Determine the Suitable Ratio of B-Glucan as an Immune Stimulant and Anti-Mycotoxigenic in Feed Additives in Mice

Mahmoud M. Suleyman*, Gihan El Moghazy, Alaa F. M. Ibrahim
The Regional Center for Food and Feed, Agricultural Research Center, Ministry of Agriculture.
*Corresponding author: moodysuleyman@gmail.com

ABSTRACT

This study aimed to determine the ideal ratio of β Glucan in feed additives as immune stimulants and anti-mycotoxigenic agents when used as a feed additive in mice in premix, as a step to reach a final composite of feed additive as a premix with an ideal ratio of β Glucan. Two different bio-assays were employed in the experiment; the Immune Globulin (IGs) test in blood and the molecular Cyto-genetic parameters (micro-nucleus test and chromosomal aberration). In order to achieve such a purpose we use 7 different groups of mice with different concentrations of β-glucan in feed premix, to compare the results 2 groups of mice were experimented with as a negative and positive control, the feed used was prepared as a typical composition of feed for the age stage of mice without any other additives to experiment the individual effect of β-Glucan only, the doses of β-glucan have been chosen as the minimum and maximum ratio of β-glucan in commercial premixes.

Keywords: β-Glucan; Immune Globulin; anti-mycotoxigenic; Mice; feed additives.

INTRODUCTION

β-Glucans (beta-glucans) are polysaccharides of D-glucose monomers linked by a β-glycosidic bond. β-glucans are a diverse group of molecules that can vary with respect to molecular mass, solubility, viscosity, and three-dimensional configuration (Ho et al. 2016). β-glucans can be found primarily in the cell wall of baker's yeast, (Burton and Fincher 2012). Some forms of β-glucan are useful in human nutrition as texturing agents and soluble fiber supplements but can be problematic in the brewing process (El Khoury et al. 2012).

Aflatoxins are naturally-occurring mycotoxins produced by several species of *Aspergillus*, a fungus, the most notable being *Aspergillus flavus* and *Aspergillus parasiticus*. Their name is derived from the early work that discovered *Aspergillus flavstones*. Aflatoxins are toxic and are among the most carcinogenic substances known. After entering the body, aflatoxin can be metabolized by the liver into a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M1 (Fratamico et al., 2008; Iqbal et al. 2014; Khalangwiset et al., 2011).

Luo et al., (2019) studied dietary β-glucan supplementation to improve growth performance, carcass traits, and meat quality of refined pigs and reported that a basic diet supplemented with 100 mg/kg *Agrobacterium* sp. ZX09 β-Glucan Improves Growth Performance, Nutrient Digestibility, Carcass Length, and Pork Quality of Refined Pigs. Wu et al., (2021) reviewed the molecular structure, antitumor immune activities, structure-activity relationship, and clinical trials of soluble β-glucans to provide a holistic view of β-glucans as immunostimulants to fight cancer. β-glucan acts as an immunostimulant, enhancing survival, growth, and protection against infectious pathogens in fishes and shellfishes (Meena et al., 2013).

The aim of the work was to determine the ideal dosage of beta-glucan in the premix to achieve the most effect on the immune system with the least effect on DNA content.

MATERIALS AND METHODS

1. Experimental Animal

The experimental study was carried out on Animal in mice (*Mus musculus domesticus*), the small mammal from the order Rodentia (Gregory et al., 2002; Meerberg et al., 2009a,b). This animal has been adopted and chosen from the Faculty of Agriculture at Alexandria university and been adopted for 5 days in the laboratory conditions before the experiment proceeds.

A total number of 70 mice have been divided into 7 groups each group contained a total number of 10 mice from the age of 5 days till the age of 35 days at prepared hatches at the Faculty of Agriculture. Through all the treatments proceeding the mice have been fed with a typical standard feed to be free of Aflatoxin and in the same farm environmental conditions. The experiment was sorted as a Group (1): Negative control, Group (2): Positive control, and 5 groups as treatments with different concentrations of β Glucan (Sigma pure) and added to the feed as shown in Table 1 with a permanent concentration of Aflatoxin (0.9 µg/kg feed) to experiment their immunostimulant and antimycotoxigenic effect as feed additives on mice.

On the 15th and the 30th day of treatment the blood was collected from the femur and was received in anticoagulants tubes in order to test the immune assays and the micro-nucleus test in blood, and then on the 30th day of treatment.

