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The Bio Test to the Effectiveness of Mycofix® Select 3.0 and Activated Charcoal to Reduce the Toxic Effects of Aflatoxin B1 in Broiler Chicks

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Abstract: ***Adding 5 g / 100 g of Mycofix® Select 3.0 (M) and g / Kg of Activated Charcoal (A) to a poultry diet contaminated with aflatoxin B1 (B1) at a concentration of 0.02 µg / kg to the superiority of treatments B1AM, B1M, and B1A significantly increased the Total Weight of the broiler at 1 day and fed on a poultry diet contaminated with B1 for 21 days before slaughter with a percentage 36.9%, 34.5% and 21.5% respectively,and increased Heart weight by 23.9%, 16.6% and 6.3%,and Liver weight increased by 33.50%, 15.07% and 7.2%and increase the weight of the Craw by 25.08%, 9.3% and 11.1%.The treatments B1AM and B1A showed significantly increased the weight of Bile by 52.4% and 1.3%.Treatment B1 reduced the value of Packed Cell Volume (PCV) by 36.3%while B1MA and B1M significantly increased PCV values by 20.5 and 14.3%.Treatment B1 reduced the value of Hb by 37.07%while B1MA and B1M significantly increased Hb values by 22.07% and 14.79%.Treatment with B1 reduced Red Blood Cell (RBC) by 13.7%while B1MA, B1A and B1M significantly increased the number of RBC by 16.37%, 15.04% and 2.63%.Treatment with B1 increased the number of white blood cell (WBC) by 27.42%while B1M, B1A and B1MA significantly reduced WBC numbers by 23.44%, 21.77%, and 6.48% compared to their value in the comparison treatment (0.0).B1A, B1M and B1MA showed a significant increase in Monocyte values of 103.3%, 60.60% and 36.36% compared to B1 treatment.Treatment B1 reduced Monocyte by 46.23% compared to the comparison treatment (0.0).Treatment B1 reduced Lymphocyte values by 27.48% compared to the comparison treatment (0.0)while B1M and B1A significantly increased Lymphocyte values by 10.945% and 5.59% compared to their values in B1treatment,The B1M treatment gave the lowest decrease in Neutrophils values of 24.87%followed by B1A treatment reduced in Neutrophils values by 18.47% compared to B1 treatment.Treatment B1 increased the value of Neutrophils by 83.7% compared to the comparison treatment (0.0).B1M showed the highest increase in Basophils values of 41.26%followed by treatment of B1A and B1MA increased by 38.09% and 34.92%.Treatment B1A showed the highest increase in Eosinophils values by 80.0%followed by treatment of B1MA and B1M by 40.0% and 20.0% compared to B1 treatment.***

Keywords: Aflatoxin B1, Mycofix® Select 3.0, Activated Charcoal and Broiler chicks

