Effect of a Specific Combination of Mannan-Oligosaccharides and β-Glucans Extracted from Yeast Cell Wall on the Health Status and Growth Performance of Ochratoxicated Broiler Chickens

M. H. H. Awaad[1], A. M. Atta[2], Wafaa A. Abd El-Ghany[1], M. Elmenawey[2], K. Ahmed[3]; A. A. Hassan[4], A. A. Nada[4] and G. A. Abdelaleem[1]

[1]Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, Egypt.
[2]Animal Production Department, Faculty of Agriculture, Cairo University, Egypt.
[3]Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt. [4]Animal Health Research Institute, Cairo, Egypt.
wafaa.ghany@yahoo.com

Abstract: The effect of a specific combination of Mannan-oligosaccharides (MOS) and β-glucans extracted form the cell wall of a specific strain of *Saccharomyces cerevisiae* (AGRIMOS®) was investigated on zootechnical performance, ochratoxicosis and immune dysfunction caused by ochratoxin in broiler chickens. Three hundred and sixty, one day-old chickens were randomly allocated in a 2x2 factorial design for 5 weeks: supplementation of 2kg/ton of MOS (presence or absence) and feed contamination (presence or absence) with 50 µg/kg of ochratoxin A (OTA) for the first 3 weeks of life was done. Obtained results revealed that OTA did affect bird's growth one week after the contamination, although the final weight gain after 5 weeks was not different from the control. The use of AGRIMOS® stimulated the overall daily gain compared to the OTA group. Feed intake and feed conversion were not affected by the dietary treatments. Cumulative mortality was similar between treatments and performance indexes significantly improved with AGRIMOS® for the OTA challenged regimes. AGRIMOS® supplementation reduced macroscopic and microscopic lesion scores associated with ochratoxicosis. Also, it corrected the depression in phagocytosis induced by ochratoxin intoxication and it had strong immunomodulation as it stimulated the immune response to vaccination. It could be concluded that administration of a specific combination of Mannan-oligosaccharides and β-glucans extracted form yeast cell wall (AGRIMOS®) to chickens improved zootechnical parameters had a potent immunomodulatory effect, evoked immune response and enhanced vaccination effectiveness. It helps not only in controlling chicken ochratoxicosis but also can play a positive role in treating chicken immune dysfunction.
[M. H. H. Awaad, A. M. Atta, Wafaa A. Abd El-Ghany, M. Elmenawey, K. Ahmed; A. A. Hassan, A. A. Nada and G. A. Abdelaleem. **Effect of a Specific Combination of Mannan-Oligosaccharides and β-Glucans Extracted from Yeast Cell Wall on the Health Status and Growth Performance of Ochratoxicated Broiler Chickens.** Journal of American Science 2011;7(3):82-96]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Mannan-oligosaccharides**,** AGRIMOS®**,** β-glucan**,** Ochratoxins, Chickens.

1. Introduction

All the antibiotic growth promoters have been banned in the European Union (EU) since the year (2006). Eventually, many other countries will have to follow these measures to export to the EU. The challenge will be to fight microbial imbalances in young animals. Feed formulators need efficient alternatives to use as commodities. Gastrointestinal tract (GIT) of poultry harbors microflora, which is formed immediately after the bird is hatched and is an important barrier against colonization of potentially pathogenic microorganisms. The bird's microflora is potentially depleted for a period of time at hatching and following any medication with an anti-microbial product. Consequently; natural alternatives concepts based on natural ingredients for non-antibiotic growth promotion and antibacterial action are highly commendable.

Mycotoxins are often found as natural contaminants in grains (Walker 2002). Ochratoxin A (OTA) is the major component of a group of secondary metabolites produced by several fungi such as *Aspergillus ochraceus* or *Penicillium verrucosum*. There have been several investigations on the mode of action of this toxin, suggesting inhibition of protein synthesis, formation of DNA adducts, and provocation of DNA single-strand breaks as a result of oxidative stress (Anke *et al*. 2003). Some of the major deleterious effects of ochratoxin in poultry include depressed growth rate, nephrotoxicity, reproductive failure and immunosuppression (Marquardt and Frohlish, 1992). The immunosuppressive effect of ochratoxins in chicken immune system will lead to immune dysfunction that can lead to exacerbation of diseases (Gill and Cross, 2002 and Wang *et al*. 2009).

The term immunosuppression is defined as a state of temporary or permanent dysfunction of the immune response, which insult to the immune system leading to increased susceptibility to diseases

(Domhs and Saif 1983). It is undoubtedly true that factors contributing to immune-suppression will lead to immunodeficiency. The latter is a hazard-anticipating causative agent of serious economic impacts in poultry industry allover the world. Recognition and scientific identification of factors encountered in immune deficiency have lead to increased perusal investigation in the counterattacking modulators to accomplish immune-stimulation. Eventually, it has already known that many diseases/disorders, that have immunomodulated components, can be modified by administration of biological compounds that activate key pathways in the immune system (Podleski 1988 and Xue *et al*. 2010). They strengthen the defense and immune mechanisms of the body and currently usable for stimulating the non-specific immune responsiveness in both the human and veterinary medical practice (Emmerich *et al*. 1988, and Awaad *et al*. 1999, 2000 a, b).

This study was conducted in an attempt to investigate the possible effect of a specific combination of Mannan-oligosaccharides (MOS) and β-glucans (AGRIMOS®) extracted from the yeast cell wall of *Saccharomyces cerevisiae* on productive performance, ochratoxicosis and immune dysfunction caused by ochratoxin in broiler chickens.

2. Material and Methods
Experimental design:

Three hundred and sixty, one day-old Arbor Acres plus broiler chickens were used in this study. The birds were allotted into 4 equal groups (I-IV) of 90 birds assigned to 3 replicates of 30 each. Those of groups I and III were fed on ration containing ochratoxin for the first 3 weeks of age (OTA and OTA+AGRIMOS® groups, respectively), while those of groups II and IV were fed on plain ration *ad libitum* (control and AGRIMOS® treated groups, respectively). Chickens of groups III and IV were fed on a standard feed containing a specific combination of Mannan-oligosaccharides and β-glucans extracted form the cell wall of a specific strain of *Saccharomyces cerevisiae* (AGRIMOS®, Lallemand SAS) supplemented at 2 kg/ ton of feed. AGRIMOS® is obtained by a specific production process consisting in the extraction of the cell wall from a unique primary grown yeast *Saccharomyces cerevisiae* (process guaranteeing consistent purity and high level of Mannans and ß-glucans). Birds of group II were kept as blank control. Chickens of all groups were vaccinated via intra-ocular route and subcutaneous route, respectively with Hitchner B1+H120 vaccine and avian influenza vaccine (Inactivated H5N2 vaccine from Intervet International, B.V. BOXMEER-Holland) at 7th and

10th day of age, respectively. La Sota vaccine (Pfizer) and 228E IBDV vaccine (freeze-dried live vaccine Noblis Gumboro 228E strain from Intervet International, B.V. BOXMEER-Holland) were given at 14 days of age via intra-ocular route.

At 35 days of age, 10 chickens from each group were challenged with velogenic viscerotropic Newcastle disease virus (VVNDv) at a dose of $10^{6.8}$ EID_{50} / ml / bird by intramuscular injection and kept under close observation for clinical signs and mortality for 2 weeks. At the end of the observation period, dead as well as sacrificed birds (at 49 days) were subjected to post-mortem examination for lesion scoring of Newcastle disease virus (NDv) (Amer 1980).

Diets:

Chicks were fed *ad libitum* commercial starter diet (23% CP and 3000 k cal ME/kg diet) during the first two weeks of age, commercial grower diet (22% CP and 3150 kcal ME/kg diet) from 2 to 4 weeks of age, and then commercial finisher diet (19% CP and 3200 kcal ME/kg diet). The diets compositions are indicated in table (1). Birds had free access to water. Semduramicin was added at a concentration of 25 p.p.m as a coccidiostat.

Ochratoxicated feed preparation:

Ochratoxin-A (OTA) contaminated raw materials were prepared according to Sansing *et al*. (1973) by artificial contamination of sterile yellow corn with *Aspergillus ochraceus*. Artificially OTA-contaminated yellow corn was then thoroughly mixed with broiler ration to provide a final concentration of 50 µg/kg of feed. The mycotoxin concentration was determined by immune affinity chromatography according to Hansen (1990) and Truckess *et al*. (1991).

