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Original Article

Effect of probiotic (lactobacillus& saccaromyces) on the immunological, biochemical and haematological changes of broiler chicken fed on ochratoxicated ration

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ABSTRACT

Eightyone day old Hubbard chicks were divided into four equal groups. First group fed on ration containing only ochratoxin A 2.5mg / 1kg, Second Group fed on ration containing probiotic and ochratoxin A 2.5 mg /1kg, third Group fed on ration containing Probiotic only a fourth group remains as a control fed on plain ration. The experiment continued for 45 days. The chicks vaccinated at 7th day with Hitchner By by ocular route and at 18th, 28th day vaccinated by Lasota vaccine via of drinking water. Every week the birds (weighted with calculation of the amount of ration which have been consumed. Whole blood was collected at 14, 28, and 42 days of age from all groups. The blood serum collected for evaluation of immune response to ND virus vaccine. Liver and kidney function assays were applied and the collected whole blood for differential leucocytic count Immunosuppression was observed in chickens fed diets containing ochratoxin A for 4 weeks, when compared with controls, in which the treated birds showed reduction in total serum protein, lymphocyte precentage weights of the thymus, bursa of Fabricius, and spleen. Urea, creatinine, cholesterol, also alkaline phosphatase, AST, and ALT exhibited a significant increase in ochratoxicated group by comparison to the control group. The addition of probiotic into the ochratoxicated ration improve both the total body weight and organ weight in comparing with the ochratoxicated ration which showed increasing the relative weight of livers and kidney, on the other hand there were a decreasein the relative weights of bursa of Fabricius and spleen. The obtained results concluded that, the addition of probiotic into the Qchratoxicated ration Improve the Immune response to NDvirus vaccine, total body weight and organ weight and also improve most biochemical and hematological parameters.

Key Words: Probiotics, Broilers, Biochemical Changes

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INTRODUCTION

Mycotoxins are among the most common contaminants in poultry feed. It causes great economic losses. Elimination or reduction of mycotoxin producing fungi in grains is not always successful. Ochratoxins are one of the most important toxins produced by moulds and affect human and animal heath and cause economic losses in animals and "poultry. Mycotoxines has been found as natural contaminants of feed stuff in many countries (Purwoko et ai, 1991). Ochratoxins A may leads to what is called vaccination failure or vaccinal immunity and may lead to occurrence of disease even in properly vaccinated flocks (Lesson et ai, 1995). There are different systems for protection against the effects of mycotoxins in poultry industries; one of these systems is the use of some chemicals additives to the food of the poultry to minimize the effect of mycotoxins.

There are varieties of physical, chemical and biological
approaches employed to counteract the mycotoxins problems such as
Probiotic, which are used for chickens to replace organisms that are not present in the alimentary tract or to provide the chickens with the effect of beneficial bacteria. Smith (1970) found that the most obvious changes in the flora of the chickens induced by dietary changes and occurred at the anterior end of the digestive tract. Churchil et al. (2001) reported that addition of live yeast culture (Saccharomyces cerevisiae)0.1% or 0.2% to diet of chickens contain aflatoxin(1ppm)resulted in counter acting the toxic effect of aflatoxin on live body weight, feed conversion and mortality rate. Khalaf-Allah et al. (2002) they studied the effect of some detoxifying compounds on broilers fed on ration containing ochratoxin with addition of Saccharomyces cerevisiae (dried yeast powder using of these compounds in addition to ochratoxicated ration leads to improvement of body weights, body weights gain.

Recent approaches for detoxification is through dietary modifications, involving the use of lipotopes as choline, and sulpher containing amino acids (Nahm 1991), vitamins (Kirn and Combs 1992).

The objects of the present study:
1-Studies on different types of toxins._produced by asperagillus ochraceus.
2- Experimental studying the effect of ochratoxin in broiler chickens.
3- Study the effect of probiotics as feed additive on chickens fed ochratoxicated ration:
a- Immunological changed of broiler chicks.
b- Biochemical and hematological changes.

MATERIAL AND METHODS

Experimental chicks: Eighty one day (unsexed) old Hubbared chicks were obtained from Alarabia company for poultry production.

Basal diet:
a. Commercial broiler starter grower ration from Cairo Company forpoultry production.
b. Probiotic antitoxin-mold clear from Ascopharme added to some groupsof chicks in a dose of: 1 g/ kg ration along the period of the experiment from one day old up to 43 day of age. Each 1 kg probiotic contain:
Lacto bacillus + saccaromyces: 50 g
Calcium propionate: 200 g
Copper sulphate: 8 g
Aluminium, calcium and Magnisium silicate up to 1000 g.

