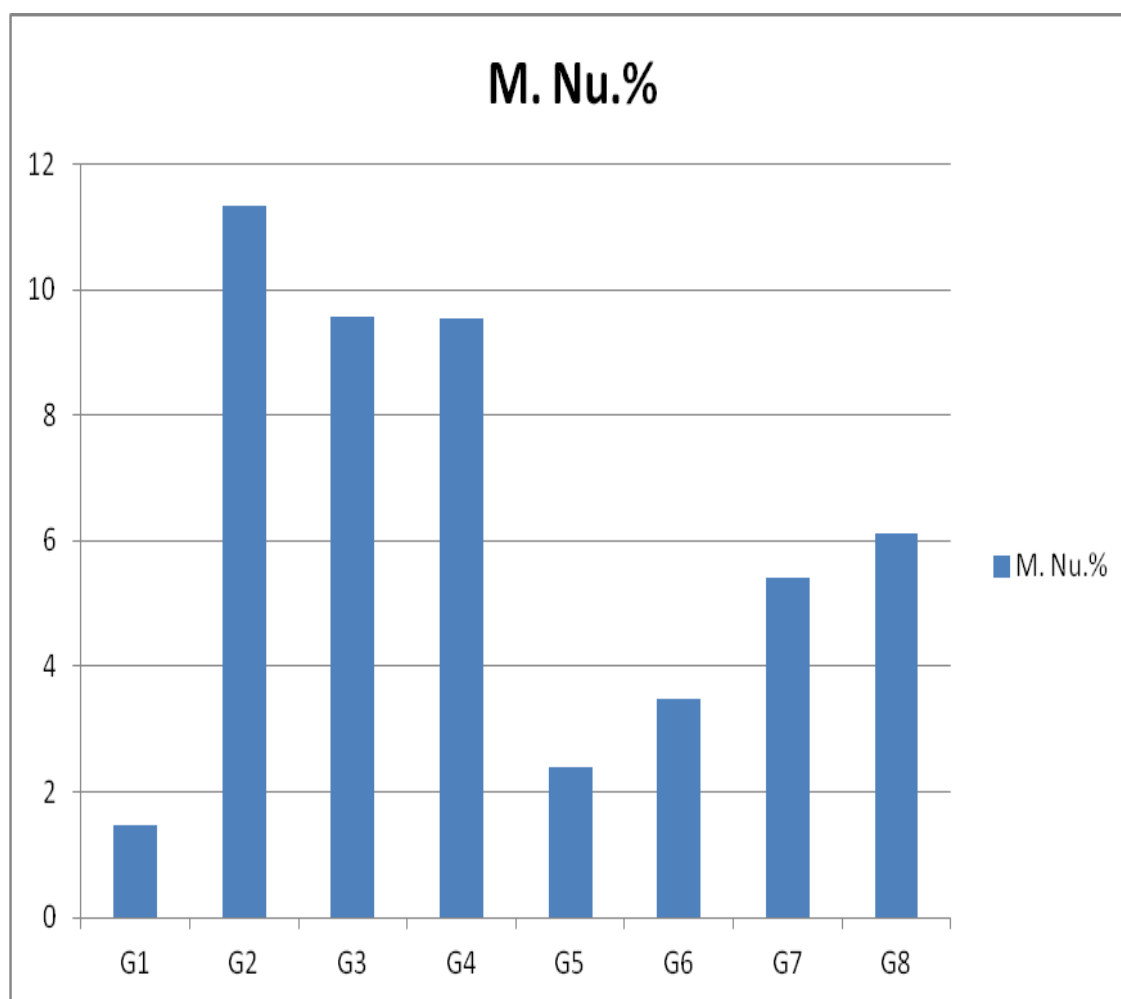
| Treatments in groups | Total Aberrant Metaphase % |
|----------------------|----------------------------|
| Group 1              | 14                         |
| Group 2              | 44                         |
| Group 3              | 40                         |
| Group 4              | 32                         |
| Group 5              | 19                         |
| Group 6              | 22                         |
| Group 7              | 29                         |
| Group 8              | 25                         |

