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EFFECT OF ENTEROCOCCUS FAECIUM DSM 7134 PROBIOTIC UPON SOME HEMATOBIOCHEMICAL VALUES IN ACUTE OCHRATOXICOSES IN BROILERS

Blagoevska Katerina[1], Dodovski Aleksandar[2], Blagoevski Andreja[3], Popovska-Percinic Florina[1], Pendovski Lazo[1], Mickov Ljupco[1], Stojkovski Velimir[1]

[1]*Institute for reproduction and biomedicine,*
[2]*Veterinary Institute, Ss. Cyril and Methodius University, 1000 Skopje, Republic of Macedonia,*
[3]*Global Feed Additives, DOOEL, Skopje*

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ABSTRACT

The effect of a single strain probiotic *Enterococcus faecium DSM 7134*, upon some biochemical and haematological parameters in broilers, was examined after a single dose application of ochratoxin A (OTA). For that purpose, a total of 160 Ross broiler chicks were randomly ditsributed into four different treatments with two replicates of 20 chicks each: 1) control; 2) probiotic 3.300 x 10^9 CFU, tested at 0.2 g/l in drinking water; 3) OTA 50 mg/l, in drinking water; and 4) probiotic plus OTA, in same amounts as in groups 2 and 3. Blood samples were collected by cardiac puncture 6 h and 12 h after ochratoxin A application. Evaluated parameters in chicks' blood were: total protein, albumin, urea, creatinine, haematocrite and haemoglobin.
Data for all parameters at 12 hours after OTA application, revealed significant ($p<0.01$) diffreneces in group receiving only mycotoxin (OTA) when compared with data from the chicks in group receiving probiotic and ochratoxin (P+OTA). Hematological analyses showed significant ($p<0.01$) decreased hemoglobin value in chicks from group OTA at 12 hours post exposure to mycotoxin, compared to those at 6 hours post exposure. Chicks from the group receiving only probiotic, at 12 hours showed significant ($p<0.01$) decrease in creatinine value compared to that of the control group. It can be concluded, that administration of probiotic Enterococcus Faecium DSM 7134 in drinking water together with ochratoxin, may well be a contribution to a healthy broiler production, especially if probiotic is used as an integral part of a larger conept including management factors.

Key words: Enterococcus faecium DSM 7134, ochratoxin A, broilers, biochemical profile, haematological values

INTRODUCTION

Ochratoxin A (OTA), a secondary metabolite mainly produced by *Aspergillus ochraceus* and *Penicillium viridicatum*, has been shown as a potent nephrotoxin to animals and human, even thought animals may vary in their susceptibility to the toxin effects (1).

Ochratoxin is the main cause for the Balkan Endemic Nephropathy (2), and according to the International Agency for Research on Cancer (IARC) (3), OTA is classified as on of the possible carcinogens in group 2B. Besides being nephrotoxic, ochratoxin is considered to cause a broad range of toxicological effects such as hepatotoxic, neurotoxic, immunotoxic, genotoxic and teratogenic effects in both human and animals (4). Ochratoxin can adversely affects animal production, especially in swine and poultry industries. Particularly in poultry production, ochratoxicoses can cause significant losses due to its effects on performance and health, by reducing the growth rate and feed consumption, reducing immune response, increasing mortality and poor feed conversion (5-8). Furthermore, OTA affects the biochemical profile in poultry, by altering the amounts of total blood protein, albumin, globulin, urea, triglycerides, uric acid and creatinine

Corresponding author: Ass. Katerina Blagoevska, MSc

E-mail address: katerinab@fvm.ukim.edu.mk
Present address: Department of biochemistry and cell biology, Institute for reproduction and biomedicine, Faculty of Veterinary medicine-Skopje, "Ss. Cyril and Methodius" University,
Lazar Pop- Trajkov 5-7, 1000 Skopje, R. Macedonia
tel: +389 2 3240 731; fax: +389 2 3114 619

(9-10). Diets contaminated with ochratoxin fed to broilers, could lead to leucopenia and lymphopenia (11), and decreased number of red blood cells and hemoglobin concentration could be observed, as well (12).