Table 1: The groups of treatments and doses.

Groups	B Glucan conc (mg/kg feed)	Aflatoxin con. (µg/kg feed)
1	0	0
2	5	0.9
3	10	0.9
4	15	0.9
5	20	0.9
6	25	0.9
7	30	0.9

The mice were slighted and the bone marrow tissue was removed from the femur bone of each side and subjected to the bio-assay of chromosomal aberration test as follows:

2. Immune Globulin Analysis:

Immune globulins G (IgGs) were tested in blood samples of mice for the determination of serum IgGs concentrations, according to Julian et al., (2002).

2.3. Micro-nucleus test:

The blood was smeared on slides and air-dried for 12 h and then fixed in methanol for 10mins, followed by 5% Giemsa (w/v) staining. Each group had 1000 erythrocytes examined to detect the micronuclei in erythrocytes, the slides were analyzed using a 1000 x oil immersion lens, and statistical analysis was performed using a student's t-test (Grisolia and Starling, 2001).

4. Chromosomal aberrations test:

Each animal received 0.5 ml (4 µg) of colcemid/100 g for the weight of the cells of the chicken. After four hours the animals were killed and marrow was removed. Chromosome complement preparations were performed according to the method described by Mukti et al. (2016). The marrow was removed by an isotonic solution and obtained in a mortar containing isotonic solution and filtered through two layers 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 37 °C. The homogenate was then centrifuged for 10 min at 2300 rpm. The supernatant was discarded and then the pellet was suspended in methanol and glacial acetic acid (3:1), and centrifuged. (repeat this 4 times). Cells in the fixative were dropped onto very clean glass slides and air-dried. Extended cells were stained with 10% Giemsa (pH 6.8) for 5 min. Scanning slides for the mitotic spread was easily accomplished with a 25X magnification objective, and analysis was performed with a 100X objective. To control for bias, all prepared slides were coded for chromosomal abnormalities before scoring; At least 100 scoreable metaphases per treatment were examined and recorded for viscosity, deletion, RCF, and fragment...etc.

RESULTS AND DISCUSSION

1. Immune Globulins

The effects of β glucan on the blood serum IgGs concentration of mice after 15 and 30 days of the treatment are given in Tables 2 and 3, respectively. The difference showed that group number 6 gives the results that meet N.C.

2. Micro-nucleus test:

The results obtained from the micro-nucleus test were summarized in Tables 4 and 5 for the test after 15, and 30 days, respectively, of treatment, where groups number 6 and 7 were shown the results of the negative control.

3. Chromosomal Aberration test:

Table 6 summarized the results obtained from the analysis of the chromosomal aberration in the bone marrow of mice after 30 days of treatment in the groups as also, shown in Figures 1 and 2. Stickiness, deletion, RCF, and fragmentation of chromosomes (Figure 3) were observed with percentages shown in Table 6, where groups 6 and 7 are the groups that meet the results of the negative control.

Table 2: Analysis of Immune Globulins of mice after 15 days of treatment.

Treatments in groups	Break Area Measuring
Group 1	$5x10^7$
Group 2	$15x10^7$
Group 3	$13x10^7$
Group 4	$8x10^7$
Group 5	$8x10^7$
Group 6	$6x10^7$
Group 7	$8x10^7$

Table 3: Analysis of Immune Globulin of mice after 30 days of treatment.

Treatments in groups	Break Area Measuring
Group 1	$6x10^7$
Group 2	$17x10^7$
Group 3	$11x10^7$
Group 4	$10x10^7$
Group 5	$12x10^7$
Group 6	$9x10^7$
Group 7	$11x10^7$

Table 4: Analysis of micro-nucleus test after 15 days of treatment.

Treatments in groups	Micro nucleated Erythrocytes %
Group 1	2±0.2
Group 2	13.7 ± 2.1
Group 3	6.4 ± 0.21
Group 4	5.7 ± 1.2
Group 5	4.12 ± .32
Group 6	4.2 ± 1.2
Group 7	3.6 ± 1.7

Table 5: Analysis of micro-nucleus test after 30 days of treatment.