1. Introduction

Mycotoxins are a group of secondary metabolites with low molecular weightwhich are produced by some fungi such as *Aspergillus, Penicillium, Fusarium* and others. Mycotoxins are secondary metabolites produced by filamentous fungi that can cause a wide variety of harmful effects to animals and humans (Zain, 2011). Fungi are ubiquitous in the environment and are a serious global concern in agriculture since they can infect crops in the field and/or during postharvest stages such as storage and transport of agricultural products (Bryden, 2012). Mycotoxins can cause harm by directly contaminating agricultural products or indirectly via a 'carry-over' effect into animal tissues, milk and eggs (Koppen *et al*, 2010). The level of mycotoxin contamination depends on the type of crop, agronomic practice and climate conditions. Food and fodder contamination occurs during the preparation of food and feed from the field to consumingas well as the susceptibility of mycotoxins to resistance to certain industrial processes (CAST, 2003; Joseph et al., 2008). Mycotoxins are transmitted directly or indirectly to humans by eating animal productswhich have already been fed to fooder contaminated with mycotoxins (Maxwell et al., 2006; Milicevic et al., 2010). Mycotoxins are frequent contaminants of human foods and animal feeds, produced by specific fungal strains. Mycotoxins are capable of affecting the health and performance of domestic animals, decrease the immune response and even cause death when their levels are high enough (Murugesan*et al*., 2015).The discovery of aflatoxins is the true beginning of fungal toxicology, then the discovery of mycotoxins continued (Wyllie and Morchouse, 1977).Studies have shown the risk of mycotoxins on human and animal health (Wogan, 1966) and on the environment with its different effects at low concentrations (Jones et al., 1982).Some species *Aspergillus* are *Aspergillusflavus, A.parasiticus, A.namius* produces aflatoxin B1(Smith et al,1992).as a result of the rapid development of the poultry industry and the importance of the fodder industry, which form for 65-70% of the cost of production,growth rates are important standard for identifying the effect of mycotoxins on the vital processes of birds,as well as their economic importance,aflatoxin B1 reduces the rate of increase in weightbecause of the feeding of contaminated fodder at the minimum level of B1,the decrease in weight is increased by an increase in the amount of aflatoxin B1 (Ibrahim et al., 2000; Kubena, 1990).Ramos et al. (1997) indicate the use of absorption compounds to remove aflatoxin toxicity from poultry diet as well as the use of other biochemical and physical. Dale and Wgatt (1995) also indicate that there was no perfect method to remove the effect of mycotoxinsor reducing them from fodder material widely. Physical methods are one of the best preventive ways to catch toxins when they pass through the digestive systemand reduce the period of survival and put them out of the body and thus reduce their absorption And reduce their passive effects on poultry production and health,Galvano et al. (1996) and Vekiru et al (2007) reported that aflatoxin toxin could be adsorbed by organic compounds such as Activated charcoal

(AC) and Hydrated Sodium Calcium Alumino-Silicates (HSCAS).

Mycofix plus 3.0®: Mycofix plus 3.0 is the product ofBiomin® GTI GmbH. Herzogenbeurg, Austria. Mycofix®Plus originally contained the components: Synergistic blendof minerals, Biological constituent, Synergistic blend ofminerals, Biological constituent, BBSH 797, phytogenic substances, and Phycophytic constituents. Mycofix, is one of the adsorbent that can be added in poultry feed and is claimed to neutralize moderate levels of aflatoxin (up to 2500-3500 ppb) in poultry feed. Biomin® (2000)reported that aflatoxin toxin could be absorbed by Mycofix deactivates aflatoxin with its polar functional group, due to AF fixation to adsorbing components in Mycofix, with stable binding capacity. Adsorption starts in the oral cavity during salivation and continues in stomach and gut. The fixed mycotoxin being unable to enter the blood and subsequently excreted in faeces after 98% adsorption of AF by Mycofix. The results of the present study also confirm previous studies showing that Mycofix is capable of counteracting the adverse effects of trichothecenemycotoxins. Diaz (2002) showed that dietary supplementation of 1.5 kg/t Mycofix completely overcame the adverse effects of 1 ppm dietary 4,15-diacetoxyscirpenol (DAS) in broiler chicks. In this latter study, 2 ppm dietary DAS caused a significant decrease in body weight (BW) gain after only 7d of exposure, in contrast to the 28 d required for 2 ppm dietary T-2 toxin to cause the same effect. This fact confirms the greater toxicity of DAS for chickens compared with T-2 toxin. In terms of LD50, DAS is the most toxic trichothecene for poultry species. The LD50 values for DAS, T-2 toxin, HT-2 toxin, and Neosolaniol in 1-d-old chicks are 2.0, 5.0, 7.2, and 24.9 mg/kg, respectively (Leeson et al, 1995).The study aims to: Comparison of the efficiency of Mycofix® Select 3.0 and Activated Charcoal (A)Inreducing the toxic effects of Aflatoxin B1.

