Bulgarian Journal of Veterinary Medicine, 2014, **17**, No 3, 191–198
ISSN 1311-1477; online at http://tru.uni-sz.bg/bjvm/bjvm.htm

Original article

BLOOD TRIIODOTHYRONINE, THYROXINE AND THYROID-STIMULATING HORMONE CONCENTRATIONS IN MULARD DUCKS WITH EXPERIMENTAL AFLATOXICOSIS

I. VALCHEV, L. LAZAROV, TS. HRISTOV, D. KANAKOV, R. BINEV & Y. NIKOLOV

Department of Internal Non-Infectious Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary

Valchev, I., L. Lazarov, Ts. Hristov, D. Kanakov, R. Binev & Y. Nikolov, 2014. Blood triiodothyronine, thyroxine and thyroid-stimulating hormone concentrations in mulard ducks with experimental aflatoxicosis. *Bulg. J. Vet. Med.*, **17**, No 3, 191–198.

The aim of the experiment was to evaluate the effect of aflatoxin B_1 applied either independently or in combination with Mycotox NG on blood plasma triiodothyronine (T_3), thyroxine (T_4) and thyroid-stimulating hormone (TSH) in mulard ducks. Four groups of 20 ten-day-old birds each were used. The control group received compound feed according to the species and the age. The feed of group II was supplemented with 0.5 mg/kg aflatoxin B_1, of group III – with 0.8 mg/kg aflatoxin B_1, whereas group IV received compound feed with 0.5 mg/kg aflatoxin B_1 and 2 g/kg Mycotox NG. Blood hormone concentrations were assayed on the 21st and 42nd day in samples collected from *v. metatarsalis medialis*. Lower blood T_3 and T_4 were established in groups II and III. The addition of 2 g/kg Mycotox NG to the feed of group IV had not a significant protecting effect against the adverse effects of aflatoxin B_1 on blood triiodothyronine and thyroxine concentrations.

Key words: aflatoxicosis B_1, ducks, Mycotox NG, thyroid-stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3)

INTRODUCTION

Aflatoxins are secondary toxic metabolites produced by *Aspergillus* (*A. flavus, A. parasiticus, A. nomius* and *A. pseudotamarii*) fungi (CAST, 2003). They contaminate naturally cereal crops such as corn, wheat, sorghum, beans including peanuts and cotton seed (Gugnani, 2000). Poultry are extremely sensitive to these mycotoxins (Robens & Richard, 1992), and among them, ducks are the most sensitive species (Ostrowski-Meissner, 1983). The commonest aflatoxins in animal feeds and animal foodstuffs are AFB_1, AFB_2, AFG_1 and AFG_2. Aflatoxin B_1 is the most toxic and most commonly encountered in poultry feeds (FAO & WHO, 1997; CAST, 2003). The share of AFB_1 from total aflatoxins in naturally contaminated

cereals is 75% (Saad, 1993). Apart from contaminating animal feeds, aflatoxins are also detected in animal foodstuffs (milk, eggs, meat) (Giray *et al.,* 2007). The optimum environmental conditions for growth and toxin production by *Aspergillus* moulds are a temperature of 25–32 °C (Lillehoj, 1983) and relative humidity 61–91% (Moss, 1991). The EC legislation and the American Food and Drug Administration (FDA) have specified that aflatoxins in feeds for growing birds should not exceed 20 ppb for compound feeds and 0.005 ppb in each of feed supplements (Anonymous, 2002; 2009).

The severity of aflatoxicosis in birds depends on several factors: the species and the age, the amount of ingested toxin, the duration of exposure, dietary protein level etc. (Kaya *et al.,* 2002). The intake of aflatoxin-contaminated feeds results in a number of undesirable effects in poultry, such as lower live body weight, weight gain, feed consumption, higher feed conversion ratio (Afzal & Saleem, 2004; Oguz *et al.,* 2004; Abosadi *et al.,* 2007; Zao *et al.,* 2010), changes in relative weights of visceral organs (Rosa *et al.,* 2001; Manafi *et al.,* 2012), altered morphology of the liver (Zao *et al.,* 2010; Yildirim *et al.,* 2011), kidneys (Mohamed & Mohamed, 2009; Yildirim *et al.,* 2011) and immune organs – thymus, bursa of Fabricius, spleen (Sakhare *et al.,* 2007), haematological and blood biochemical changes (Zao *et al.,* 2010; Mohamed & Mohamed, 2009; Yildirim *et al.,* 2011), as well as changes in thyroid hormones (T_3 and T_4) concentrations (Eraslan *et al.,* 2005; 2006). Aflatoxins are potent immunosuppressors which impair humoral and cellular immunity (Ibrahim *et al.,* 2000) and increase the susceptibility to bacterial, viral and protozoan diseases (Qureshi *et al.,* 1998; Shashidhara & Devegowda, 2003).

