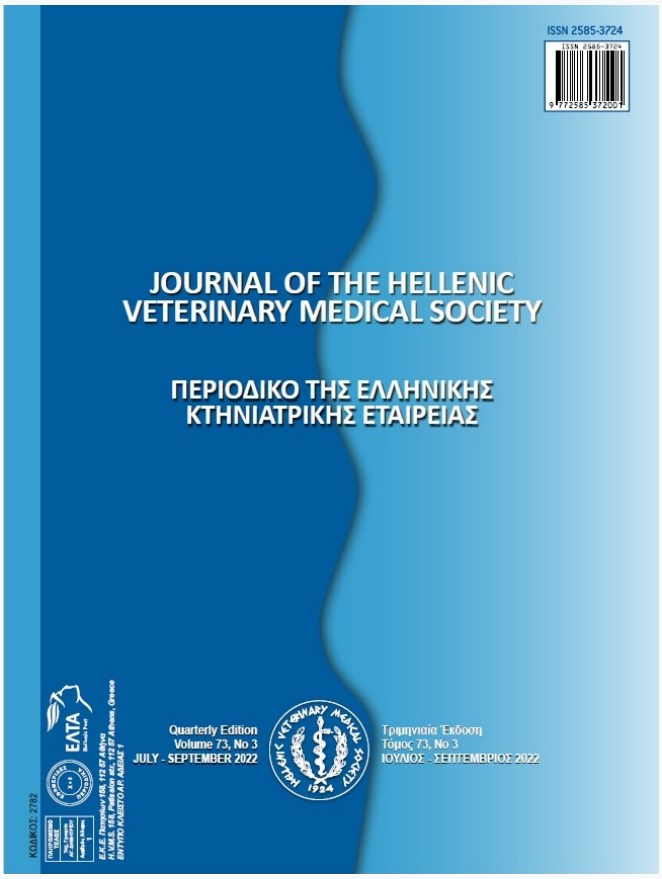
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 3 (2022)



Effects of fumonisin and Salmonella infection in the expression of Toll-like receptors in chicken ovary

V Gkiorsos, G Michailidis, M Anastasiadou, I Giannenas

doi: 10.12681/jhvms.27476

To cite this article:

Gkiorsos, V., Michailidis, G., Anastasiadou, M., & Giannenas, I. (2022). Effects of fumonisin and Salmonella infection in the expression of Toll-like receptors in chicken ovary. *Journal of the Hellenic Veterinary Medical Society*, *73*(3), 4477–4484. https://doi.org/10.12681/jhvms.27476

J HELLENIC VET MED SOC 2022, 73 (3):4477-4484
ΠΕΚΕ 2022, 73 (3): 4477-4484

Research article
Ερευνητικό άρθρο

Effects of fumonisin and *Salmonella* infection in the expression of Toll-like receptors in chicken ovary

V. Gkiorsos[1], G. Michailidis[1], M. Anastasiadou[2], I. Giannenas[3]

[1]*Laboratory of Physiology of Reproduction of Farm Animals, Department of Animal Production, School of Agriculture, Aristotle University of Thessaloniki, Greece*

[2]*Institute of Animal Science, Hellenic Agricultural Organisation-DEMETER, Paralimni Gannitsa, Greece.*

[3]*Laboratory of Nutrition, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece*

ABSTRACT: Mycotoxins are secondary metabolites produced mainly by fungi that contaminate animal feed and basic food products throughout the world. Currently, more than 500 mycotoxins are reported and one of the most important concern to public health and agriculture is Fumonisin B1 (FB1). FB1, a mycotoxin produced by *Fusarium moniliforme*, is a contaminant of animal feed with various and complex cellular effects. Although FB1 has been associated with various diseases in animals, to date few studies have been performed to evaluate the endocrine disrupting effects of FB1 and more particularly the effects in the innate immune mechanisms of farm animals and more specifically in poultry species. As the family of Toll-like receptors (TLRs) is one of the key molecules of the innate immune system, the aim of the present study was to evaluate the transcriptional changes ofTLRs in the chicken ovary *in vivo*, in chicks fed with FB1 and in response to Salmonella Enteritidis (SE) infection. RNA was extracted from the ovaries of sexually mature and aged birds, treated with FB1 and SE. Quantitative real-time PCR analysis revealed that FB1 and SE infection resulted in a significant down regulation of 3 TLR genes in the ovary of sexual mature and 4 TLR genes in the ovary of aged birds. These findings suggest that the mycotoxin FB1 suppresses the innate immune system of the chicken reproductive organs, through alteration in the expression of certain TLR genes.