2. Materials and Methods

Ability of fungus *Aspergillusflavus sp. Link ex Fries*on the production of aflatoxin B1

The mycotoxins were prepared by using rice to produce the aflatoxin B1 after estimating the moisture content,then, we took 200 g of rice in petri dish (diameter 25 cm)with 100 ml of distilled waterand sterilization in the autoclave device at 120 ° Cand pressure 1.5 bar / cm² for 20 minutes and two consecutive times within 48 hoursto ensure the sterilization process,inoculation of petri dish with the fungus isolation *Aspergillusflavus sp. Link ex Fries*developing on maize grain, which producing aflatoxin B1, shake the petri dish for four minutes daily and for four days respectivelyto ensure homogeneous distribution of the fungus vaccine in the medium,petri dish were incubated at 25 ± 1 ° C for 21 days.Drying the contents of the petri dish at 40 ° C,the amount of aflatoxin B1 was estimated inHigh Performance Liquid Chromatographsy (HPLC) device.

Poultry feed

Usefodder type Ivan / Ivan Feed Company / Arbil, Iraq ,primary stage,feed consists of protein, wheat, maize, soybean meal, dicalcium lysine, vitamins, choline chloride, fine metals, food salt, soybean oil, antifungal,anti-toxin, anti-coccidiosis, anti-bacterial and viral, anti-oxidant.

Broiler chicks

In the experiment, were used 210 chicks of the broiler chicks as a coob type,she weighed the chicksbefore distributing them to treatment, the weights of the chicks should be equal to ± 5 grams,distribution of chicks on 7 treatments, each treatment consists of 3 replicates, by 10 chick / replicate (Table 1).

Table 1: Treatments

Treatment	1	2	3	4	5	6	7
B1(mg/Kg)	0.0	0.2	0.2	0.2	0.2	0.0	0.0
A (g/100g)	0.0	0.0	5.0	0.0	5.0	5.0	0.0
M (g/kg)	0.0	0.0	0.0	1.0	1.0	0.0	1.0

Calculating the feed conversion ratio (FCR) per week, calculating the amount of feed consumed, and dead chicks daily.

Total Body weight

Weighed chicks before slaughter and 3 weeks old

Internal organs weight

Eradication of internal organs such as liver, heart, craw, bile and spleen after slaughtering,organs were directly weighed, estimate the relative weight of each organs to body weight.

Blood biochemical properties

Using the haemocytometer in counting the red blood cell (RBC) and the White Blood Cell (WBC) directly (Natt and Herrck, 1952),calculate the differential count of white blood cells by placing a blood smear on a glass slide, then pigmentation it with WrigGiemsa after fixation (Shane and Patterson, 1983). The slides were examined under a microscope (Burton and Guion, 1968).Calculate the number of Heterophlis and Lymphocytes cells and calculate the H / L ratio, calculation of Packed Cell Volume (PCV) values by capillary tubes (Archer, 1965), calculate the concentration of Hb according to the method of Varley et al., (1980).

Blood

Blood samples from the humoral vein were taken from 3 chicks / replicate (9 chicks / treatment)with 3 weeks age in tubes container on anticoagulant K-EDTA to calculate the number of red blood cells (RBC) and white blood cell (WBC) and the size of the Packed Cell Volume (PCV) and the concentration of hemoglobin (Hb) and the proportion of Heterophlis into Lymphocytes cells (H / L ratio).

Blood Serum

Blood samples were taken in the same way above and placed in tubes free of anticoagulation (K-EDTA) to obtain blood serum after coagulation and separated by centrifugation at 3000 rpm and for 15 minutes, the tubes were kept frozen (-18 ° C) until use.

Statistical analysis

The results of the experiment were analyzed according to the statistical program(SPSS) the 14th edition, using the Completely Randomized Design (CRD) of 7x3 x3 and

extraction the least significant difference (LSD)at a significant level (P <0.05).