Measured parameters:

Chicken zootechnical performances were determined according to North and bell (1990) for Body Weight (g), Body Weight Gain (g), Feed Consumption (g/day/bird), Feed Conversion (g feed/g live body weight), and Performances Indexes defined as follows:
- Point Spread= (Live body weight in pounds) - (Feed conversion) X 100.
- Performance Index = Live body weight (Kg) / Feed conversion X 100.
- European Performance Efficiency Factor (EPEP): A / B x 10000
Where:
A= Average live weight (kg) X Livability.
B= Marketing age (days) X Feed conversion.
Birds were individually weighed.

Immunoassays:

The possible effect of AGRIMOS® on the cell mediated immunity was investigated using phagocytic activity of macrophages, lysozyme and Nitric oxide activities (Muller *et al*. 1995) on blood samples taken at 3 and 5 weeks of age on 5 birds randomly chosen per group. The possible effect of AGRIMOS® on the humoral immunity was assessed through haemaglutination inhibition (HI) test for determining antibody titers against ND (Amer 1980). Blood samples were collected from 10 randomly chosen birds at weekly intervals (0-7 weeks of age) from all groups, centrifuged for 10 minutes at 1,000 x g and serum samples were subjected to HI test.

As the primary lymphoid organ, Bursa of Fabricius is of particular interest to look at for cell mediated immune parameters, this organ was withdrawn from 5 birds / replicate at weeks 3 and 5 of age, sacrificed and the bursa / body weight ratio was calculated according to Sharma *et al*. (1989) as follows: Organ weight (g) x 1000 / Total body weight (g), expressed as the arithmetic mean for each group of birds.

Histopathology assay:

Liver, kidneys, Bursa of Fabricius, spleen and thymus glands were collected from the sacrificed 5 chickens per replicate at 3 and 5 weeks of age and fixed in 10% buffered formalin. Paraffin-embedded sections were routinely prepared and stained with Hematoxylin and Eosin (Bancroft *et al*. 1996), and scored for histopathological lesions according to the method described by Rosales *et al*. (1989).

Statistical analyses:

Two-way analysis of variance was used using SAS software general liner models procedure (SAS Institute 1999). The main factors were ochratoxin contamination and yeast cell wall supplementation. Mean values were assessed for significance using Duncan's multiple range test with significance set at $P<0.05$.

3. Results
Zootechnical performance:

As shown in table (2), no significant ($P<0.05$) difference was recorded between the control and the AGRIMOS® treated group at any time for all the zootechnical parameters concerned. However OTA contamination had a significant ($P<0.05$) deleterious effect on growth from week 2 and the body gain slowed down until the end of the OTA challenge. At the end of the experiment; control and OTA compromised chickens had not statistically different body weights even if the OTA group exhibits the lowest final body weight. Finally, no significant ($P<0.05$) difference was detected between the control and the OTA group for any of the other zootechnical parameters. Interestingly, OTA+ AGRIMOS® treated chicken did not exhibit the same trend compared to control than the OTA group according to body weight and body weight gain. Indeed AGRIMOS® supplementation compensated the growth delay due to OTA contamination. Chickens from the OTA+ AGRIMOS® group maintained similar body weight and body weight gain than the control and the AGRIMOS® groups. Furthermore, body weight was found to be significantly ($P<0.05$) higher in the OTA+ AGRIMOS® group compared to the OTA group two weeks after the first feeding to the end of the trial, while body weight gain was also higher but only during the second and the third week of the trial.
Point Spread and Performance Index were also significantly ($P<0.05$) higher in the OTA+ AGRIMOS® group versus OTA.

Finally, the cumulative mortality rate of AGRIMOS® group recorded the lowest value, but without significant ($P<0.05$) differences as compared to other groups. Only Control group recorded the highest mortality at week four of the study.

Immune status assessment:

Cell mediated immunity parameters are reported in table (3). No significant ($P<0.05$) difference was measured between the two examined dates (3rd and 5th weeks of age) for phagocytic percentage and phagocytic index in group II (control), whereas lyzozyme and Nitric oxide increased significantly ($P<0.05$) from the third to the fifth week of rearing. However this phenomenon was not observed in group IV (AGRIMOS®) as cell mediated immune parameters were already high after 3 weeks of rearing. Indeed, Nitric oxide, as well as phagocytic indexes were found significantly ($P<0.05$) higher in group IV (AGRIMOS®) compared to group II (control) at 3 weeks of age. These differences remained significant ($P<0.05$) at 5 weeks of age for the macrophage activity indexes. No effect of OTA contamination has been reported in this study on cell mediated immune parameters when compared to control (group II), even if phagoctytic index and phagocytic % tended to be systematically affected by ochratoxicosis at both sampling times. Indeed, lower values for these parameters were systematically recorded for group I (OTA); while almost no difference between group III (OTA+ AGRIMOS®) and group II (control) was detected whatever the parameter concerned even if a higher lyzozyme activity was measured in group III after 3 weeks of rearing. Concerning Nitric oxide activity, at 3 weeks

of age, group I (OTA) produced the lowest figure with significant (P<0.05) differences compared to groups III (OTA+ AGRIMOS®) and IV (AGRIMOS®). On the other hand, the AGRIMOS® group produced the highest amount of Nitric oxide at week three. At five weeks of age, no difference was recorded between groups.

As regards humoral immunity, the haemaglutination inhibition (HI) test (Figure 1) indicated that the AGRIMOS® treatment in group III corrected the depression in humoral immunity induced by OTA. As a matter of fact, after one week of feeding the OTA contaminated diet, the chicken that did not receive AGRIMOS® showed a significant (P<0.05) decrease in HI, while this drop was attenuated in the group III (OTA+ AGRIMOS®). The same phenomenon was recorded during week 4, and lasted over the fifth week of rearing.

Bursa of Fabricus live weight and Bursa / Body weight index were not affected by the treatments after 3 weeks of rearing. However, at 5 weeks of age, chickens from group I (OTA) showed significant (P<0.05) lower Bursa weight and Ratio index compared to groups II (control) and IV (AGRIMOS®), while group III (OTA+ AGRIMOS®) showed intermediate results (Figure 2).

Histopathological changes:

In ochratoxicated (immunocompromised) chickens, the main organs such as liver and kidneys were differently affected according to the AGRIMOS® supplementation or not.

Concerning liver; OTA group, showed vacuolar degeneration of hepatocytes, hyperplasia of epithelial lining bile duct associated with appearance of newly formed bile ductuoles and fibroplasia in the portal triad. Some examined sections revealed chronic cholangitis manifested by fibrous connective tissue proliferation and massive inflammatory cells infiltration (mainly heterophils, macrophages and lymphocytes) in the wall of bile duct (Photo 1). Focal area of hepatic necrosis replaced by mononuclear leucocytes (Photo 2) was observed in all examined sections. Regarding liver of chickens of AGRIMOS® treated group (IV) showed no histopathological changes except vacuolar degeneration of centrolobular hepatocytes (Photo 3). Chickens of group III (OTA+AGRIMOS®) showed improvement in the histopathological picture as the examined sections revealed few vacuolar degeneration of hepatocytes, slight thickening in the wall of bile duct associated with leucocytic cells infiltration mainly heterophils (Photo 4) together with small focal area of hepatic necrosis replaced by leucocytic cells.

At the kidneys level, chickens of group I (OTA) revealed vacuolar degeneration of epithelial lining of the renal tubules, congestion of intertubular blood vessels and capillaries, subcapsular hemorrhage and interstitial hemorrhage (Photo 5) accompanied with multiple focal areas of tubular necrosis which completely replaced by massive leucocytes (Photo 6). Those of group IV (AGRIMOS®) revealed no histopathological changes. Chickens of group III (OTA+AGRIMOS®) showed congestion of intertubular blood vessels and capillaries, peritubular leucocytic cells infiltration (Photo 7) together with focal area of tubular necrosis replaced by leucocytic inflammatory cells infiltration. As regards the major immune organs, Bursa of Fabricus of chickens of group I (OTA) showed vacuolation of lymphoid follicles (Photo 8), lymphocytic necrosis and depletion especially in the medulla of lymphoid follicles as well as hyperplasia and vacuolation of follicle associated epithelium. While in groups III and IV examined sections revealed no histopathological alterations (Photo 9). Concerning spleen; OTA group revealed focal splenic hemorrhage, lymphocytic necrosis and depletion, hyperplasia of reticular cells associated with atrophy of the lymphoid follicles (Photo 10). While in groups III and IV examined sections revealed no histopathological alterations (Photo 11). Thymus glands of group I (OTA) showed focal thymic hemorrhage (Photo 12) associated with lymphocytic depletion in the medulla. While in groups III and IV examined sections revealed no histopathological alterations (Photo 13).