The removal of aflatoxins from contaminated feeds is the main problem when seeking efficient means of decontamination. During the last years, a number of studies aimed at finding appropriate methods to minimise production losses due to the presence of aflatoxins in compound poultry feeds. The methods for reduction of aflatoxins' adverse effects include use of mould growth inhibitors, microbial inactivation, mechanical separation, heat and chemical inactivation, grinding, usage of nutrient supplements and mycosorbents (Yildiz *et al.,* 2004). Most of these techniques are expensive, take a lot of time, and are unfeasible, inefficient and potentially dangerous. At present, the most popular method by reason of its easy application is the use of inorganic adsorbents. They bind aflatoxins and thus, decrease their absorption in the alimentary tract.

The purpose of the present study was to determine the effect of aflatoxin B_1 independently or together with Mycotox NG on blood concentrations of some hormones (triiodothyronine, thyroxine and thyroid-stimulating hormone) in mulard ducks.

MATERIALS AND METHODS

The experiment was performed with eighty 10-day-old female mulard ducks divided in four groups of 20 birds in each:

- Group I – control. Mulards from the control group were fed pelleted starter, grower and finisher feeds according to their age, produced at Zoohraninvest, Stara Zagora.
- Group II – experimental. Mulards received the standard feed supplemented with 0.5 mg/kg aflatoxin B_1.

- Group III – experimental. Mulards received the standard feed supplemented with 0.8 mg/kg aflatoxin B_1.
- Group IV – experimental. Mulards received the standard feed supplemented with 0.5 mg/kg aflatoxin B_1 and 2 g/kg Mycotox NG (Ceva Santé Animale, France).

Aflatoxin B_1 was produced by *Aspergillus flavus* (99% purity; Sigma-Aldrich, Germany). In experimental groups, the feed was ground before being mixed with aflatoxin for better homogenisation. The mulards were reared under optimal microclimatic conditions, equal for all groups (Anonymous, 2006).

Blood samples were collected from *v. metatarsalis medialis* by the 21st and 42nd day of the trial in sterile heparinised vacutainers (FL medical, Italy) for analysis of blood triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (TSH). Blood was centrifuged within 30 min from collection at 1500×g for 10 min. Plasma was separated, immediately frozen and kept at –20°C until analysed. The blood hormones were assayed on an automated analyser Elecsys 2010 (Roche Diagnostics) via electro-chemiluminescence immunoassay (ECLIA) and commercial test kits (Roche Diagnostics).

The experiment was approved by the Animal Ethics Commission at the Faculty of Veterinary Medicine, Trakia University (Permit 42.10.10.2011).

Data were statistically processed by one-way analysis of variance (ANOVA), and the level of statistical significance was determined by the Tukey-Kramer test ($P<0.05$).

RESULTS

The changes in blood concentrations of studied hormones (T_3, T_4 and TSH) are

Table 1. Effect of aflatoxin B_1 (AFB_1) only or co-administered with Mycotox NG on blood plasma concentrations of triiodothyronine, thyroxine and thyroid stimulating hormone in mulard ducks. Group I – control; group II – 0.5 mg/kg AFB_1; group III – 0.8 mg/kg AFB_1; group IV – 0.5 mg/kg AFB_1 + Mycotox NG. Data are presented as mean ± SEM; n=20

Groups	Triiodothyronine (T3, nmol/L)		Thyroxine (T4, nmol/L)		Thyroid stimulating hormone (TSH, mU/L)	
	21 days of age	42 days of age	21 days of age	42 days of age	21 days of age	42 days of age
I	4.20± 0.079	3.97± 0.083	46.06± 1.17	47.32± 1.01	0.81± 0.024	0.78± 0.046
II	3.81± 0.057[1b]	3.62± 0.072[1b]	40.64± 1.26[1a]	37.28± 1.94[1c]	0.77± 0.035	0.81± 0.039
III	3.54± 0.074[1c,2a]	3.47± 0.053[1c,2a]	32.90± 1.36[1c,2c]	28.71± 1.07[1c,2c]	0.79± 0.040	0.77± 0.035
IV	4.07± 0.055[2a,3c]	3.89± 0.043[2a,3c]	45.75± 1.40[,2a,,3c]	43.69± 0.88[2b,3c]	0.77± 0.040	0.75± 0.040