Keywords:Mycotoxins;Fumonisin B1; innate immunity; Toll-like receptors

Corresponding Author:
Ilias Giannenas, Laboratory of Nutrition, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece
E-mail address: igiannenas@vet.auth.gr

Date of initial submission: 11-07-2021
Date of acceptance: 27-09-2021

INTRODUCTION

Mycotoxins are secondary metabolites of filamentous fungi, which under appropriate temperature and humidity conditions may develop on various foods and feeds (Fernández-Blanco et al., 2016). Currently, more than 500 mycotoxins are reported, which are mainly produced by fungi belonging to the genera *Aspergillus,Penicillium, Fusarium, Alternaria* and *Claviceps*(Fung et al., 2004). *Fusarium* species are contaminants of wheat, maize, and other grains worldwide, capable of producing high levels of fumonisins mycotoxins; Fumonisin B1 (FB1) being a main concern to agriculture, livestock and public health. FB1 is the most prevalent of thefumonisins, accounting for approximately 70% of total detected fumonisins (Martins et al.,2012)and is a secondary metabolite produced by the filamentous fungi Fusarium moniliforme. It can be developed on various plants and animal feeds,with various and complex cellular effectson animal species (Fernández-Blanco et al., 2016).

Recent studies have shown evidence of global and extent contamination of corn and animal feed with FB1 and its implication for animal health and productivity (Boutignyet al., 2012; Garrido et al., 2012; Moretti et al., 2019). Mycotoxins are known to induce toxic effects in farm animals, causing distress and reduced productivity. In detail, exposure to FB1 can cause immunotoxicity and impair reproductive function in farm animals(Bryden 2012; Haque et al., 2020). Although the toxic effects of FB1are well established, these studies were primarily focused on performance, hematology, biochemistry, and pathology.

To date few studies have been performed to evaluate the endocrine disrupting effects of FB1 and specifically the effects on innate immune parameters. The immune system is an important defensive mechanism against invading organisms, moreover impaired immune functions will decrease resistance to infectious diseases. Several mycotoxins have been shown to suppress immune responses and cause immunomodulation in domestic animals (Oswald et al., 2005).FB1 has also been linked with various diseases in domestic animals, but the target tissue or organ for its toxicity differs among the animal species. Monogastric livestock, such as poultry, are more sensitive to mycotoxins because of the high percentage of cereals in their diet and because they lack a rumen with a microbiota that is able to degrade mycotoxins before their intestinal absorption (Pierron et al., 2016a).

In recent years host antimicrobial peptides and proteins have been recognised as key mediators of the innate immune response in many vertebrate species. One of the key molecules of the innate immune system is the family of Toll-like receptors (TLRs). TLRs constitute a family of highly conserved molecules, that play a major role in pathogen detection and disease resistance through their recognition of pathogen associated molecular patterns (Akira and Takeda 2004). These molecules, which initiate the innate immune response and evoke an anti-microbial response in both vertebrates and invertebrates, have recently been identified in the chicken genome.

The impact of the mycotoxins on the immune system of exposed animals is a matter of concern because, by this way, these natural-occurring toxins may make susceptible farm animals to the infectious diseases.There is clear evidence that FB1 in feed or food cause animal mycotoxicosis, which could result in economic losses for the livestock industry by reducing the value of contaminated feed and affect both animal health and productivity as well as the transmission of pathogens such as Salmonella spp. (Fink-Gremmels, 2008; Yang et al., 2020). Salmonellosis is a major cause of food-poisoning worldwide with outbreaks usually associated with *Salmonella enteriditis* (SE) and connected to the consumption of contaminated poultry meat or eggs (Guard-Petter, 2001). Infection of the reproductive organs and especially the chicken ovary is often the major cause of the production of contaminated eggs mainly due to the transovarian transmission of SE in laying hens. We have previously reported that 8 members of the TLRs are expressed in the chicken ovary and that the expression of 3 members was altered in response to *Salmonela enteritidis* (SE) infection (Michailidis et al., 2010). However the effects of FB1 on the expression of these genes in the chicken ovary has not been reported.