In the past decades, research was focused on finding solutions for mycotoxin detoxification, by binding it to different adsorbents, adding antioxidants to the diet and other feed supplements (13-16). Results have showed that not all binders have the same capacity to protect animals against the detrimental effects of mycotoxins. Some experiments (17) showed that, most of the adsorbents, by beeing beneficial to the host organism by binding the mycotoxins from the feed, in the same time they may impair the use of the essential nutrients from the feed, as well. For that reason, it is very important that the methods used for mycotoxin decontamination should not influence or contribute to losses of nutritional feed value or palatability.

Recently more attention is given to the use of specific bacterial strains which are able to biotransform mycotoxins (18) . Untill now, several bacterial strains have been proven for *in vitro* and *in vivo* biotransformation of different mycotoxins such as: aflatoxins, ochratoxin, trichotecenes and zearalenone (19-26). These beneficial bacteria are so called probiotics. They are defined as nonpathogenic live microbial food supplements, which beneficially influence human (27) and animals health by reducing pathogen colonization and therefore contribute to human and animal welfare (28-30).

Based on the data on field outbreaks of ochratoxicosis resulting in poor growth rate and poor feed efficiency (31), particularly being quite pronounced in young broiler chicks (9), this research was conducted in order to study the effect of acute experimental OTA toxicity upon hematological and biochemical profile in young broiler chicks.

MATERIAL AND METHODS

Experimental birds and management

This study was carried out on 160 day-old Ross broiler chicks, received from a commercial hatchery, from a maternal flock appropriately vaccinated according to the established immunoprophylaxis program. During the exepriment and afterwards, animals were tretated according to the "Guide for the care and use of laboratory animals" approved by the National Research Council (ILAR)(32) in USA.

Experimental design

One hundred and sixty 1-day-old Ross broiler chicks, after weighing were equally assigned in two groups of 80 chicks: 1) control (C); 2) probiotic (P), receiving 0.2 g/l in drinking water, with concentration of 3.300 x 10^9 cfu. Both groups were kept on a floor system under standard hygienic and ambiental condidtions, and were supplied feed and water ad *libitum*, throughout the experimental period of 14 days. During this period chicks were fed commercial starter ration (23.5 % crude protein and 3025 kcal metabolizable energy (ME)/kg). At 14 days of age, chicks were randomly distributed into four different treatments with two replicates of 20 chicks each: 1) control (C); 2) probiotic 3.300 x 10^{12} cfu, tested at 0.2 g/l in drinking water (P); 3) OTA 50 mg/l, in drinking water (OTA); and 4) probiotic plus OTA, (P+OTA), in same amounts as in groups 2 and 3. Calculated OTA intake was 5,85 mg per chick in a single dose, while calculated probiotic intake concetration was 0,023 g with concnetration of 3.300 x 10^9 cfu/chick/day. Detailed layout of the experiment is presented in Table 1.

Blood samples were collected by cardiac puncture at 6 and 12 hours after toxin application. After blood was taken, small aliquot was immeadiately transferred into a 500 ml tubes with anticoagulant, and the rest was transferred into serum tubes. Evaluated parameters in chicks' blood were: total protein, albumin, urea, creatinin, haematocrite and haemoglobin.

Table 1: Chick's grouping and treatment

Groups	Diet and treatment
C - control	Basal diet
OTA (Ochratoxin A)	Basal diet + OTA, 50 mg/l, in drinking water
P - probiotic	Basal diet + probiotic, 0.2 g/l in drinking water, with concentration of 3.300×10^9 cfu
P+OTA Probiotic and ochratoxin A	Basal diet + probiotic, 0.2 g/l in drinking water, with concentration of 3.300×10^9 cfu and OTA, 50 mg/l, in drinking water

Hematological parameters

For hematological analyses blood samples with anticoagulant (K_3EDTA) were taken from fifteen randomly selected birds from each group, 6 and 12 hours after OTA application. Hemoglobin and hematocrit concentration were determined by automated cell counter hematology analyser, Celly (*Hycel Diagnostics, France*).