*Difference from control group I; [a]P<0.05; [b]P<0.01; [c]P<0.001; 1 – vs control group; 2 –vs group II; 3 – vs group III.

shown on Table 1. Blood T_3 and T_4 were statistically significantly lower in ducks from groups II and III by 21 and 42 days of age ($P<0.05$–$P<0.001$). On the 21st day, triiodothyronine attained 3.81±0.057 nmol/L in group II ($P<0.01$) and 3.54±0.074 nmol/L in group III ($P<0.001$) vs controls (4.20±0.079 nmol/L). Thyroxine concentrations on day 21 were 40.64±1.26 nmol/L ($P<0.05$) and 32.90± 1.36 nmol/L ($P<0.001$) in groups II and III, respectively as compared to untreated group I (46.06±1.17 nmol/L).

By 42 days of age, the observed changes were more marked. Average blood T_3 in group II was 3.62±0.072 nmol/L ($P<0.01$) whereas in group III – 3.47±0.053 nmol/L ($P<0.001$). Respective T_4 concentrations were 37.28±1.94 nmol/L (group II; $P<0.001$) and 28.71± 1.07 nmol/L (group III; $P<0.001$). T_3 and T_4 levels in 42-days-old control ducklings were 3.97±0.083 nmol/L and 47.32±1.01 nmol/L, respectively. In experimental group IV, the combined treatment with AFB_1 and 2 g/kg Mycotox NG resulted in insignificantly lower blood T_3 and T_4 levels compared to controls ($P>0.05$). At 21 days of age, the values were 4.07±0.055 nmol/L and 45.75±1.40 nmol/L, and at 42 days of age – 3.89± 0.043 nmol/L and 43.69±0.88 nmol/L, respectively.

There were no statistically significant differences in blood TSH concentrations between control and experimental groups for both studied ages.

DISCUSSION

Thyroid gland hormones are essential for maintenance of systemic physiological equilibrium of living beings (Nakamura & Nakao, 1993; Bozakova & Popova-Ralcheva, 2007). The reduced secretion rate of these hormones has a direct effect on the general condition of organisms (Rose, 2000). Our results demonstrated that AFB_1 could influence blood T_3 and T_4 concentrations by decreasing them. Nevertheless, these changes were not directly related to TSH as seen from the insignificant alterations in TSH levels in all treated groups compared to controls. The changes in T_3 and T_4 should result in TSH alteration, even indirectly. Lower T_3 and T_4 concentrations stimulate thyroid gland T_3 and T_4 receptors, stimulating the synthesis and release of TSH (Noyan, 1993). Increased TSH levels stimulate the utilisation of iodine by the alimentary tract and its diffusion in the thyroid gland (Noyan, 1993; Markou *et al.,* 2001). Iodine is accumulated in the thyroid and forms a complex with thyroglobulin molecules. Afterwards, each thyroglobulin molecule binds to one or two molecules and forms T_3 and T_4. This way, blood T_3 and T_4 levels are sustained (Noyan, 1993; Dunn & Dunn, 1999; Markou *et al.,* 2001). The lack of substantial increase in blood TSH levels observed in this study could be probably due to the lower sensitivity of thyroid receptors caused by aflatoxin B_1 (Graczyk *et al.,* 2002; Eraslan *et al.,* 2006). Aflatoxins are reported to induce lipid peroxidation in cells (Rastogi *et al.,* 2001). The damage of thyroid receptors has probably resulted from the aflatoxins-induced enhanced generation of reactive oxygen species, provoking lipid peroxidation.

Lower thyroid hormone levels indicate development of metabolic disturbances. The relationship between feed quality and blood thyroid hormone concentrations has been extensively studied. An essential regulatory function of these hormones on growth, energy utilisation and a number of vital functions is reported in chickens (Carew *et al.,* 1998). Quails, treated orally

with 2.5 mg total aflatoxin (B_1, B_2, G_1 and G_2) per 1 kg feed over 21 days exhibited reduction of T_3 and increase in T_4 concentrations (Eraslan *et al.,* 2006). Probably, these changes occurred consequently to the delayed conversion of T_4 into T_3 in peripheral tissues (only a small part of blood T_3 is synthesised in the thyroid gland; the major part comes from T_4, in particular 5-deiodinase liver T_4 conversion (Berry & Larsen, 1992). According to the same authors, aflatoxins limit this process by inhibition of 5-deiodinase, resulting in lower blood T_3 together with higher blood T_4 levels.