Media:
Sabourd's dextrose agar medium: was prepared according to Cruickshank et al., (J975).
Yeast exteact sucrose broth: It used for testing the toxicity andproduction of ochratoxins and was prepared according to Davis (1969).
Yeast extracts: 20 g
Sucrose: 40 g
Dist water: up to 1000 ml
Then autoclaved at 121 C° for 15 minutes.

New castle disease virus veccines: Hitchner B_1 produced by Izovac Company and Lasota vaccine produced by Lohman animal health GmbH.

Chemicals for thin layer chromatography: Kit. For ochratoxin obtained from Biochemical Hrt No. 70040 1 mg each.

Kits for determination of total serum protein, albumin and globulin according to (Henary, 1964).

Testing the toxicity of. Ochraceus isolates which obtained from the department of Bacteriology, "Mycology and Immunology; Faculty of veterinary Medicine Sadat City, Minufiya University: according to (Davis etal., 1966)

Extraction of ochratoxin from liquid medium: The culture filtrate through Whatman filter paper into culture filtrate and mycellial mats. The filtrate was acidified by adding 0.1 N hydrochloric acid and the ochratoxin extracted from the acidifed filtrate by adding chloroform twicely and separate the chloroform layer by seperatory funnel. Equal amount of 0.1M sodium bicarbonate solution was added to the

acidified chloroform extract and shaken in separatory funnel. Thus the chloroform contained noochratoxins. The sodium bicarbonate extract was acidified with 0.1 N hydrochloric acid and retracted by chloroform again. The purified chloroform extract was evaporated to dryness to remove chloroform using evaporator or water bath then the residuse taken to detamine the ochratoxin.

Qualitative determination of the ochratoxin: according to, (Scott et al, 1970)
Confirmatory tests for the presence of ochratoxin: according to, (Scott et al., 1970)

Quantitative determination of ochratoxin A: according to Shannon et al., 1970).

Preparation of ochratoxicated ration:A known amount of prepared ochratoxin A were dissolved in 95% ethanol and added to the remained of the feed.

Experimental design: 80 one day old Hubbard chicks were divided into 4
groups each group contain 20 birds.
Group No 1: Fed on ration contain only ochratoxiniA 2.5mg /1kg
Group No.2: Fed on ration contain probiotic and ochratoxin A 2.5mg /1kg
Group No 3: Fed on ration containing Probiotic only.
Group No 4: Control fed on ration without any additives.
The experiment continued for 45 day. The chicks vaccinated at 7th day with Hitchner B_1 by occular route and boostered at 18th, 28th day vaccinated by Lasota vaccine via of drinking water. Every week the birds (weighted with calculation of the amount of ration which have been consumed.

Collection of blood: Whole blood was collected from shank and /or wing vein at 14, 28, and 42 day of age from all groups. The blood serum collected for evaluation of immune response to ND virus vaccine and liver and kidney function assays and collected whole blood with heparin as anti coagulant for differential leucocytic count.

Methods for evaluation of immune response.
Evaluation of cell mediated immune response (Differential leucoytic count).

Haemagglutineation test (HA): according to (Anon, 1971) and Haemayglutination inhibition test (HI) according to (Allan and Gough1974).

Serum Biochemical Analysis:
Total protein: were estimated according to Henary, (7974)by using Kits from Biomereux co.)

Albumin: Was colorimetrically determined in serum according to Domas et al., (1971).
Urea: Was colorimetrically determined in serum according to Patton and Crauch (1977). using Kits from Biomereux co.)
Creatinine: Was colorimetrically determined in serum according to Yong.etal., (1975). using Kits from BM Egypt co.)
Cholesterol: Was colorimetrically determined in serum according to Melatonini, et al., (1987). Using Kits from Diamond co.)

ALT and AST: According to Reitman and Frankel (1957). using Kits from BM. Egypt co.)

Globulines: According to Mancini et al., (1965), Immunoglobulin G, A and M (IgG, I g A, I g M,) were measured in serum by using Endoplate immunoglobulin test kit obtained from BioMereux co.

Nutritional parameters:
Relative organ weight: According to Huffetal., (1986).
Growth rate: According to (Broody, 1945)

RESULTS AND DISCUSSION

The probiotics act as organisms and substances that contribute to intestinal microbial balance and can be used to stimulate microbial growth. In addition, Fuller (1973) defined probiotics as a live microbial feed supplement, which beneficially affects the host by improving its intestinal microbial balance. Martin, (1996) the addition of probiotics to poultry ration improves the production of vitamin and short chain fatty acids from the food substances, besides keeping the integrity of the intestinal epithelium, stimulation of the immune response and protection against entero-pathogenic microorganisms.