All examined sections from group II (blank control) revealed no histopathological changes.

Concerning the macroscopic lesion scoring after the challenge with velogenic viscerotropic Newcastle disease virus (VVNDv) conducted at day 35 on ten birds per treatment, the results indicated a significant (P<0.05) higher lesion score in the OTA group (group I) (2.6) while no significant (P<0.05) difference was observed among the three other groups (0.9, 0.7 and 0.8; for groups II, III and IV, respectively) (Figure 3).

4. Discussion

Our results indicate that OTA contamination has a negative effect on growth parameters of broiler chicken. Interestingly we showed that a contamination of the feed with OTA at 50 µg / kg induced a negative effect that can be detected after two weeks of feeding. The reduction in growth parameters due to OTA in the present study is consistent with the previous reports of Raju and Deveyowda (2000), Kumar et al. (2003), Verma et al. (2004) and Elaroussi et al. (2006). Huff et al. (1974) reported that OTA impaired protein metabolism system. Hsieh (1987) showed that OTA

inhibits the activity of phenylalanine t-RNA synthetase, an enzyme involved in the initial step of protein synthesis. Marquardt and Frohlich (1992) explained that OTA affects DNA, RNA and protein synthesis as well as carbohydrate metabolism. Elaroussi *et al*. (2006) showed that the reduction in growth parameters, feed conversion and feed consumption of OTA treated broiler chickens resulted from the decrease in serum T4 and increase in serum T3 hormones concentration. They also found that body weight and T4 concentrations in the control group were significantly higher than in the OTA-treated group. Wentworth and Ringer (1986) reported that T3 and T4 had a significant effect on growth of broiler chickens. However, it is important to emphasize that our results indicated that at the level of contamination used in the present study, the induced delay in growth can be catch up one week after feeding back an uncontaminated diet. It can be then concluded that the contamination level applied in this study was possibly too low to induce important and irreversible damages on chicken performances.

Interestingly, the AGRIMOS® supplementation had compensated the growth delay induced by OTA at week two and three, and resulted in a higher final body weight compared to the OTA group (I). Previous researches demonstrated that MOS and β-glucans could enhance broiler performance especially under unhygienic conditions (Tzianabos 2000, Huff *et a*l. 2006, Tokic *et al*. 2007 and Yang *et al*. 2007). The enhancement of these agents can be explained in part by the improvement of intestinal function or gut health through the increase of villi height, uniformity and integrity (Huff *et al*. 2006). Surprisingly, at the end of the trial the OTA+AGRIMOS® group (III) showed a significant higher final body weight compared to the other treatments. This result is somewhat difficult to explain at this stage, but it can be assumed that the low contamination level in OTA and the associated effects on immune modulation has been responsible for an increase in the animal health status providing a benefit over the uncontaminated group.

Furthermore, no significant differences were observed throughout the experimental period between groups for both feed consumption and feed conversion. Opposite results were reported by Kubena *et al*. (1989), Raina *et al*. (1991) and Elaroussi *et al*. (2006). They mentioned that OTA contamination reduced feed consumption and deteriorated the feed conversion ratio. Indeed; the difference between the present and previous results might be due to the dose of OTA, then the age of the birds and the period of administration used in our study. Finally, the cumulative mortality rate of the AGRIMOS® group (IV) recorded the lowest value, but without significant differences as compared to other groups. During the experimental weeks, no significant differences were recorded between groups, except after four week of age when, control group recorded the highest mortality rate.

The OTA group had the worst economic parameters: both Point spread and Performance index of OTA group (I) were significantly lower than OTA+AGRIMOS® group (III), while control (II) and AGRIMOS® group (IV) had almost similar value without any significant differences. However, no significant differences were found between groups in European performance index. It appears from these experiments that the use of AGRIMOS® in the feed of broiler chickens can significantly improve chicken zootechnical performance.

Boorman *et al*. (1984) already reported the immunotoxic effects induced by ochratoxin on cell mediated immunity with respect to both structural and functional alterations: decreased splenic cell count, reduce thymus size and increase bone marrow depression. Ochratoxin had also been shown to interfere with natural killer cell activity (Luster *et al*. 1987). Our results confirmed the immunotoxic effect of OTA on macrophage activity. This result agrees with Muller *et al*. (1995) who stated that ochratoxin has a non selective suppressive effect on various immune and defense reaction. Dhuley (1997) recorded significant decrease of chemotactic activity of murine macrophages, marked reduction in production of interleukin-1 (IL1) and tumor necrosis factor (TNF) alpha in mice intoxicated with ochratoxin. Singh *et al*. (1990) recorded significant reduction in cell mediated immune response as well as depression in phagocytic activity of splenic macrophages in broiler chickens fed with diet intoxicated with OTA.

Our results also indicated that group IV (AGRIMOS®) birds revealed significantly higher phagocyte percentage and phagocytic index over all the other groups at both 3 and 5 weeks of age; while OTA group (I) had the inferior values but without significant differences than OTA+AGRIMOS® (III) and control (II) groups. Additionally, group III chickens did not show any significant change as compared with control group (II). This may traduce that AGRIMOS® corrected the depression in phagocytosis induced by ochratoxin. Williams *et al*. (1999) suggested that the protective effect of β-glucans might be due to the antioxidant capacity as well as the inhibition of early activation of tissue muscular nuclear factor-KB (NF.KB) and NF-IL6. Mowat (1987) and Stokes *et al*. (1987) reported that β-glucans are known to possess antitumor, antioxidant, and antimicrobial activities by enhancing

the host immune functions. Chae *et al*. (2006) stated that β-glucans are beneficial for growth performance in broilers and increased CD8 cell. Vetvicka *et al*. (2007) recorded significant increase in phagocytosis of peripheral blood cells and antibody production induced by oral administration of β-glucans in balb/C mice. Macrophages are known to play an important role in resistance to infection. They are part of the non-specific first line of defense because of their ability to engulf and degrade invading microorganisms (Sharma and Tizard, 1984). Enhancement of phagocytic function is even expected to be applicable for therapy of microbial infection and cancer (Popov *et al*. 1999). Tizard (1996) and Stafford *et al*. (2002) reported that macrophages perform a variety of functions other than phagocytosis; they act as secretor cells, produce Nitric oxide that kill intracellular microorganisms, secrete many different proteins such as lysosomal enzymes and cytokines that play a key role in regulating immunity. With respect to lyzozyme activity, control group (II) at 3 weeks of age had the lowest values when compared to OTA+AGRIMOS[®] group (III). At 5 weeks of age, no differences were found between all groups. Lysozymes are proteins of low molecular weight found in polymorphonuclear cells and synthesized also in mononuclear cells. They are present in most tissue fluid except cerebrospinal fluid, sweet and urine. Lysozymes are considered as a number of innate humoral factors that elaborated from the body and showed domestic increase in their concentration (Weir 1983). Similar results were obtained by Karkowski *et al*. (1999) who recorded non significant differences in serum lysozme activity of pregnant mares injected with β-glucans.

It has been shown that ochratoxin exposure affects humoral immunity by reducing antibody production which results in significant decrease in haemagglutination inhibition (HI) antibody titers (Creppy *et al*. 1983, Stoev *et al*. 2000, Verma *et al*. 2004, Elaroussi *et al*. 2006 and Xue *et al*. 2010). In the present investigation, AGRIMOS[®] enhanced the humoral immunity in both immunocompromized and non-immunocompromized chickens. Karakowski *et al*. (1999) recorded significant increase in gamma globulin fraction, level of IgG in pregnant mares injected with β-glucans. Shashidhara and Devegowda (2003) in studying the effect of dietary MOS on broiler breeder production traits and immunity reported that the antibody responses against infectious bursal disease virus (IBDV) were significantly higher in MOS treated group and that the maternal antibody titers in progeny were also influenced by MOS supplementation.