3. Results

Total weight of chicks (g)

In thetotal weightof the chicks,table 2 & figure 1 shows significant differences between B1MA, B1M and B1A in the total weight of the chicks before slaughtering at the age of 21 days 720.199, 707.514, 639.258 g compared to the treatment of B1 (Aflatoxin B1) 525.939 g, with an increase of 36.9%, 34.5% and 21.5%.These results are consistent with Al-Saidy et al. (2013), who pointing to that Activated Charcoal (A) is added by 5% gled to the reduction of toxic effects of aflatoxin B1 (0.02 micrograms / kg)with an increase rate of 599.84 g / chick and a change of 17.49% compared to control treatment,as well as adding Mycofix® Select 3.0 by 1% g / kgled to a reduction of toxic effect of aflatoxin B1 (0.02 µg / kg)with an increase of 636.63 g / chickwith an increase of 12.43% compared to the comparison treatment, and the addition of Activated Charcoal and Mycofix® Select 3.0 to Aflatoxin B1 (0.02 µg / kg)to increase the weight of 655.23 g / chick withan increase of 9.87% compared to the comparison treatment.B1M treatment showed significant differences in the total body weight of the chicks, this results consist with Biomin® (2000) that Mycofix, is one of the adsorbent that can be added in poultry feed and is claimed to neutralize moderate levels of aflatoxin (up to 2500-3500 ppb) in poultry feed. Mycofixdeactivates aflatoxin with its polar functional group, due to AF fixation to adsorbing components in Mycofix, with stable binding capacity. Adsorption starts in the oral cavity during salivation and continues in stomach and gut.The fixed mycotoxin being unable to enter the blood and subsequently excreted in faeces after 98% adsorption of AF by Mycofix,and withDiaz et al. (2005) that no significant differences in body weight (BW) were observed among the different groups during the first 3 wk of age, although groups 1 and 3 (control and 2.0 kg/t Mycofix) consistently had greater BW than theother 4 groups.

Treatment of B1 (aflatoxin B1) at concentration 0.02 µg/g caused a decrease in the body weight by 39.212% compared to the treatment comparison 0.0, these results are consistent with what Al.Saidy and Samir (2015) thatfeed broiler chickens for 21 days on fodder contaminated with B1 at a concentration of 4.7 µg / g caused a decrease in weight by 66.25% compared to the treatment comparison 0.0,and with the results of Ibrahim et al. (1997); Al- Jubory (2002) who indicated that feeding the chicksOn a feed contaminated with aflatoxin B1 with a concentration of 2.5 µg / kg and for 21 days leads to a decrease in the live body weight.The reason for reducing body weight to the effect of AFA B1 is to reduce the effectiveness of digestive enzymes of protein, lipids and starch which leads to a reduction in the weight of the chicks (Al-Jubory, 2001)or the effect of aflatoxin B1 on dietary conversion efficiency.Adding activated charcoal in the fodder to reduce the effect of the poison T- 2toxin and significantly increases the weight of animals (Al-Hadithi, 2005).

Table 2: Total weight of chicks before slaughter

Treatment			Total Weight before Slaughter (g)
B1(mg/kg)	A (g/100g)	M (g/kg)	
0.0	0.0	0.0	865.210
0.2	0.0	0.0	525.939
0.2	5	0.0	639.258
0.2	0.0	1.0	707.514
0.2	5	1.0	720.199
0.0	5	0.0	748.666
LSD (P <0.05)			37.32

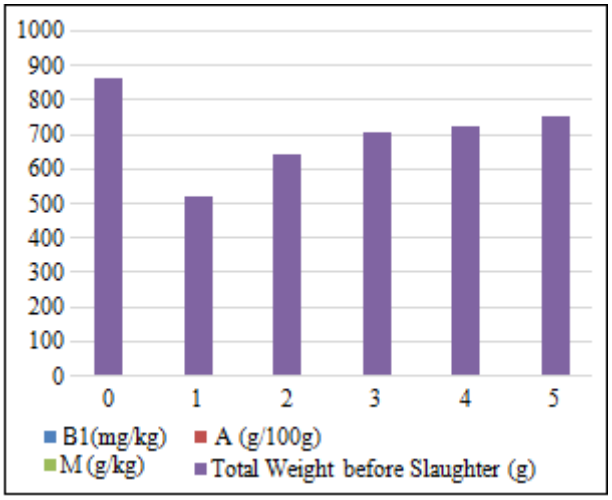

Figure 1: Shows total weight of chicks before slaughter

Weight of the internal organs of the chicks (g / 100 g)