The lower blood T_3 and T_4 could be further attributed to lower iodine concentrations, participating in the synthesis of hormones (Dunn & Dunn 1999; Markou *et al.,* 2001). The AFB_1-caused damage of alimentary tract epithelium could reduce the absorption of dietary iodine (Johri *et al.,* 1990). Thus, blood serum iodine levels decrease and this could indirectly influence thyroid T_3 and T_4 synthesis.

The reduction of plasma proteins, involved in T_3 and T_4 transportation, has been outlined as a reason for lower blood levels of studied hormones (Jassar & Balvant 1993; Graczyk *et al.,* 2002).

Altered concentrations of thyroid gland hormones (T_3 and T_4) could be also attributed to the impaired morphology of the gland. The experimental patulin intoxication of rats (Selmanoglu & Kockaya, 2004) has resulted in impaired thyroid gland morphology (lymphoid cell infiltration with enlarged interfollicular interstitial tissue and degenerated colloid).

The supplementation of feeds with adsorbents is considered as one of the most reliable methods for reduction of adverse effects of mycotoxins on animals (Dakovic *et al.,* 2005). The different clays, bentonites, zeolites, synthetic aluminosilicates and phyllosilicates are able to bind mycotoxins and thus, to reduce their deleterious effects on animals (Abdel-Wahhab *et al.,* 1999). Adsorbents exhibit a high affinity towards binding aflatoxins in the gastrointestinal tract of birds decreasing their toxicity (Kececi *et al.,* 1998; Eraslan *et al.,* 2005, 2006). The adsorbent Mycotox NG contains bentonite (montmerillonite) and micronised yeast (*Saccharomyces cerevisiae*). Our data confirm previous data (Celyk *et al.,* 2003; Eraslan *et al.,* 2005; Shi *et al.,* 2009) showing that these mycosorbents could decrease at a significant extent the toxic effects of aflatoxins and protect growing birds.

In conclusion, the results from the present study demonstrated clearly that the supplementation of the compound feed of mulards with increasing doses of aflatoxin B_1 (0.5 mg/kg or 0.8 mg/kg) resulted in lower blood plasma triiodothyronine and thyroxine concentrations. The addition of a mycosorbent had a partially protecting effect against the observed toxic effects.

REFERENCES

Abdel-Wahhab, M. A., S. A. Nada & H. A. Amra, 1999. Effect of aluminosilicates and bentonite on aflatoxin-induced developmental toxicity in rat. *Journal of Applied Toxicology,* **19**, 199–204.

Abousadi, A. M., E. Rowghani & M. Ebrahimi Honarmand, 2007. The efficacy of various additives to reduce the toxicity of aflatoxin B_1 in broiler chicks. *Iranian Journal of Veterinary Research,* **8**, 144–150.

Afzal, M. & Z. Saleem, 2004. Effects of addition of a mycotoxin detoxifier in poultry feed containing different levels of aflatoxins on the performance of broilers. *Asian Australian Journal of Animal Science,* **17**, 990–994.

Anonymous, 2002. Directive 2002/32/EC of the European Parliament and of the Coun-

cil of 7 May 2002 on undesirable substances and products in animal feed. *Official Journal of the European Communities*, 30 May 2002.

Anonymous, 2006. Ordinance 44/20.04.2006 for veterinary medical requirements to animal rearing facilities, *Official Gazette*, 41, Appendix 9/II, pp. 61–62 (BG).

Anonymous, 2009. Ordinance 10/03.04.2009 on the maximum allowable concentrations of undesirable substances and products in feeding stuffs. *Official Gazette*, 29, 17 April 2009.

Berry, M. J. & P. R. Larsen, 1992. 'The role of selenium in thyroid hormone action. *Endocrine Reviews,* **13**, 207–219.

Bozakova, N. & S. Popova-Ralcheva, 2007. Thyroid hormones level and relative liver weight in male turkeys in relationship with their welfare. *Biotechnology in Animal Husbandry*, **23**, 1511–1518.

Carew, L.. B., K. G. Evarts & F. A. Alster, 1998. Growth, feed intake, and plasma thyroid hormone levels in chicks fed dietary excesses of essential amino acids. *Poultry Science,* **77**, 295–298.