The influence of OTA on relative Bursa weight was observed only at the fifth week of age. At this age OTA group had significantly lower relative Bursa weight than control. This result is concordant with previous studies looking at this indicator (Campbell *et al*. 1983, Chang *et al*. 1981, Huff and Doery, 1981 and Wang *et al*. 2009). For instance, Hatab (2003) reported that OTA contamination resulted in a significant reduction in relative Bursal weight. However, in this case, the reduction was observed as early as one week of age.

We also showed that AGRIMOS[®] lowered the macroscopic lesion score after VVNDv challenge and limited the histopathological lesions attributed to ochratoxicosis. Hanif *et al*. (2008) fed OTA and yeast (*Trichosporon mycotoxinivorans)* based toxin deactivator for broilers and found lowered lesion scores by 4. These results strengthen that AGRIMOS[®] is able to implement the innate-cell mediated immunity as well as the humoral immune response in both OTA intoxicated and non intoxicated chickens. Zou *et al*. (2006) studied the effect of MOS on growth performance and immunity of broilers and reported that Mannan oligosaccharides are natural oligosaccharides, composed of various mannose chains that block pathogens colonization within the digestive tract of the animal, while β-glucans are essential components of yeast cell walls that are particularly important to support the non-specific immune system of the animal. They concluded that MOS limits pathogenic bacterial development within the gastro intestinal tract, favors beneficial intestinal flora without being used as a substrate for pathogens, and stimulates the immune response. MOS and β-glucans have been shown to improve immune response and to block bacterial adhesion (especially enteric pathogens) to gut lining (Volman *et al*. 2008).

In conclusion; administration of a specific combination of Mannan-oligosaccharides and β-glucans extracted form yeast cell wall (AGRIMOS[®]) to chickens improved zootechnical parameters and had a potent immunomodulatory effect in the form of evoking immune response and enhancing vaccination effectiveness. Also, it helps not only in controlling chicken ochratoxicosis but also can play a positive role in treating chicken immune dysfunction.

Corresponding Author:
Wafaa A. Abd El-Ghany.
Poultry Disease Department, Faculty of Veterinary Medicine, Cairo University, 12613 Giza, Egypt.
E-mail: wafaa.ghany@yahoo.com

Table 1. Composition of the broilers 3-phase diets (g/kg as fed) and their calculated chemical composition (on as fed basis)

Ingredients	Starter	Grower	Finisher
Yellow corn	524.5	544.2	628.5
Soybean meal 44%	332.4	299.1	221.1
Corn gluten meal 60%	70	70	66.5
Oil	30	43.8	40
Di-calcium phosphate	18	18	18
Lime stone	13	13	13
D.L. Methionine	2.2	2.1	2.3
Lysine hydrochloride	2.9	2.8	3.6
Sodium chloride	4	4	4
Premix[1]	3	3	3
Calculated analysis			
Crude protein %	230	220	190
Metabolizable energy (kcal/kg)	3000	3150	3200

[1]Each gram of mineral mixture contained: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopheryl acetate), 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron (FeSO4 H_2O), 40 mg; copper (CuSO$_4$ 5H_2O), 7 mg; iodine [Ca(IO$_3$)$_2$], 0.7 mg; selenium (Na$_2$SeO$_3$), 0.3 mg.

Table 2. Average body weight, body weight gain, feed intake, feed conversion, mortality and performance indexes (two-way ANOVA)

	I OTA	II Control	III OTA+AGRIMO	IV AGRIMOS	SEM	P value
Body weight (g)						
on d 1	40.4	39.8	40.7	39.6		
wk 1	149.7[b]	153.2[a]	152.1[a]	156.9[a]		
wk 2	360.0[b]	404.1[a]	405.4[a]	414.7[a]		
wk 3	746.2[b]	813.0[a]	838.4[a]	823.6[a]		
wk 4	1454.2[b]	1507.3[ab]	1542.3[a]	1549.4[a]		
wk 5	2022.7[c]	2061.2[bc]	2157.2[a]	2120.4[ab]		
Body gain (g)						
wk 0-1	109.3	113.2	111.4	117.3		
wk 1-2	210.7b	250.9[a]	253.4a	258.3a		
wk 2-3	381.0[b]	408.9[ab]	427.1[a]	409.3[ab]		
wk 3-4	691.9	660.8	688.5	710.8		
wk 4-5	578.4	554.0	586.2	571.0		
wk 1-5	1982.3[c]	2021.5[bc]	2116.9[a]	2080.7[ab]		
Daily feed intake (g/head)						
d 1-35	157.1	145.5	148.3	157.1		
FCR						
d 1-35	1.577	1.477	1.480	1.537		
Mortality (%)						
wk 1	0.0	0.0	3.03	0.0		
wk 2	0.0	3.03	3.03	0.0		
wk 3	0.0	6.07	0.0	6.07		
wk 4	6.07b	0.0a	0.0a	0.0a		
wk 5	3.03	0.0	0.0	3.03		
wk 1-5	9.1	9.1	6.07	9.1		
Point spread (%)	288.2[b]	306.5[ab]	329.2[a]	314.2[ab]		
Performance Index	283.5[b]	308.3[ab]	322.8[a]	305.0[ab]		
EPEF	317.0	342.9	359.9	351.7		

FCR: Feed conversion rate; EPEF: European Performance Efficiency Factor
Values in the same row with different superscripts a,b,c were significantly (P<0.05) different. Number of samples per group = 90

Table 3. Macrophage activity, serum lysozyme activity and Nitric oxide content at 3 and 5 weeks of age.

	Age	I OTA	II Control	III OTA+AGRIMOS	IV AGRIMOS	SEM	P value
Phagocytic %	3 wk	58.33[b]	61.25[b]	61.00[b]	65.50[a]		
	5wk	59.00[b]	60.50[b]	63.75[b]	71.00[a]		
Phagocytic index	3 wk	0.080[b]	0.123[b]	0.133[b]	0.253[a]		
	5wk	0.100[b]	0.140[b]	0.160[b]	0.258[a]		
Lysozyme (µg/ml)	3 wk	9.85[ab]	2.73[b]	17.00[a]	9.85[ab]		
	5wk	9.85[a]3	6.28[a]	9.85[a]	7.53[a]		
Nitric oxide (µg/ml)	3 wk	10.75[c]	13.25[bc]	17.75[ab]	19.50[a]		
	5wk	17.50[a]	21.25[a]	24.50[a]	17.50[a]		