**Figure 1. Graph showing the percentage of total Chromosomal Aberration**

**Table 2.** the results obtained from the analysis of micronucleus obtained from the blood erythrocytes cells of Broilers (*Gallus gallus domesticus*) after treatment of the 8 Group in precede

| Treatments in groups | Micronucleated Erythrocyte % |
|----------------------|------------------------------|
| Group 1              | 1.47 $\pm$ 0.31              |
| Group 2              | 11.35 $\pm$ 2.1              |
| Group 3              | 9.58 $\pm$ 1.8               |
| Group 4              | 9.54 $\pm$ 1.9               |
| Group 5              | 2.39 $\pm$ 0.9               |
| Group 6              | 3.47 $\pm$ 1.3               |
| Group 7              | 5.41 $\pm$ 1.7               |
| Group 8              | 6.12 $\pm$ 1.5               |

1000cells were counted



**Figure 2.** Graph showing the percentage of Micronucleated Erythrocyte

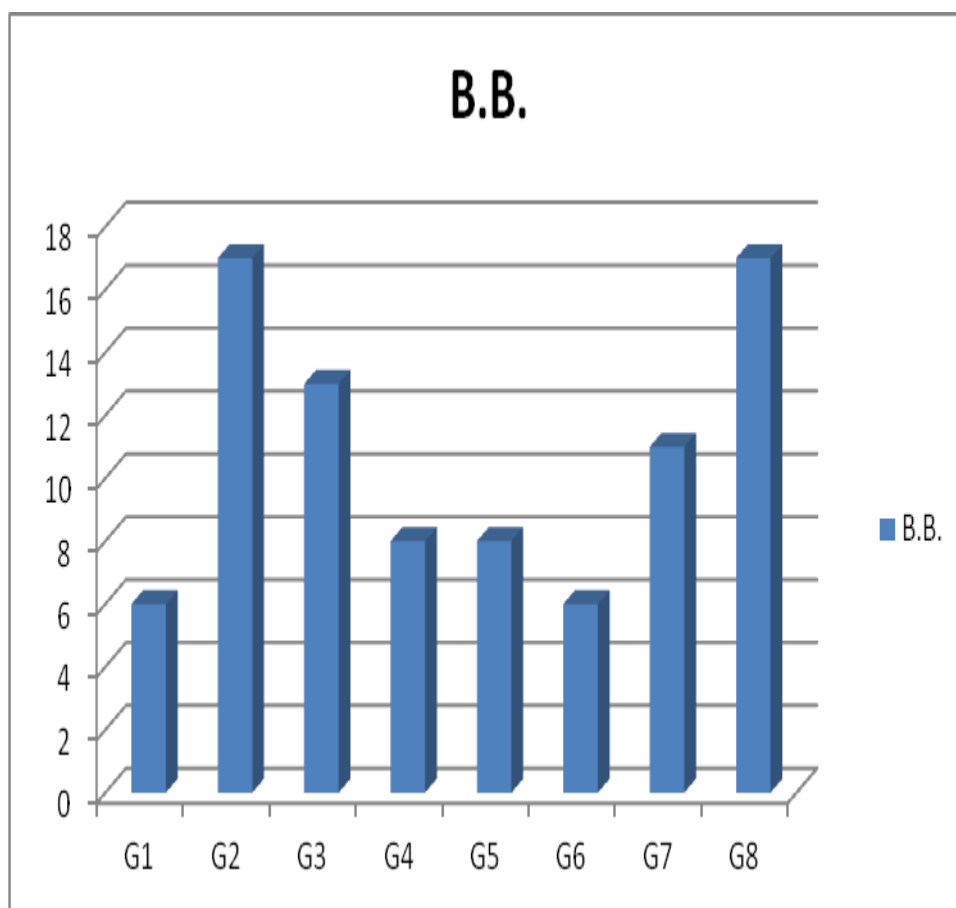
#### C- Immune Globulin:-

The effects of the prebiotic on serum IgGs concentrations of broilers are given in Table (4) the difference in IgGs concentrations between the control

and experimental groups was not significant, also shows also that Group (6) gives the same results as well as the negative control.