Biochemical parameters

Blood was collected from 15 chicks without anticoagulant and serum was extracted by centrifugation at 3000rpm for 10min, followed by serum storage at −20 °C till analyses were done. Commercially available colorimetric kits from *Human* (Germany) were used for the determination of total protein, albumin, urea and creatinine, according to the manufacturer's instruction. All analyses were performed on spectrophotometer Cecil 2000 series, (*Cecil Instrument 2000 England),* according to the manufacturer's protocol.

Statistical analysis

Data obtained from all parameters in this study were subjeted to descriptive statistics, where mean and standard deviation were calculated. For comparison within the groups, t-dependent test was used, and differences between groups were analysed with Tukey, ANOVA by using SPSS sofware. For all parameters, differences with $p<0.01$ were considered statisticaly significant.

RESULTS AND DISCUSSION

Effect on hematological parameters

Data from the analyses of hemoglobin and hematocrit are presented in Table 2. Data presented in Figure 1 show significant decrease in hemoglobin concentration 12 hours after toxin application, in group fed with ochratoxin compared with the results at 6 hours in the same group. Lower values for hemoglobin were detected in group P+OTA compared with the group that received only probiotic, but were not significant. However, values from group P+OTA compared to those from group OTA were statisticaly ($p<0,01$) higher at 12 hours after toxin application (Figure 2).

Values for hematocrit concentration showed in Table 2, were decreased in group OTA, when compared to control and P+OTA, after 6, while 12 h after toxin application, the decrease of the hematocrit concentration in this group was statisticaly significant ($p<0,01$). Also in group P+OTA, decreased values for hematocrit were noticed, compared with those in group fed with only probiotic, both 6 and 12 h, after toxin application. Gradually with time, changes in values of hematocrit concentration within all groups were not detected, except for the group fed with probiotic, where value at 12 h was increased compared to the same at 6h. This was not a significant increase. Decreased values for hemoglobin and hematocrit in broilers fed ochratoxin A, are in correlation with findings carried out by (33-34, 12). Reason for decreased Hb values could be impaired protein synthesis by ochratoxin, which is confirmed in this study by lower values for serum albumin in OTA group (33,35).

Table 2. Values (M±SD)[1] of hemoglobin (Hb), hematocrit (Hct), total proteins (TP), albumins (ALB), urea (UREA) and Creatinin (Creatinin) in four different groups (Control; OTA; Probiotic; P+OTA) measured after 6 and 12 hours from the application of ochratoxin A in the OTA and P+OTA groups.

Group	Control		OTA		Probiotic		P+OTA	
Variables	**6h**	**12h**	**6h**	**12h**	**6h**	**12h**	**6h**	**12h**
Hb (%)	9,20±0,15	9,27±0,14	7,31^{a} ±0,19	6,99^{ad} ±0,14	9,12±0,16	9,22±0,18	8,15±0,24	8,25^{ad}±0,22
Hct (%)	26,07±1,16	26,73±0,98	20,37±1,68	20,17^{e}±1,33	26,73±1,10	27,27±1,21	23,13±1,46	23,68^{e}±0,95
TP (g/dl)	3,25±0,26	3,39±0,25	2,34±0,15	2,29^{f}±0,14	3,41±0,41	3,49±0,26	2,93±0,12	2,99^{f}±0,16
ALB(g/dl)	1,93±0,17	1,97±0,15	1,38±0,13	1,33^{g}±0,15	1,96±0,23	1,96±0,18	1,59±0,12	1,66^{g}±0,17
UREA(mg/dl)	1,09±0,28	1,08±0,11	1,73±0,54	1,91^{h}±0,22	0,90±0,22	0,86±0,12	1,41^{c}±0,10	1,05^{ch}±0,13
Creatinin(mg/dl)	0,26±0,02	0,27±0,04	0,38^{b} ±0,02	0,45^{bi} ±0,06	0,25±0,04	0,23±0,03	0,34±0,03	0,37^{i}±0,05

[1]Data are means ± standard deviation, a-i Values for each criteria in a row with same superscript are significantly different at p<0,01

Effect on biochemical parameters

Mean values with standard deviation of serum total protein (TP) and albumin (ALB) concentrations are presented in Table 2. Significant reduction (p≤0,01) was observed for borth parameters in group OTA at 12 hours after application, when compared to P+OTA group (Figure 2). Total protein and albumin values in group P+OTA, 12 hours after toxin apllication are higher than 6h after application. On the contrary from this results, TP and ALB values in OTA group, 12h after mycotoxin application are lower than those measured at 6h. These are not significant results.