Celyk, K., M. Denly & T. Savas, 2003. Reduction of toxic effects of aflatoxin B1 by using baker yeast (*Saccharomyces cerevisiae*) in growing broiler chicks diets. *Revista Brasiliera de Zootecnia*, **32**, 615–619.

CAST (Council for Agriculture, Science and Technology): Mycotoxins: Risks in plant, animal and human systems. In: *Task Force Report No. 139,* Ames, Iowa, USA, 2003.

Dakovic, A., M. Tomasevic-Canovic, V. Dondur, G. E. Rottinghaus, V. Medakovic & S. Zaric, 2005. Adsorption of mycotoxins by organozeolites. *Colloids Surf. B Biointerfaces,* **46,** 20–25.

Dunn, J. T. & A. D. Dunn, 1999. The importance of thyroglobulin structure for thyroid hormone biosynthesis. *Biochimie*, **81**, 505–509.

Eraslan, G., D. Essiz, M. Akdogan, F. Sahindokuyucu, L. Altintas & S. E. Hismiogullar, 2005. Effects of dietary aflatoxin and sodium bentonite on some hormones in broiler chickens. *Bulletin of the Veterinary Institute in Pulawy,* **49**, 93–96.

Eraslan, G., M. T Akdoúan, B. C. Lüman, M. Kanbur & N. Ýk Delübas, 2006. Effects of dietary aflatoxin and hydrate sodium calcium aluminosilicate on triiodothyronine, thyroxine, thyrotrophin and testosterone levels in quails. *Turkish Journal of Veterinary and Animal Science,* **30,** 41–45.

FAO & WHO, 1997. Joint FAO/WHO Expert Committee on Food Additives, June 17–26. Rome.

Giray, B., G. Girgin, A. B. Engin, S. Aydin & G. Sahin, 2007. Aflatoxin levels in wheat samples consumed in some regions of Turkey. *Food Control,* **18,** 23–29.

Graczyk, S., B. Kotoński, A. Malicki, J. Orda, & W. Zawadzki, 2002. The thyroxine levels and blood serum profiles in ducklings after chronic aflatoxin B_1 administration. *Medicina Veterinaria,* **1,** 21–29.

Gugnani, H. C., 2000. Ecology and taxonomy of pathogenic aspergilli. *Frontiers in Bioscience,* **8**, 346–357.

Ibrahim, I. K., A. M. Shareef & K. M. T. Al-Joubory, 2000. Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. *Research in Veterinary Science*, **69**, 119–122.

Jassar B. S. & S. Balvant, 1993. Biochemical changes in experimental aflatoxicosis in broiler chicken. *Indian Journal Animal Science,* **63**, 847–848.

Johri T. S., V. R. Sadagopan, M. A. Shrivastava & S. Jumdar, 1990. Effect of dietary aflatoxin on the performance of purebred broiler chicks. *Indian Journal Animal Science,* **60**, 1246–1248.

Kaya, S., 2002. Mikotoksinler. Ücinden: Kaya, S., Pirincci & I., Bilgili, A. ed: Veteriner ekimliÛinde Toksikoloji. Medisan Yayõn Serisi 53, Ankara, 544-573.

Kececi, T., O. Demet & H. Oguz, 1995. Single and combination effects of dietary aflatoxin and adsorbent (Mycofix Plus) on some hematological and serum bioche-

mical parameters of broiler chickens *Journal of Veterinary Science,* **11,** 95–101.

Lillehoj, E. B., 1983. Effect of environmental and cultural factors of aflatoxin contamination of developing corn kernels. In: *Aflatoxin and A. flavus in Corn,* eds V. L. Diener, R. L. Asquith & J. W. Dickens, Southern Coop Serv. Bull. 279, Craftmaster, Opelika, Ala, 112P.

Manafi, M., B. Umakantha, M. Noor Ali & H. D. Narayana Swamy, 2012. Study of the Combination Effects of Aflatoxin and T-2 Toxin on Performance Parameters and Internal Organs of Commercial Broilers. *Global Veterinaria*, **8**, 393–396.

Markou, K., N. Georgopoulos, V. Kyriazopoulou & A. G. Vagenakis, 2011. Iodine-induced hypothyroidism. *Thyroid,* **11**, 501–510.

Mohamed, A. H. & M. H. Mohamed, 2009. Haemato-biochemical and pathological studies on aflatoxicosis and treatment of broiler chicks in Egypt. *Veterinaria Italiana*, **45**, 323–337.