Values in the same row with different superscripts a,b,c were significantly (P<0.05) different. Number of samples per group = 10.
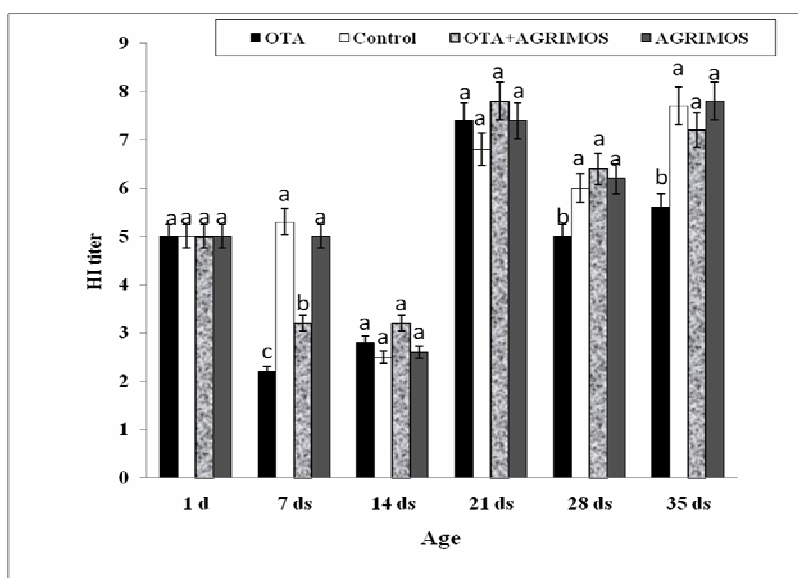
Figure 1. Haemaglutination inhibition (HI) against Newcastle disease virus (NDv) during the first 35 days of chickens' life.
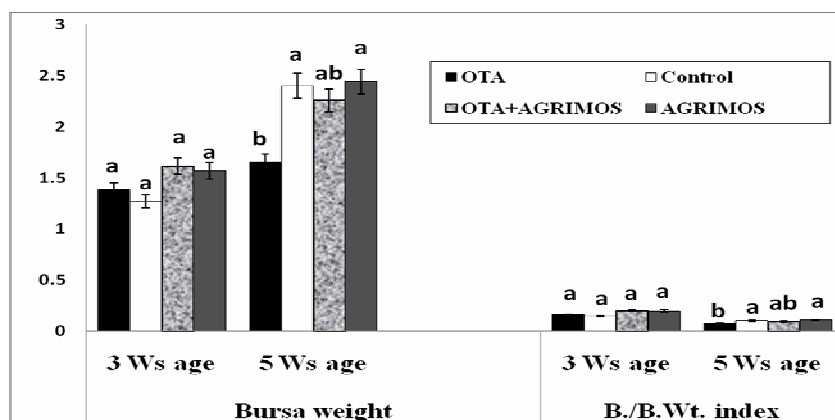
Figure 2. Bursa weight and Bursa/Body weight indexes of ochratoxicated and non-ochratoxicated, AGRIMOS® treated and untreated chickens versus blank control chicken groups.
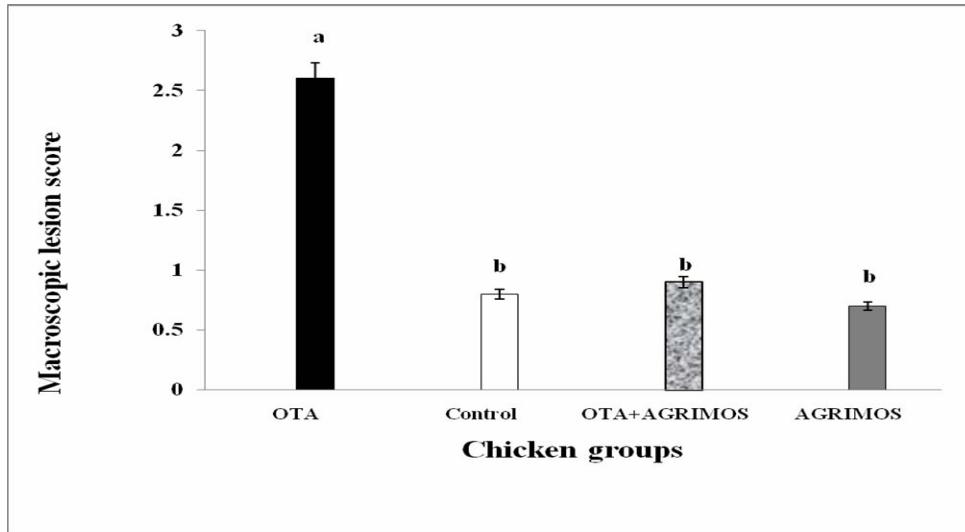
Figure 3. Results of macroscopic lesion scores of velogenic viscerotropic Newcastle disease virus (VVNDv) challange of ochratoxicated and non-ochratoxicated AGRIMOS® treated and untreated chickens versus blank chicken group.
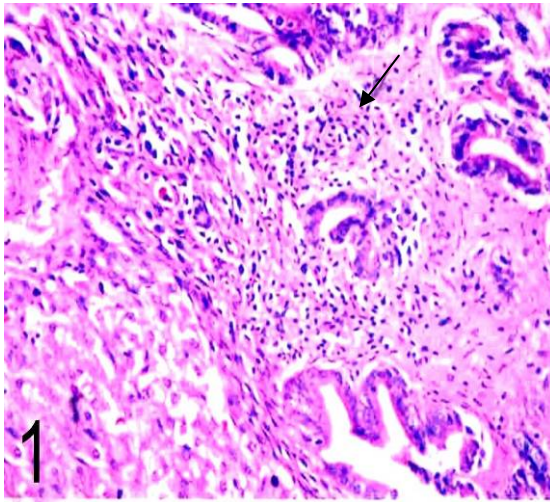
Photo 1: Liver (gr.I) showing chronic cholangitis. Notice the fibrous connective tissue proliferation and massive inflammatory cells infiltration in the wall of bile duct (arrow) (H&E x200)
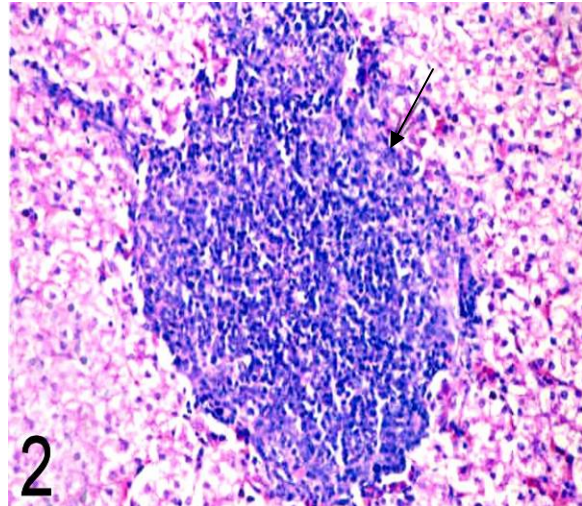
Photo 2: Liver (gr.I) showing focal hepatic necrosis replaced by mononuclear leucocytes (arrow) (H&E x200)
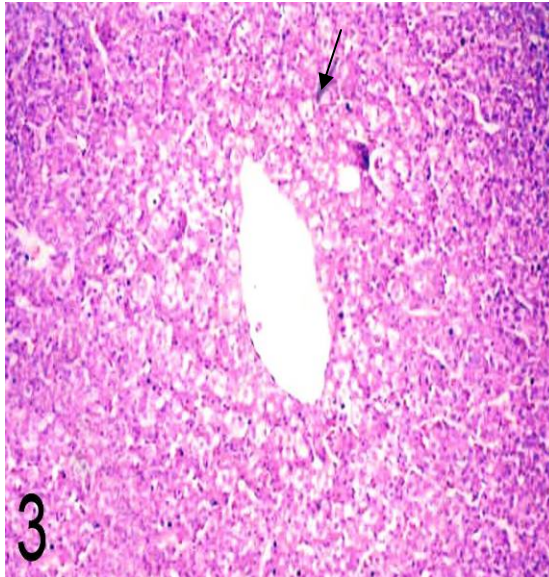
Photo 3: Liver (gr.IV) showing vacular degeneration of centrolobular hepatocytes (arrow) (H&E x200)
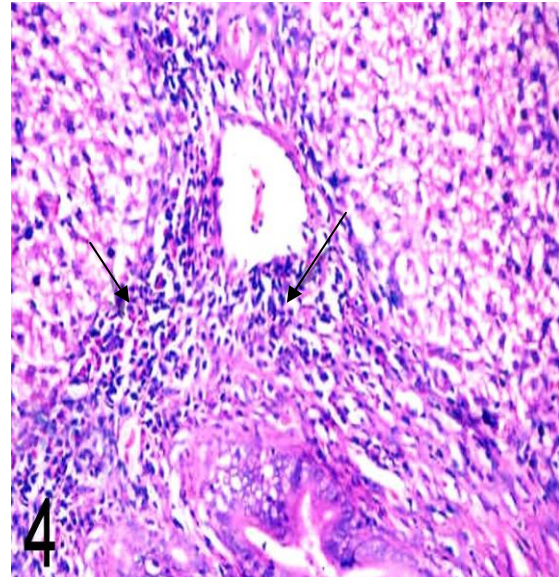
Photo 4: Liver (gr.III) showing vacular degeneration of hepatocytes, slight thickening in the wall of bile ducts associated with leucocytic cells infiltration (arrow) (H&E x200)
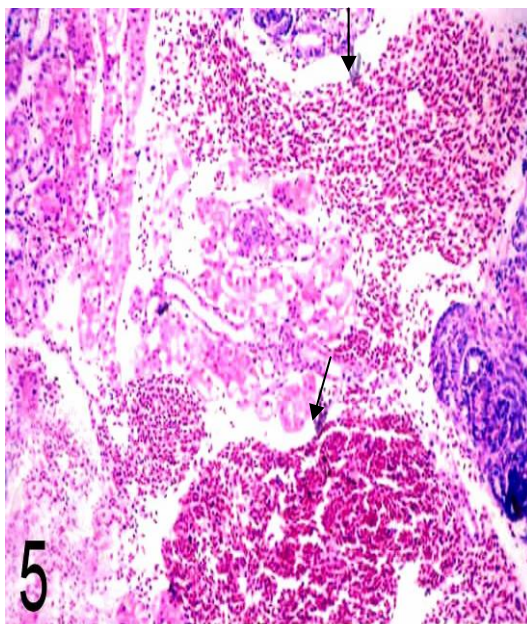
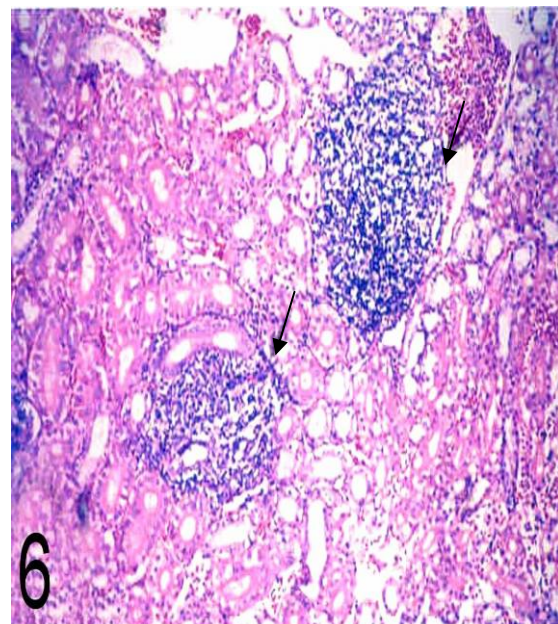**Photo 5: Kidney (gr.I) showing massive interstitial haemorrhage (arrow) (H&E x100)**