Our results are in accordance with similar findigs reported by several authors (9,10,34). Decreased values of total protein and albumin could be due to liver histopathological changes observed in this experiment, but not presented in this paper. Reduction of protein and albumin values is a result of an impaired protein synthesis by the toxicological effect of ochratoxin A, as well. Higher values for TP and ALB in P+OTA group, compared to OTA group, confirm the protective role of probiotic, as far as protein synthesisi is concerned.

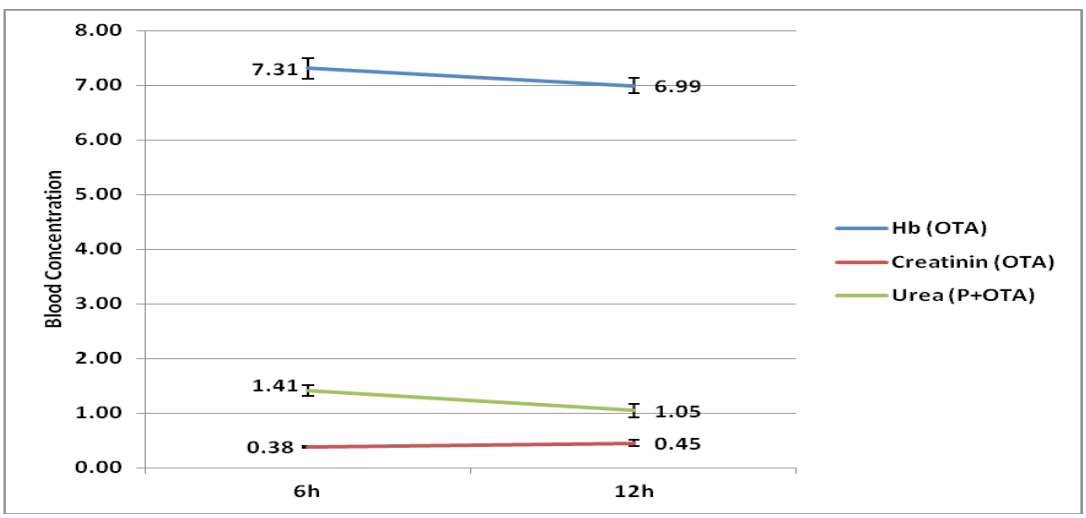

Figure. 1. Changes (p<0.01) on hemoglobin (Hb), creatinin and urea concentration in groups with mycotoxin (OTA and P+OTA) after 6 and 12 hours after the application of the mycotoxin.

Mean values with standard deviation of serum urea and creatinine, 6 and 12 h after ochratoxin A application are showed in Table 2. We found that, creatinine values in group OTA, 12 hours after toxin application, are significantly higher ($p<0.01$) compared to those obtained at 6 hours in same group (Figure 1). However, urea concentration in group P+OTA, was found to be statisticaly lower at 12 hours after toxin applicatioon, compared to the values obtained at 6 hours (Figure 1). Concerning the concentration of both urea and cretinine, higher values are noticed in OTA group compared to control and P+OTA group at 6 and 12h after toxin application, but were not significant, except for the values at 12 hours compared to P+OTA group ($p<0.01$). These findigs are in agreement with those by (10, 34, 35). Decreased creatinine and urea values in group fed with probiotic compared to control group, are a comfirmation of the ameliorating effect of the probiotic in counteracting the toxic effect in experimental ochratoxicoses.

Increased serum creatinine and urea concentrations in ochratoxin group, are an indicator of a inflammatory or degenerative changes in the kidney (36), and it is assumed that ochratoxin induced nephrotoxicity could be due to interference with transport function in collecting tubule cells together with diffused impairment of the proximal tubules function.