Treatments in groups	Micro nucleated Erythrocytes %
Group 1	2.21±1.2
Group 2	11.4± 0.24
Group 3	4.9 ± 1.4
Group 4	5.6 ± 0.9
Group 5	5.54 ± 1.24
Group 6	3.9 ± 0.72
Group 7	3.81 ± 0.64

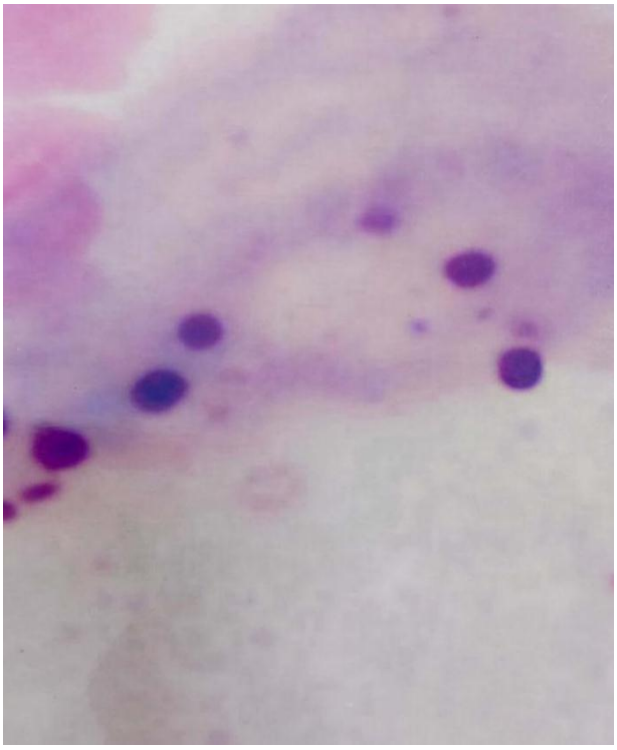

Figure 1: Nucleus in Lysis

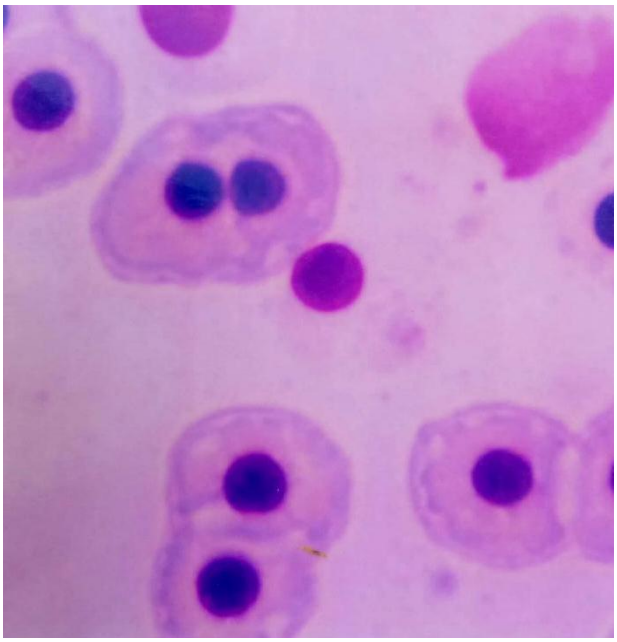

Figure 2: Bi Nucleated

Table 6: Analysis of chromosomal aberration test after 30 days of treatment.

Treatments in Groups	Stickiness	Deletion	RCF	fragmentation	Total Aberrant Meta-phases %
Group 1	5	3	0	7	15
Group 2	19	8	3	14	44
Group 3	18	7	3	12	40
Group 4	14	7	1	12	34
Group 5	10	8	2	9	29
Group 6	8	4	0	7	19
Group 7	9	4	0	9	22

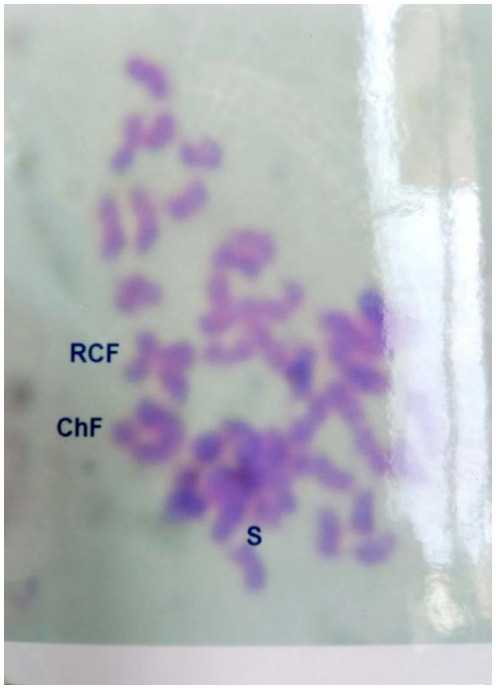

Figure 3: Shows some types of aberrations observed (Stickiness, fragmentation and RCF)