In the **internal organs** weight**,**the results in table 3 showed a significant effect of B1MA, B1M and B1A treatments in the rates of the internal organs weight of the chicks,and in the **Heart** weight 5.140, 4.838, 4.410 g, compared to the treatment of B1 4.147 g with an increase of 23.9%, 16.6% and 6.3%.Then the **Liver** 25.470, 21.941, 20.444 g compared to the B1 treatment 19.066 g with an increase of 33.5%, 15.07% and 7.2%.These results are consistent with Al-Hadithi (2005) that added bentonit and charcoal to the fodder contaminated in T-2 toxinlead to restores liver normal weight with significant differences between treatments compared to the T-2 toxin treatment.Results showed that the addition of 0.25% mycofix to the feeds contaminated with 2.5, 3.5 ppm were responsible for reducing liver residual AFM1 levels.And **Craw** 20.161, 17.633, 17.850 g compared to the B1 treatment 16.118 g with an increase of 25.08%, 9.3% and 11.1%. In the **Bile** weight, the results in Table 3 also showed a significant effect of B1AM and B1A 1.134, 1.703 g in the Bile weight with an increase of 1.3% and 52.4%. The B1M treatment 0.939 g did not show any significant differences in the weight of the Bilecompared to the treatment B1 1.117 g. **Spleen** did not appear any differences of treatment B1 0.621 g, this results consist withDiaz (2005) that the relative weights of liver, spleen, heart, proventriculus, gizzard, and bursa of Fabricius. No significant differences in the relative weight of liver, spleen, heart, proventriculus, or bursa of Fabricius were observed among the 6 experimental groups. However, chickens receiving Mycosorb, MycoAd, and Zeolex had significantly greater relative gizzard weights than the control group and those receiving Mycofix. These results are consistent with **Al-saidy and Samir** (2015) who pointing to the effect of aflatoxin B1 (4.7 µg / g) on the

internal organs of broiler chicks, as it leads Aflatoxin B1 to increase significantlyin the weight of the liver, spleen and gizzard 4.08, 0.19 and 8.96 g / 100 g, respectivelycompared to control treatment of 3.84, 0.08 and 6.53 g / 100 g, respectively.There were no significant differences in heart weight, and with Kubena et al. (a1997) who indicated increased weight of the internal organs of the broiler chickenswhen fed on feed contaminated with Aflatoxin B1 only and with Ibrahim et al. (1997); Al-Jubory (2002) who referred to feeding the chicken broiler for 21 days when fed on feed contaminated with Aflatoxin B1at a concentration of 2.5 and 3.5 µg / kg lead to increased weight of the liver, spleen and gizzardandreduce the weight of the bursaand reduce the rate of body weight in chicks.

Table 3: Effect of Aflatoxin B1 (B1) and Activated Charcoal (A) and Mycofix® Select 3.0 (M) in the rate of weightof internal organs in the broiler chicks