Still, further research need to be done in order to better understand the dose and time dependent toxicological effect of OTA and to confirm the probiotic effect upon broiler biochemical profile and performances, by using different probiotic bacteria as single strains or in a mixture.

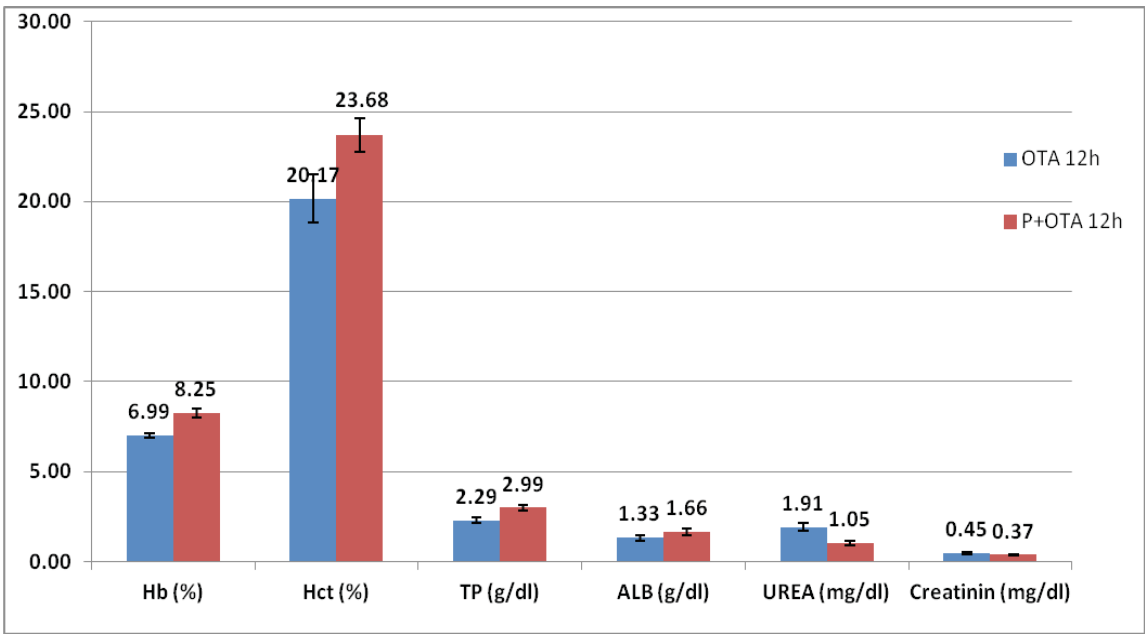

Figure 2. Statisticaly significant changes ($p<0.01$) on hemoglobin (Hb), hematocrit (Hct), total protein (TP), albumin (ALB), urea and creatinine in groups with mycotoxin (OTA and P+OTA) after 12 hours after the application of the mycotoxin. For all parameters mean value and standard deviation (M±SD) is shown.

CONCLUSIONS

The objective of the present study was to evaluate the acute toxic efect of a single dose application of OTA in broiler chicks, upon certain blood parameters. Obtained results confirm that OTA is an important mycotoxin in broiler production, due to the induced changes in hematobiochemical analyses.

Based on the reviewed literature, as well as on data obtained in this study, we can conclude that single dose application of ochratoxin A in concnetration of 50 mg/l in drinking water, affects broil-

er's hematological and biochemical parametres. It resulted in significant reduction in hemoglobin and hematocrit percentage, decrease in total serum proteins and albumin, but increase in concentration of urea and creatinine.

We think that administration of probiotic Enterococcus Faecium DSM 7134 may well be a contribution to a healthy broiler production at least if they are used as integral part of a larger concept including management factors.