For Immune Globulins, the blood serum IgG concentration of rats after 15 days of treatment and after 30 days of treatment showed that treatment group 6 was the highest group that meets the results of the negative control group, and diagrams (1) and (2) clears this result and means that when the concentration of B glucan in feed was 25 mg/kg of feed, that was the ideal concentration for IgG to have an effect in the blood.

Cao et al. (2022) found that the Immunomodulatory effect of polysaccharides from yeast cell walls has been demonstrated effective to change immune response and altered gut microbiota composition in chickens. In our previous study, a yeast cell wall product containing β-glucan and MOS was found effectively to enhance intestinal IgA response to NDV vaccination and modulated the cecum microbiota by oral route.

The results obtained from micro-nuclei test analysis after 15 days of treatment showed that group (7) was the most fulfilling the negative control's results, whereas the results obtained after 30 days of treatment showed that Groups (6 and 7) most closely resembled the negative control results when the concentration of B glucan in the feed additive was 25 and 30 mg/kg of feed.

The results obtained from the chromosomal aberration test showed that group (6) when the concentration of B glucan was 25 mg/kg feed treated the negative control's results as 19 aberrant meta-stages compared to the negative control of 15 aberrant meta-stages. Completes in, group number (7) results even when the concentration of B-glucan in the feed was 22 of the total abnormal meta-stages compared to the negative control in the 30 mg/kg feed as shown in diagram number (5).

To compare these results, Morales-Lopez et al., (2009) and Cox et al., (2010) reported that the feed consumption of birds with a ratio of β glucan 15mg/kg to 30 mg/kg feed was not different from that of the control. Hahn et al. (2006) also reported that β-glucan did not show any effects on average daily feed intake and gain to feed ratio (G: F ratio) as the β-glucan level of the diet (0, 0.1, 0.2, 0.3, and 0.4 g/kg) increased in weanling pigs.

CONCLUSION

The results obtained from all parameters showed that the ratio between 25-30 mg/kg feed of pure B glucan is the best ratio for B glucan to be added to the feed and this ratio can affect the inhibition of the toxins' affections in the feed biologically and can uses also to activate the immune system.

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الملخص العربي

تحديد الجرعة المثلى من البيتاجلوكان كمحفز مناعى ومضاد للسمية الوراثية فى إضافات الاعلاف

محمود محمد سليمان، جيهان المغازى، علاء فتحى ابراهيم

المركز الإقليمي للأغذية والأعلاف، مركز البحوث الزراعية ، وزارة الزراعة

الهدف من اجراء البحث هو تحديد افضل نسبة من مادة البيتاجلوكان عندما يستخدم كاضافات علفية لتحفيز الجهاز المناعة وكذلك مضاد للسمية الوراثية ولاثبات الغرض تم استخدام الفئران كحيوان تجربي لهذا الغرض حيث انه تم اقلمة عدد ٧٠ فار فى الظروف المعملية وتقسيمهم الى ٧ مجموعات وهى مؤشر سالب ومؤشر موجب و٥ معاملات تم تثبيت نسبة الافلاتوكسين المستخدم مع تغيير نسبة البيتاجلوكان فى العلف وقد تم استخدام اختبارت لتاكيد الغرض و هى اختبار الشذوذات الكروموسمية، اختبار النواة الصغيرة، وتحليل الاجسام المناعية. وقد اظهرت النتائج ان المجموعات رقم(٦) و(٧) هى افضل النتائج المثلى وعلية فانه طبقا للبحث نجد ان النسبة الافضل للبيتاجلوكان فى العلفة تتراوح بين ٢٥ الى ٣٠ مل جرام/كيلو علف.

الكلمات المفتاحية: β-Glucan؛ الجلوبيولين المناعي مضاد للسموم الفطرية، الفئران، إضافات مغذيه.