The obtained results for the detection of ochratoxin production by Aspergillus ochraceus using HPLC techniques, the same results was developed by Soares and Rodriguez Amaya (1985), they developed a simple, economical and rapid method for screening and quantitation of Ochratoxin A in cereals. Screening was carried out by using a silica gel aluminum oxide minicolumn with adetectipn limit of 80 ug / kg. The detection limit was lo ug/kg for TLC "quantitatioryjKfhere are two groups of probiotic preparations: those, which primarily intended to be effective in the crop and the anterior regions of the alimentary tract. Among the first group are various lactobacillus cultures and preparations, which are thought to colonize the crop and small intestine.

Homma and Hamaoka (1998) reported that birds supplements with Bacillus subtilis culture as

probiotic feed additive improved' health, productivity and management problems.
In chickens, fed diets containing ochratoxin A at a concentration of 2-4mg/kg for 20 daysv the lymphoid cell population of immune organs was decreased (Dwivedi and Burns, 1984a).

The results obtained in table (1) concluded that, the addition of probiotic into the ochratoxicated ration improve both the total body weight and organ weight in comparing with the ochratoxicated ration without any probiotic which showed increasing the relative weight of livers, decrease the relative weights of bursa of Fabricius and spleen and increase the relative weights of kidneys. Imrnunosuppression was observed in chickens fed diets containing ochratoxin A at (0.5) or 2 mg/kg for 21 days. When compared with controls, the treated animals had reduced total serum protein, lymphocyte "counts, and weights of the thymus, bursa of Fabricius, and spleen (Singh et al., 1990). Ochratoxins A has carcinogenic, teratogenic, mutagenic and immuno-suppresive effect. In addition, the same findings were noticed by (El-Shewy, 1999), Groups of 20 Hubbard broiler chickens were fed diets containing ochratoxin A alone at 1.25 or 2.5 mg/kg of diet or in combination with propionic acid for 3 weeks" A significant reduction in body-weights gain were seen by the second week of feeding and was still present at the third week (by 19%). Also, the relative kidney weight was increased in the group given ochratoxin A.

Table (2) showed the effect of ochratoxinA and probiotics on total serum protein, albumin and globulines were higher in their levels in comparing with the chicks fed ochratoxicated diets only noticed that, the levels of total serum protein, albumin and globulines. These results agree with those ofTungetai., (1975), Huff et at., (1986), El Shewey (1999)} Salwa et al., (2000) and Flourage (2005,).The reduction of levels of total serum protein, albumin and globulines during ochratoxication are indicators of impaired protein synthesis. The reduction of serum immunoglobulins may be attributed to the significant depression of immunoglobulin containing cells in all lymphoid organs, atrophy of lymphoid organs Dwivedi and Burns, (1984). Also the same results of blood parmeters noticed by El-Shewy et al., (1979) they found that ochratoxin A caused leucocytopenia in chickeris which were characterized by lymphocytopenia and monocytopenia with normal heterophils count.

Also, the dietary ochratoxin A leads to decrease antibody titre against Newcastle disease virus vaccine and decrease differential leucocytic count: decrease number of lymphocytes, monocytes, basophiles and eosinophils, Florage, (2000 and 20005) reported that ochratoxin A alone and in combination with aflatoxin 81 in chickens leads to immuno suppression effect in which there were a decrease in serum immunoglobulins and leucocytic count. Khalaf-Allah at al., (2002), they found that using of these compounds in addition to ochratoxicated ration leads to improvement of body weights, body weights gain. Weights of liver, spleen, bursa and proventriculus became near to control values prevented ochratoxin residues in various organs of chickens also the level of total proteins and globulins were restored to nearly the normal ranges. Paul flora and Malathy (2004) found that broilers fed on ochratoxin A contaminated ration were suffered from anemia and decrease in total erythrocyte count, packed cell volume and hemoglobin concentration values. Also, Florage (2000 and 2005) noticed that ochratoxinA reduced body weight serum total albumin, protein and cholesterol levels in broilers fed on Ochratoxin A contaminated ration. Awaad et al., (2005) they used probiotic in prevention of chicken ochratoxicosis immune dysfunction and found that using of this probiotic in a dose of 100g /ration with broiler basal diet containing 5 ppb ochratoxin A resulted in decrease mortality rate in broiler chickens, improved mean body weights, decrease histopathologicl changes in lymphoid organs (bursa and thymus) and increase heamagglutinetin inhibition geometric mean titer against Newcastle and Gumboro.

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