**Table 4. the results obtained from the analysis of Immune Globulins of Broilers (*Gallus gallus domesticus*) after treatment of the 8 Group in preceede**

| Treatments in groups | Break Area Measuring |
|----------------------|----------------------|
| Group 1              | $6 \times 10^7$      |
| Group 2              | $17 \times 10^7$     |
| Group 3              | $13 \times 10^7$     |
| Group 4              | $8 \times 10^7$      |
| Group 5              | $11 \times 10^7$     |
| Group 6              | $6 \times 10^7$      |
| Group 7              | $11 \times 10^7$     |
| Group 8              | $17 \times 10^7$     |



**Figure 3. the results obtained from the analysis of Immune Globulins of Broilers (*Gallus gallus domesticus*) after treatment of the 8 Group in preceede**

#### CONCLUSION

The results obtained from all the parameters shows that Mannan and B-Glucan have an Immuno-stimulants, Prebiotics and Antimycotoxigenic effect when use as a Feed Additives On Broilers, the results shows that he different concentrations of Mannan and B-Glucan in the premix can affect the inhibition of toxins effactions in the feed and can make repair when mixed with feed in

case if this case are toxificated, also the results shows that group (5) which is 17% Mannan and 25% B-Glucan in premix as a feed additives and group (6) which is 25% Mannan and 25% B-Glucan in premix as a feed additives give the most results which are meets the negative control results in general, that means that the best ratio for Mannan in the premix in between 17% and 25% and the best ratio for B-Glucan in the premix is 25%.

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## الملخص العربي

## تأثير المنان والبيتاجلوكان كمحفز مناعى وبادىء حيوى ومضاد للسمية الوراثية كإضافات اعلاف للدواجن

نُجْد الصيحي، جيهان المغازى، احمد العكازى، محمود سليمان

واضحة ان المجموعات (5) و (6) هم اقرب ما يكون للكنترول السالب.

نتائج اختبار النواة الصغيرة اظهر العدد النسبى للنوية الصغيرة فى المجموعة (1) 1.47% والمجموعة (2) 11.35% والمجموعة (3) 9.58% والمجموعة (4) 9.45% والمجموعة (5) 2.39% والمجموعة (6) 3.47% والمجموعة (7) 5.41% والمجموعة (8) 6.12%. وهذا له دلالة واضحة ان المجموعات (5) و (6) هم اقرب ما يكون للكنترول السالب.

بتلخيص النتائج الكلية المتحصل عليها نجد ان المجموعة (5) والمجموعة (6) هم افضل النتائج مقارنة مع الكنترول السالب ونجد ايضا من النتائج ان مخاليط الاعلاف المحتوية على المنان والبيتاجلوكان لها تأثير مناعى وحيوى ومزيل لسمية الوراثية عند استخدامها كإضافات اعلاف للدواجن.

المهدف من اجراء الدراسة هو اختبار دراسة تأثير المنان والبيتاجلوكان كمحفز مناعى وحيوى ومضاد للسموم الفطرية عند استخدامه كإضافات علفية للدواجن فى صورة مخلوط. ولاجراء هذا الاختبار تم تقسيم العدد الكلى ل 80 فرخ صغير الى 8 مجموعات تم تربيتها حتى عمر 45 يوم.

وقد تم اجراء اختبارات اساسية لهذا الغرض و هى:

1- اختبار الشذوذات الكروموسومية.

2- اختبار النواة الصغيرة.

تحليل الكروموسومات اوضح ان المجموعات تحتوى على العديد من الشذوذات التركيبية للكروموسومات وكانت النسبة الكلية للشذوذات فى المجموعة (1) 14% والمجموعة (2) 44% والمجموعة (3) 40% والمجموعة (4) 32% والمجموعة (5) 19% والمجموعة (6) 22% والمجموعة (7) 29% والمجموعة (8) 25%. وهذا له دلالة