Photo 6: Kidney (gr.I) showing multiple focal areas of necrosis completely replaced by massive leucocytes (arrow) (H&E x100)
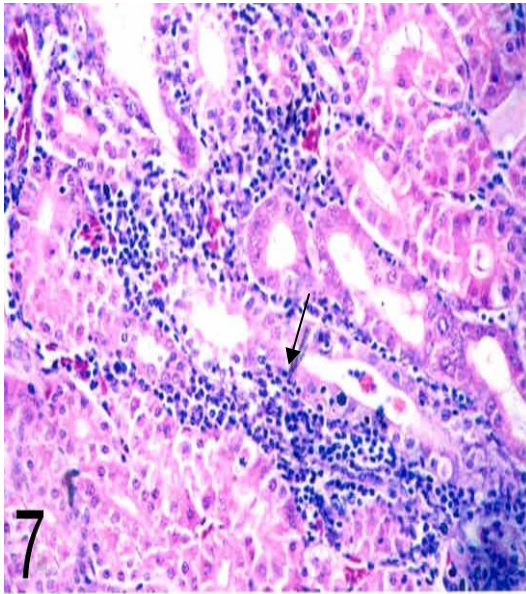
Photo 7: Kidney (gr. III) showing peritubular leucocytic cells infiltration (arrow) (H & E x200)
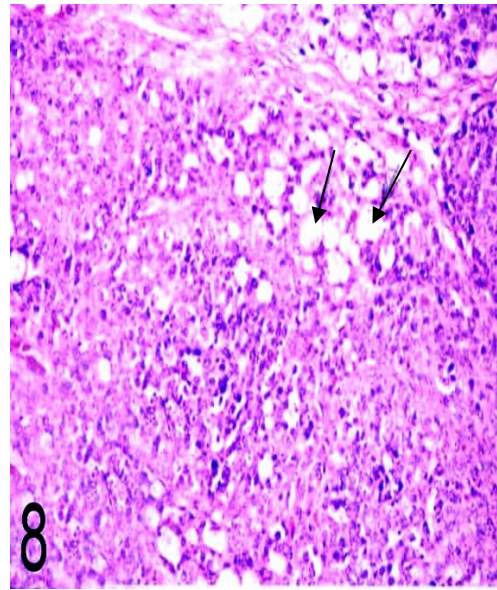

Photo 8: Bursa of Fabricius (gr. I) showing vaculations of lymphoid follicles (arrow) (H & E x200)
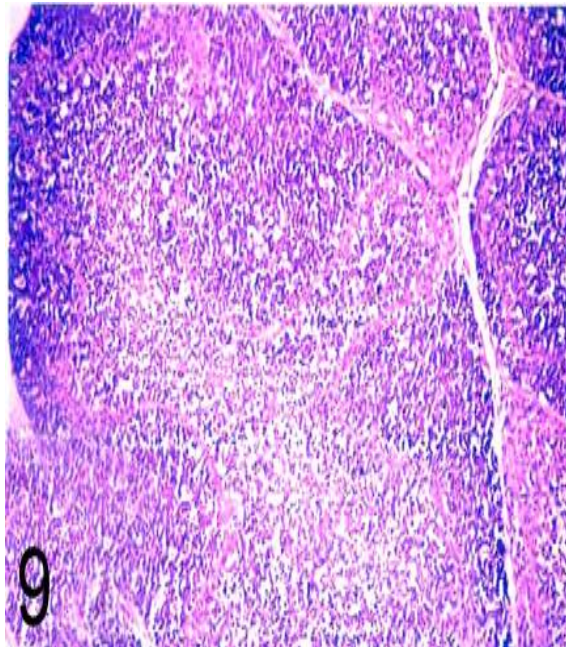

Photo 9: Bursa of Fabricius (gr. III & IV) showing no histopathological changes (H & E x100)
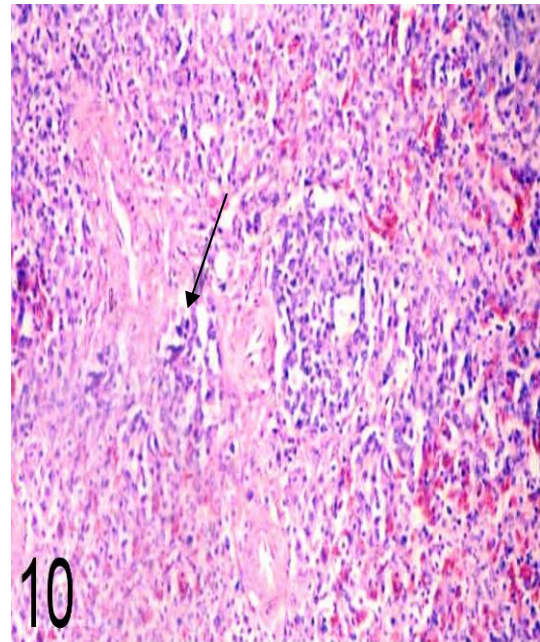

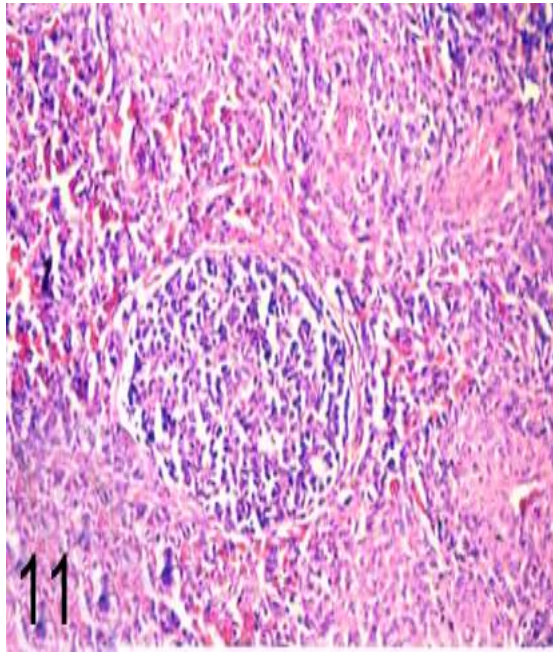**Photo 10: Spleen (gr. I) showing atrophy of lymphoid follicles (arrow) (H & E x200)**