Treatment			Weight Entrails (g/100g of body weight)				
B1 (mg/kg)	A (g/100g)	M (g/kg)	Heart	Liver	Craw	Bile	Spleen
0.0	0.0	0.0	5.306	5.306	22.674	1.510	0.817
0.2	0.0	0.0	4.147	4.147	16.118	1.117	0.621
0.2	5	0.0	4.410	4.410	17.850	1.730	0.831
0.2	0.0	1.0	4.838	4.838	17.633	0.939	0.600
0.2	5	1.0	5.140	5.140	20161	1.134	0.693
0.0	5	0.0	5.157	5.157	20.644	1.550	0.480
0.0	0.0	1.0	5.084	5.084	21.771	1.188	0.759
LSD (P <0.05)			0.722	0.611	1.640	0.285	N.S

mplete Blood Count

In PCV, results in table 4 show that treatment B1 is 29.2it has caused the reduction of PCV by 36.3%, Treatment B1MA significantly exceeded 35.2 on treatment B1 29.2 and an increase of 20.5% in **PCV**, followed by treatment B1M 33.4 which was significantly exceeded with an increased by 14.3% in PCV, M treatment 39.4 did not show any significant differences from the comparison treatment (0.0) 39.8 in PCV. **In Hb**, treatment B1 9.06 reduced Hb by 39.07%. The B1MA treatment was 11.06 significantly exceeded in Hb with an increased by 22.07% followed by treatment B1M 10.4 with an increase by 14.79%and B1A treatment 9.2 did not show any significant differences from treatment B19.06 in Hb. This results agree with Al-Hadithi (2005), which he noted was added by the Physical adsorbentsincluding activated charcoal and bioactive, to a significant increase in the hemoglobin rate compared to the T-2 toxin aloneespecially T2_toxin +2% (Bentonit + charcoal) and (*L.rhamnosus* + T2_toxin)which gave the Hemoglobin rate 8.3.8.4 g / 100 ml, respectively. Mughallis (2004) found that he added 2% activated charcoal for broiler fodder contaminated with AFB1 with a concentration of 400-300 mg / kglead to a significant increase in the level of Hemoglobin and lymphocytes.**In RBC**, treatment B1 9.06 caused the reduction of the RBC number by 13.7%. The B1MA treatment 2.64 was significantly exceeded in RBC and increased by 16.37% compared to treatment B1 2.26, followed by B1M treatment 2.6 and increased by 15.04%, Then B1A treatment is 2.32and increased by 2.65%,The treatment of M 2.64 significantly exceeded on the comparison treatment (0.0) 2.62 in the number of RBC.**in table 5**, in **WBC,** B1 treatment 31.78 caused an increase in WBC number.These results are consistent with **Al-saidy and Samir** (2015) who pointing to the effect of aflatoxin B1 (4.7 µg / g)causing a significant reduction in Hemoglobin and the total number of red blood cells was 5.29 g / 100 ml and 1.81 × 106 cells / ml³ respectivelycompared to the comparison treatment 8.78 g / 100 ml and 2.39 × 106 cells / ml³,Aflatoxin B1 also significantly increases the number of white blood cells and In the proportion of Heterophyll cells to Lymphocytes 22.96 × 103 cells / ml³و and 0.4722.96 × 103 cells / ml³ و and 0.47 compared to the comparison treatment 19.48 and 0.24, respectively.The reason is due to the effect of aflatoxin B1 on iron absorption in the gut of broiler chickswhich leads to a significant reduction in Hemoglobin and the number of red blood cells, or to the effect of aflatoxin B1 in the bone marrow that affects most of these standard (Lanza, 1979),or to the effect of aflatoxin B1 on the iron-transmitting protein and on the ability of iron to correlation,or to Hemolytic anemia caused by the consumption of chicks for aflatoxin B1which leads to reduced hemoglobin and the number of red blood cells (Ibrahim et al., 1997; Ibrahim et al., 1998b),or to the effect of aflatoxin B1, which leads to the induction of increased white blood cells in the broiler chicks (Shareef et al., 1998).The B1MA treatment 29.72 significantly exceeded in WBC reductionby 6.48% compared to treatment B1 24.94, followed by treatment B1A 24.86 by 21.77% reduction, then B1M treatment 24.33 with the highest reduction rate of 23.44% in WBC number,and the treatment A 29.55, treatment M 30.71did not appear any significant difference in reducing the number of WBC. Al-Hadithi (2005), pointed out that the addition of physical adsorbentsincluding ActivatedCharcoal and Bioactive, to contaminated fodder with T-2 toxinto a significant increase in the number of red blood cells in the blood,their number was 2.7×10^{12} and 3×10^{12} / liter for treatmentsrespectively,and no significantly from comparison treatment.while addition of physical adsorbents including activated charcoalalone and bioactivealoneto asignificant increase in the number of white blood cellsTo 24.1×10^{9} cell / g(0.05) compared with T-2 toxin(Ziprin,1990). Mughallis (2004) found that he added 2% activated charcoal for broiler fodder contaminated with Fumonisin B1 with a concentration of 400-300 µg / kg lead to a significant increase in the number of red blood compared with Fumonisin B1 toxin alone. In **Monocyte**, all treatment showed significant differences compared to B1, where treatment B1 3.3 leads to reduce the Monocyte count by 46.34%, and the treatment B1A 6.7 gave the highest increase in Monocyte 103.03%, followed by B1M 5.3 treatment, with an increase of 60.60%.B1MA 4.5 showed the lowest 36.36% increase Monocyte compared with B1.Treatment A 7.2 significantly exceeded on the comparison treatment (0.0) 6.15 Monocyte. In **lymphocytes**, Treatment B1 47.5 reduced Lymphocyte by 27.48% compared to the comparison treatment 65.5%,this is consistent with what Li et al.,(2000) foundthat feeding the broiler chicks on a moniliformin-contaminated feed with a concentration of 100 µg / kg, leading to a significant decrease in Lymphocytes of blood plasma.Treatment B1M 52.7 showed a significant increase in Lymphocyte on treatment B1 with an increase of 10.945, followed by treatment B1A 50.3 and an increase of 5.59%. Treatment B1MA 48.10 showed no significant differences in Lymphocytes from treatment B1 47.5.A and M treatment 67.3 and 68.2 were significantly exceeded in Lymphocytes compared tothe comparison treatment 65.5.T-2 toxin caused