REFERENCES

1. Iheshiulor, O.O.M., Esonu, B.O., Chuwuka, O.K, Omede, A.A., Okoli, I.C., Ogbuewu, I.P. (2011). Effects of mycotoxins in animal nutrition: A review. Asian J.Animal Sci 5: 19-33.
2. Puntaric, D., Bosnir, J., Smit, Z., Skes, I., Baklaic, Z. (2001). Ochratoxin A in corn and wheat: geographical association with endemic nephropathy. Croat Med J 42: 175-180.
3. International Agency for research for cancer (IARC) http://www.naturalrussia.com/pdfs/Carcinogen_list.pdf
4. Abdel-Wahhab, M.A., Kholif, A.M. (2008). Mycotoxins in animal feeds and prevention strategies: A review. Asian J. Anim. Sci 2: 7-25.
5. Gibson, R.M., Bailey, C.A. (1989). Ochratoxin A and dietary protein. 1. Effects on body weight, feed conversion, relative organ weight and mortality in three-week-old broilers. Poult Sci 68: 1658-1663.
6. Santin, E., Paulillo, A.C., Maiorka, P.C., Alessi, A.C., Krabbe, E., Maiorka, A. (2002). The effects of ochratoxin/aluminosilicate interaction on the tissues and humoral immune response of broilers. Avian Pathology 31, 73-79.
7. Verma, J., Johri, T.S., Swain, B.K., Ameena, S. (2004). Effect of graded levels of aflatoxin, ochratoxin and their combination on the performance and immune response of broilers. Br Poult Sci 45: 512-518.
8. Politis, I., Fegeros, K., Nitsch, S., Schatzmayr, G., Kantas, D. (2005). Use of Trichosporon mycotoxinivorans to suppress the effects of ochratoxicosis on the immune system of broiler chicks. British Poultry Science 46, 58-65.
9. Huff, W.E., Kubena, L.F., Harvey, R.B. (1988). Progression of ochratoxicosis in broiler chickens. Poultry Science 67 , 1139 -1146.
10. Sreemannarayana, O., Marquardt, R.R., Frolich, A.A., Abramson, D., Phillips, G.D. (1989). Organ weights, liver constituents and serum componenets in growing chicks fed ochratoxin A. Arch Environ Contam Toxicol 18: 404-410.
11. Moura, M.A., Machado, C.H., Porfírio, L.C., Freire, R.B. (2004). Effects of ochratoxin A on broiler leukocytes. Rev. Bras. Cienc. Avic. 6, 3, 187-190.
12. Elaroussi, M.A., Mohamed, F.R., El Barkouky, E.M., Atta, A.M., Abdou, A.M., Hatab, M.H. (2006). Experimental ochratoxicosis in broiler chickens. Avian Pathol 35(4):263-269.
13. Baudrimont, I., Betbeder, A.M., Creppy E.E. (1997). Reduction of the ochratoxin A-induced cytotoxicity in Vero cells by aspartame. Arch Toxicol 71(5): 290-298.
14. De La Cruz, J.P., Quintero, L., Villalobos Auxiliadora M., Sánchez de la Cuesta, F.(2000). Lipid peroxidation and glutathione system in hyperlipemic rabbits: influence of olive oil administration.1485 (1): 36-44.
15. Aravind, K.L., Patil, V.S., Devegowda, G., Umakantha, B. Ganpule, S.P. (2003). Efficacy of esterified glucomannan to counteract mycotoxicosis in naturally contaminated feed on performance and serum biochemical and hematological parameters in broilers. Poult Sci 82: 571-576.
16. Placha, I., Borutova, R., Gresakova, L., Petrovic, V., Faix, S. Leng, L. (2008). Effects of excessive selenium supplementation to diet contaminated with deoxynivalenol on blood phagocytic activity and antioxidative status of broilers. Journal of Animal Physiology and Animal Nutrition 92(4): Early view.
17. Chung, T.K., Erdman J.W., Baker Jr., D.H. (1990). Hydrated sodium calcium aluminosilicate: effects on zinc, manganese, vitamin A, and riboflavin utilization. Poultry Sci 69:1364-1370.
18. Biagi, G. (2009). Dietary Supplements for the reduction of mycotoxin intestinal absorption in pigs Dimorfipa. University of Bologna, Italy. Biotechnology in Animal Husbandry 25 (5-6): 539-546.

19. Haskard, C.A., El-Nezami, H.S., Kankaanpää, P.E., Salminen, S. J., Ahokas, J.T. (2001). Surface binding of aflatoxin B1 by lactic acid bacteria. Applied and Environmental Biology 67: 3086-3091.