Photo 11: Spleen (gr. III & IV) showing no histopathological changes (H & E x200)
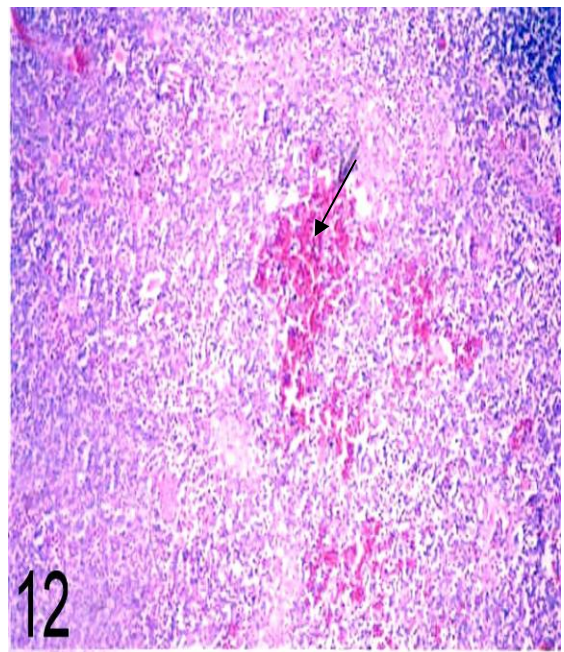

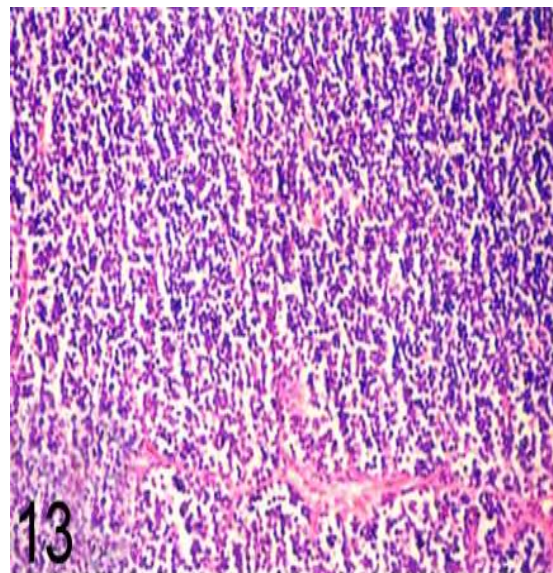**Photo 12: Thymus gland (gr. I) showing focal thymic haemorrhage (arrow) (H & E x100)**

Photo 13: Thymus gland (gr. III & IV) showing no histopathological alterations (H & E x00)

References

1. Amer, M.M. (1980). Studies on the effect of the different diluents used with of Newcastle vaccines on the immune status of birds. M.V.Sc. Thesis, Faculty of Veterinary Medicine, Cairo University.

2. Anke, L., Hildebrand, H., Bach, U., Dingermann, T. and Ahr, H.J. (2003). A new approach to studying ochratoxin A (OTA) - induced nephrotoxicity: expression profiling *in vivo* and *in vitro* employing CDNA.

3. Awaad, M.H.H., Kutkat, M.A. and El-Shobaki, F.A. (2000)b. Immunipotentiation of infectious bursal disease (IBD) vaccination. Veterinary Medicine Journal Giza, 48(1): 27-33.

4. Awaad, M.H.H., Saad, F.E., Elshazly, O.A., Afify, M.A., Zouelfkar, S.A. and El-Miniawy, H.M.F. (2000)a. Immunomodulation of acidifiers in cyclophosphamide treated chickens. Veterinary Medicine Journal Giza, 48(1): 145-160.

5. Awaad M.H.H., Saad, F.E., Elshazly, O.A. and El-Miniawy, H.M.F. (1999). mmunomodulation of organic acids in chickens. Suez Canal Veterinary Medicine Journal, II (1) 103-114.

6. Bancroft, J.D., Stevens, A. and Turner, D.R. (1996). Theory and Practice of Histological Techniques. 4th Ed. New York, Churchill, Livingstone.

7. Boorman, G.A., Hong, H.L., Dieter, M.B., Hates, H.T., Poland, A.E., Stack, M. and Luster, M.I. (1984). Myelotoxicity and macrophage alteration in mice exposed to ochratoxin A. Toxicology and Applied Pharmacology, 72: 304-312.

8. Campbell, M.L.Jr., May, J.D., Huff, W.E. and Doerr, J.A. (1983). Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis. Poultry Science, 62: 2138-2144.

9. Chae, B.J., Lohakare, J.D., Moon, W.K., Lees, S.L., Park, Y.H. and Hahn, T.W. (2006). Effects of supplementation of B-glucan on the growth performance and immunity in broilers. Research in Veterinary Science, 80: 291-298.

10. Chang, C.F., Doerr, J.A. and Hamilton, P.B. (1981). Experimental ochratoxicosis in turkey poults. Poultry Science, 60: 114-119.

11. Creppy, E.E., Stormer, Roschentaler, F. R. and Grunhalgh, R. (1983). Effects of two metabolites of ochratoxin A (4R) 4-hydrochratoxin and ochratoxin A on immune response in mice. Infection and Immunity, 39: 1015-1018.

12. Dhuley, J.N. (1997). Effect of some Indian herbs on macrophage functions in ochratoxin A treated mice. Journal of Ethnopharmacology, 58(1): 15-20.

13. Domhs, J.E. and Saif, Y.M. (1983). Criteria for evaluating immunosuppression. Avian Diseases, 28(2): 305-309.

14. Elaroussi, M.A., Mohamed, F.R., El-barkouky, E.M., Atta, A.M., Abdu, A.M. and Hatab, M.H. (2006). Experimental ochratoxicosis in broiler chickens. Avian Pathology, 35(4):263-269.

15. Emmerich, B., Pachmann, K., Hallek, M., Milatovic, D., Lohmann-Mathes, M.L., Busch, R. and Emsiander, H.P. (1988). Immunomodulation with bacterial extracts: Effects on gamma interferon, macrophages, T-lymphocytes and S-IgA in bronchoalveolar lavage fluid patients with chronic bronchitis. 7th. Congress of the European Society of Pneumology (SEP), Budapest, Hungary.

16. Gill, H.S. and Cross, M.L. (2002). In: Nutrition and Immune Function, Calder, P.C., Fild, C.J. and Gill, H.S. (Eds), pp. 251-272 (Wallingford, UK, CAB International).

17. Hanif, N.Q., Muhammed, G., Siddique, M., Khanum, A., Ahmed, T., Gadahai, J.A. and Kaukab, G. (2008). Clinico-pathomorphological, serum biochemical and histological studies in broilers fed ochratoxin A and a toxin deactivator (Mycofix® Plus). British Poultry Science, 49(5): 632-642.

18. Hansen, T.J. (1990). Affinity column cleanup and direct fluorescence measurement of aflatoxin M1 in raw milk. Journal of Food Protection, 53: 75-77.

19. Hatab, M.H.M. (2003). Determination of the toxic effect of ochratoxin A on broiler performance and immune system response by the use of nuclear techniques. M.Sc Thesis. Faculty of Agriculture, Cairo University.

20. Hsieh, D.P.H. (1987). Mode of action of mycotoxin, in: Krogh, P. (Eds) Mycotoxin in food, pp. 149-176 (London, Academic press).

21. Huff, W.E. and Doery, J.A. (1981). Synergism between aflatoxin and ochratoxin A in broiler chickens. Poultry Science, 60: 550-555.

22. Huff, G.R., Huff, W.E., Rath, N.C. and Tellez, G. (2006). Limited treatment with β-1, 3/1, 6-Glucan improves production values of broiler chickens challenged with *Escherichia coli*. Poultry Science, 85: 613-618.

23. Huff, W.E., Wyatt, R.D., Tuker, T.L. and Hamilton, P.B. (1974). Ochratoxin in broiler chickens. Poult Sci., 53: 1585-1591.

24. Karakowski L., Krzyzanowski, J., Wrona, Z. and Siwicicki, A. (1999). The effect of nonspecific immunostimulation of pregnant mares with 1,3/1,6 glucan and Levamisole on the immunoglobulins levels in colostrums, selected indices of nonspecific cellular and humoral

immunity in foals in neonatal and postnatal period. Veterinary Immunology and Immunopathology, 68: 1-11.