a significant increase in the proportion of Heterophilic cells to Lymphocytesin comparison with comparison treatmentreaching 0.29 and 0.20 respectively (Al-Hadithi, 2005). In **Neutrophils**, Treatment B1 40.6 caused increased the number of neutrophils, with an increase of 83.7% compared to the comparison treatment (0.0) 22.10. These results differ from the results obtained by Mughallis (2004)who pointed out that the broiler chicks fed with Fumonisin B1at concentration 300-400 µg / kg leads to a decrease in the rate of Lymphocytes to the Neutrophils.The treatment B1M 30.50 showed the best reduction rate in Neutrophil 24.87%, followed by treatment B1A 33.1 with a reduction rate of 18.47%.Treatment B1MA 40.30 did not show any significant differences in Neutrophil from treatment B1 40.60. Treatment A 23.10 gave the highest reduction rate of 43.10%in Neutrophil, followed by treatment M 24.10 with a reduction of 40.64%. In the **Basophils**, there were no significant differences in the number of Basophilsbetween the treatment B1 6.2 and comparison treatment 6.3. Treatment B1M 8.9 gave the highest increase rate 41.16% in the Basophils, followed by treatment B1A 8.70 increased by 38.09%, then B1MA treatment 8.50 with an increase of 34.92% compared with B1 treatment 6.20. The M treatment 5.30 decreased significantlycompared to the comparison treatment (0.0) 6.30 for the Basophils. In the **Eosinophils**, there were no significant differences between treatment B1 0.50 and the comparison treatment of 0.50 in the number of Eosinophils, while treatment B1A 0.90 gave the highest increase rate 80.0% in the number of Eosinophils, followed by treatment B1MA 0.70, an increase of 40.0%, then the B1M 0.60 treatment increased by 20.0%.Treatment M 0.50 did not show a significant difference compared to the comparison treatment 0.50 in the Eosinophils.

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