20. El-Nezami, H.S., Chrevatidis, A., Auriola, S., Salminen, S., Mykkänen, H. (2002). Removal of common Fusarium toxins in vitro by strains of Lactobacillus and Propionibacterium. Food Additives and Contaminants 19: 680-686.

21. Lahtinen, S.J., Haskard, C.A., Ouwehand, A.C., Salminen, S.J., Ahokas, J.T. (2004). Binding of aflatoxin B1 to cell wall components of Lactobacillus rhamnosus strain GG. Food Additives and Contaminants 21: 158-164.

22. Gratz, S., Täubel, M., Juvonen, R.O., Viluksela, M., Turner, P.C., Mykkänen, H., El-Nezami, H. (2006). Lactobacillus rhamnosus strain GG modulates intestinal absorption, fecal excretion, and toxicity of aflatoxin B1 in rats. Applied and Environmental Biology 72: 7398-7400.

23. Schatzmayr, G., Zehner, F., Täubel M., Schatzmayr, D., Klimitsch, A., Loibner, A.P., Binder, E.M. (2006). Microbiologicals for deactivating mycotoxins. Molecular Nutrition and Food Research 50: 543-551.

24. Niderkorn, V., Morgavi, D.P., Pujos, E., Tissandier, A., Boudra, H. (2007). Screening of fermentative bacteria for their ability to bind and biotransform deoxynivalenol, zearalenone and fumonisins in an in vitro simulated corn silage model. Food Additives and Contaminants 24: 406-415.

25. Trufanov, O.V., Kotyk, A.M. Bozhok, L.V. (2008). Effect of probiotic preparation based on Bacillu subtilis (BPS-44) in experimental mycotoxicoses of chickens. Mikrobiol Z. 70(1):52-8.

26. Kodali, VP, Sen, R. (2008). Antioxidant and free radical scavenging activities of an exopolysaccharide from a probiotic bacterium. Biotechnol J 3(2):245-251.

27. Songisepp, S., Kals, J., Kullisaar, T., Mändar, R., Hütt, P., Zilmer, M., Mikelsaar, M. (2005). Evaluation of the functional efficacy of an antioxidative probiotic in healthy volunteers. in Nutrition Journal 4: 22.

28. Nováková, I., Fikselová, M., Kačániová, M., Haščík, P. (2008). The influence of biological preparations on microbial stabilization in gastrointestinal tract of broiler chickens. Acta Biochimica Polonica 55: 126.

29. Capcarova, M., Hascik, P., Kolesarova, A., Kacaniova, M., Mihok, M., Pal, G. (2011). The effect of selected microbial strains on internal milieu of broiler chickens after peroral administration. Research in Veterinary Science 91, 132-137.

30. Gratz, S.W. Mykkanen, H., El-Nezami, H.S. (2010). Probiotics and gut health: a special focus on liver diseases. World Journal Of Gastroenterology, 16: 403-410.

31. Hamilton, P. B., Huff, W. E., Harris, J. R., Wyatt, R. D. (1982). Natural occurrences of ochratoxicosis in poultry. Poultry Science, 61, (9): 1832–1841.

32. http://oacu.od.nih.gov/regs/guide/guide.pdf Welfare guide

33. Mohiuddin, S.M., Warasi, S.M.A., Reddy, M.V. (1993). Haematological and biochemical changes in experimental ochratoxicosis in broiler chicken. Ind Vet J 70: 613-617.

34. Ramadevi, N.R., Gopal Naidu, R., Ravikumar, P. (2000). An assessment of the protective effect of bentonite on ochratoxicosis in broiler with reference to certain haematological profile. Ind Vet J 77:303-306.

35. Doerr, J.A. Huff, R.B. (1980). Interactive effects of aflatoxin and ochratoxin A on some blood constituents in broiler chickens. Poult. Sci 59: 1600-1602.

36. Benjamin, M.M. (1978). Outline of Veterinary Clinical Pathology, 2nd Ed. The Iowa State University Press, Ames Iowa, 61-69.