25. Kubena, l.F., Harvery, R.B., Huff, W.E., Corrier, D.E., Phillips, T.D. and Rottinghaus, G.E.R. (1989). Influence of ochratoxin A and T-2 toxin singly and in combination on broiler chickens. Poultry Science, 68:867-872.

26. Kumar, A., Jindal, N., Shukla, C.L., Pal, Y., Ledoux, D.R. and Rottinghaus, G.E.R. (2003). Effect of ochratoxin A on *Escherichia coli*-challenged broiler chicks. Avian Diseases, 47: 425-424.

27. Luster, M.I., Germolec, D.R., Burleson, G.R., Janson, Ch.W., Ackermann, M.F., Lamm, R.R. and Hyes, H.T. (1987). Selective immunosuppression in mice of natural killer cell activity by ochratoxin A. Cancer Research, 47: 2259-2263.

28. Marqwardt, R.R. and Frohlich, A.A. (1992). A review of recent advances in understanding ochratoxicosis. Journal of Animal Science, 70: 3968-3988.

29. Mowat, A. (1987). The regulation of immune responses to dietary protein antigens. Immunology Today, 8(3): 93-98.

30. Muller, G., Kielstein, P., Kohler, H., Berdt, A. and Rosner, H. (1995). Studies on the influence of ochratoxin A on immune and defense reactions in the mouse model. Mycosis, 38(1-2): 85-91.

31. North, M. and Bell, D. (1990). Commercial Chicken Production Manual. 4th ed. AVI Book. New York.

32. Podleski, W.K. (1988). Immunomodulatory properties of Broncho-Vaxom and hypersensitivity diseases. 7th. Congress of the European Society of Pneumology (SEP), Budapest, Hungary.

33. Popov, S.V., Popova, G.Y., Ovodova, R.G., Bushneva, A.O. and Ovodov, Y.S. (1999). Effects of polysaccharides from silene vulgaris on phagocytes. International Journal of Immunopharmacology, 21: 617-624.

34. Raina, J.S., Roy, K.S.A. and Singh, B. (1991). Biochemical and histochemical studies in experimental mycotoxicosis in chicks. Indian Journal of Animal Sciences, 61(12): 1276-1281.

35. Raju, M.V. and Devegowda, G. (2000). Influence of esterified-glucomanan on performance and organ morphology, serum biochemistry and hematology in broiler exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). British Poultry Science, 41: 640-650.

36. Rosales, A.G., Villegas, P., Lukert, P.D., Fietcher, D.J., Mohamed, M.A. and Brown, J. (1989). Isolation, identification and pathogenicity of two field strains of infectious bursal disease virus. Avian Diseases, 33: 35-41.

37. Sansing, G.A., Davis, N.D. and Diener, U.L. (1973). Effect of time and temperature on ochratoxin A production by *Aspergillus ochraceus*. Canadian Journal Microbiology, 19: 1259-1263.

38. SAS® 1999. User's Guide, Release 8th ed. (Cary, NC, SAS Institute). Sharma, J.M. and Tizard, I. (1984). Avian cellular immune effector mechanisms a review. Avian Pathology, 13(3): 357-376.

39. Sharma, J.M., Dohms, J.E. and Metz, A.L. (1989). Comparative pathogenesis of serotype 1 and variant serotype 1 of infectious bursal disease virus and their effect on specific pathogen free chickens. Avian Diseases, 33: 112-124.

40. Shashidhara, R.G. and Devegowda, G. (2003). Effect of dietary mannan oligosaccharide on broiler breeder production traits and immunity. Poultry Science, 82(8): 1319-1325.

41. Singh, G.S., Chauhan, H.V., Jha, G.J. and Singh, K.K. (1990). Immunosuppression due to chronic ochratoxicosis in broiler chicks. Journal of Comparative Pathology, 103(41): 399-410.

42. Stafford, J.L., Neumann, N.F. and Belosevic, M. (2002). Macrophage-mediated innate host defense against protozoan parasites. Critical Reviews in Microbiology, 28: 187-248.

43. Stoev, S.D., Anguelov, G., Ivanov, I. and Paviv, D. (2000). Influence of ochratoxin A and an extract of artichoke on the vicinal immunity and health in broiler chicks. Experimental and Toxicologic Pathology, 52: 43-55.

44. Stokes, C.R., Miller, B.G., Bailey, M., Wilson, A.D. and Bourne, F.J. (1987). The immune response to dietary antigens and its influence on disease susceptibility in farm animals. Veterinary Immunology and Immunopathology, 17(1-4): 413-423.

45. Tizard, I. 1996. "An Introduction to Veterinary Immunology" 5th Ed., pp. 30-38 (Philadelphia, Saunders publishers).

46. Tokic, V., Lazarevic, M., Sinovic, Z. and Tokic, A. (2007). The influence of different feed additives to performance and immune response in broiler chicken. Acta Veterinaria (Beograd), 57: 217-229.

47. Truckess, M.W., Stack, M.E., Nesheim, M.E., Page, S., Albert, S.W., Hansen, R.H. and Donahve, K.F. (1991). Immunoaffinity column coupled with solution fluorometry or liquid chromatography postcolumn derivatization for determination of aflatoxin in corn, peanut and butter: collaborative study. Journal of the

Association of Official Analytical Chemists (AOAC), 74: 81-88.

48. Tzianabos, A.O. (2000). Polysaccharide immunomodulators as therapeutic agents: Structural aspects and biologic function. Clinical Microbiology Reviews, 13: 523-533.

49. Verma, J., Johri, T.S., Swain, Dr.B.K. and Ameena, S. (2004). Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers. British Poultry Science, 45(4): 512-518.

50. Vetvicka, V., Dvorak, B., Vetvickova, J., Richter, J., Kizan, J., Sima, P. and Yvin, J. (2007). Orally administrated marine (1-3)-β-D-glucan Phycarine stimulates both humoral and cellular immunity. International Journal of Biological Macromolecules, 40: 291-298.

51. Volman, J.J., Ramakers, J.D. and Plat, J. (2008). Dietary modulation of immune function by β–glucans. Physiology and Behaviour, 94(2): 276-284.

52. Walker, R. (2002). Risk assessment of ochratoxin: Current views of the European Scientific Committee on Food, the JECFA and the Codex Committee on Food Additives and Contaminants. Advances in Experimental Medicine and Biology, 504: 249-255.

53. Wang, G.H., Xue, C.Y., Chen, F., Ma, Y.L., Zhang, X.B., Bi Y.Z. and Cao, Y.C. (2009). Effects of combinations of ochratoxin A and T-2 toxin on immune function of yellow-feathered broiler chickens. Poultry Science, 88(3):504-510.

54. Weir, D.M. (1983). Immunology: an outline for students of medicine and biology: 5th Ed. pp. 15-16 (Churchill Livingstone, London. Melbourne, New York).

55. Wentworth, B.C. and Ringer, R.C. (1986). Thyroid, in: Sturkie P.D. (Ed.). Avian Physiology 4th Ed. pp. 452-465 (New York, Springer-Verlag).

56. Williams, D.L., Ha, T., Li, C., Kalbfeisch, J.H., Laffan, J.J. and Ferguson, D.A. (1999). Inhibiting early activation of tissue nuclear factor-κB and nuclear factor interleukin 6 with (1-3) β-D-glucan increases long term surgical in polymicrobiol Sepsis. Surgery, 126(1): 54-65.

57. Xue, C.Y., Wang, G.H., Chen, F., Zhang, X.B., Bi, Y.Z. and Cao, Y.C. (2010). Immunopathological effects of ochratoxin A and T-2 toxin combination on broilers. Poultry Science, 89(6):1162-1166.

58. Yang, Y., Iji, P.A., Kocher, A., Mikkelsen, L.L. and Chotc, M. (2007). Effects of mannan oligosaccharide on growth performance, the development of gut microflora, and gut function of broiler chickens raised on new litter. Journal of Applied Poultry Research, 16(2): 280-288.

59. Zou, T., Qiao, J. and Xu, Z.R. (2006). Effect of beta-mannanase (Hemicell) on growth performance and immunity of broilers. Poultry Science, 85(12): 2176-2179.