Moss, M. O., 1991. Influence of agricultural biocides on mycotoxin formation in cereals. In: *Cereal Grain: Mycotoxins, Fungi and Quality in Drying and Storage*, ed J. Chełkowski, pp. 281–295.

Nakamura, H. & K. Nakao, 1993. Mechanism of regulation of TSH biosynthesis and secretion. *Nippon Rinsho*, **51**, 2611–2617.

Noyanm, A., 1993. Hormonlar. In: *Yasamda ve Hekimlikte Fizyoloji*, Ankara, pp. 977–1033.

Oguz, H. & S. S. Parlat, 2004. Effects of dietary mannanoligosaccharide on performance of Japanese quail affected by aflatoxicosis. *South African Journal of Animal Science*, **34**, 3, 144–148.

Ostrowski-Meissner, H. T., 1983. Effect of contamination of diets with aflatoxins on growing ducks and chickens. *Tropical Animal Health and Production,* **15**, 161–168.

Qureshi, M. A., J. Brake, P. B. Hamilton, W. M. Hagler & S. Nesheim, 1998. Dietary exposure of broiler breeders to aflatoxin results in immune dysfunction in progeny chicks. *Poultry Science*, **77**, 812–819.

Rastogi, R., A. K. Srivastava & A. K. Rastogi, 2001. Long term effect of aflatoxin B_1 on lipid peroxidation in rat liver and kidney: Effect of picroliv and silymarin. *Phytotherapy Research,* **15**, 307–310.

Robens, J. F. & J. L. Richard, 1992. Aflatoxins in animal and human health. *Reviews of Environmental Contaminant and Toxicology,* **127**, 69–94.

Rosa, C. A. R., R. Miazzo, C. Magnoli, M. Salvano, S. M. Chiacchiera, S. Ferrero, M. Saenz, E. C. Q. Carvalho & A. Dalcero, 2001. Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. *Poultry Science,* **80,** 139–144.

Rose, S. R., 2000. Disorders of thyrotropin synthesis, secretion, and function. *Current Opinion in Pediatrics*, **12**, 375–381.

Saad, M. M., 1993. Preliminary study on formulation and application of aflatoxins(s) – antidote. *Veterinary Medical Journal (Giza)*, **41**, 33–37.

Sakhare, P. S., S. D. Harne, D. R. Kalorey, S. R. Warke, A. G. Bhandarkar & N. V. Kurkure, 2007. Effect of Toxiroak polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. *Veterinarski Arhiv,* **77**, 129–146.

Selmanoglu, G. & E. A. Kockaya, 2004. Investigation of the effects of patulin on thyroid and testis and hormone levels in growing male rats. *Food and Chemical Technology*, **42**, 721–727.

Shashidhara, R. G. & G. Devegowda, 2003. Effect of mannan oligosaccharide on broiler breeder production traits and immunity. *Poultry Science,* **82**, 1319–1325.

Shi, Y., Z. Xu, Y. Sun, C. Wang & J. Feng, 2009. Effects of two different types of montmorillonite on growth performance and serum profiles of broiler chicks during aflatoxicosis. *Turkish Journal of Veterinary and Animal Science,* **33**, 15–20.

Yildirim, E., İ. Yalchinkaya, M. Kanbur, M. Çnar & E. Oruc, 2011. Effects of yeast lucomannan on performance, some biochemical parameters and pathological changes in experimental aflatoxicosis in broiler chickens. *Révue de Médicine Vétérinaure,* **162**, 413–420.

Yildiz, A. O., S. S. Parlatand & I. Yildirim, 2004. Effect of dietary addition of live yeast (*Saccharomyces cerevisiae*) on some performance parameters of adult Japanese quail (*Coturnix japonica*) induced by aflatoxicosis. *Révue de Médicine Vétérinaire,* **155**, 38–41.

Zhao, J., R. B. Shirley, J. D. Dibner, F. Uraizee, M. Officer, M. Kitchell, M. Vazquez-Anon & C. D. Knight, 2010. Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. *Poultry Science,* **89**, 2147–2156.

Paper received 05.06.2013; accepted for publication 11.09.2013

Correspondence:

Assist. Prof. Ivan Valchev
Department of Internal
Non-Infectious Diseases,
Faculty of Veterinary Medicine,
Trakia University,
6000 Stara Zagora, Bulgaria,
e-mail: valtchev@abv.bg