Due to the emerging importance of TLRs in innate immunity and in reproductive physiology and the ability of mycotoxins to suppress immune responses, the aim of this study was to investigatewhether FB1 alters the expression levels of TLRs in the chicken ovary in healthy and SE infected birds. The investigation of genes and the various signaling pathways involved in inflammation of cells and tissues exposed to mycotoxins is of crucial importance in order to elucidate the role that these toxins can play in the innate immune system of vertebrates.

MATERIAL AND METHODS

Collection of tissues

Birds of the Rhode Island Reds hybrid (n=48) were used in this study and were supplied by a commercial supplier. The birds were housed in cages under a light regimen of 14 h light: 10 h dark. Feed and water were provided*ad libitum*. The management of experimental animals was in accordance with the institutional accepted welfare guidelines and the chickens were handled according to the principles for the care of animals in experimentation (National Research Council, 1985). Before the experiment was initiated, fecal samples were cultured and were confirmed to be negative for *Salmonella* organisms. Experimental groups consisted of sexual mature (28 weeks old) and aged (104 weeks old, decline in reproductive function) female chickens (n=6 at each age). Birds were sacrificed by cervical dislocation. Total ovary from each bird was removed, snap frozen in liquid nitrogen and stored at -80°C until analysed. Large yolky preovulatory follicles from the ovary of sexually mature chickens were removed before storing in liquid nitrogen.

Dietary treatment

A dietary treatment containing100 mg FB1/kg of diet for 3 weeks wereused in two groups of 28 and two groups of 104 weeks old female birds was used in this study, as previously described (Li et al., 1999). Diets were formulated to beisocaloric, isonitrogenous, and either met or exceeded thenutrient requirements of broiler chicks as recommendedby the National Research Council (1994). Diets werescreened for the presence of mycotoxins by the methodof Rottinghaus et al. (1982) and were found to be free of aflatoxin, citrinin, vomitoxin, sterigmatocystin, zearalenone,ochratoxin A, and moniliformin.

Experimental infections

Two groups of 28 and two groups of 104 weeks old female birds (n=6 per group) were orally gavaged with 0.1 ml of inoculum containing approximately 5 x 10^6 organisms of *Salmonella enteritidis* (SE). Age-matched, non infected control birds were housed under similar environmental conditions, and 0.1 ml of phosphate buffered saline (PBS) was introduced by gavage. The *in vivo* bacterial challenge of experimental animals was in concordance with the institutional accepted welfare guidelines. Chicks were sacrificed on the fourth day after infection and the ovary of each bird was collected, snap-frozen in liquid nitrogen, and stored at -80°C until analyzed.

RNA isolation and quantitative real-time PCR analysis

Total RNA was isolated from chicken ovaries stored at -80°C. Initially, the tissues were ground to a fine powder, and the RNA was extracted using the Total RNA Isolation (TRI) Reagent (Ambion), according to the instructions provided by the manufacturer. The preliminary quantity and purity of the extracted RNA was measured at 260 and 280 nm using the BioPhotometer (Eppendorf) and RNA integrity was verified by agarose gel electrophoresis and visualization of the 28S and 18S ribosomal RNA. To reduce degradation, RNase inhibitor (Invitrogen) was added to each sample (1 Unit per μg of RNA) before storage at -80°C. All samples were pretreated, before reverse transcription (RT), with DNAse (Fermentas) at a concentration of 1 Unit per μg of RNA. One μg of total RNA was reverse transcribed to cDNA using the SuperScript II Reverse Transcriptase kit (Invitrogen) according to the manufacturer instructions.

Quantitative expression analysis of TLR genes was performed with real-time PCR, using a Light-Cycler real-time PCR machine (Roche Molecular Biochemicals), as previously described (Michailidis et al., 2010), using the primers ilustrated in Table 1. PCR was performed using the KAPA SYBR FAST qPCR kit (Kapa Biosystems) and 0.2 pmol of each primer in a final volume of 20 μl using as template 1/10 of the initial cDNA synthesis reaction. Gene expression levels were quantified using the β-actin as an internal standard for cDNA normalization. The cycling parameters were: incubation at 95°C for 10 min, followed by 45 cycles of incubation at 95°C for 10 sec, 56-59°C (Table 1) for 8 sec, 72°C for 8 sec, read at 60°C. For identification of the PCR products a melting curve was performed from 65 to 95°C with read every 0.2°C and 5 sec hold between reads. All the reactions were performed four times using cDNA synthesized from RNA extracted from the ovary of different birds. The threshold cycle (Ct) values of the PCRs were averaged and relative quantification of the transcript levels was performed using the comparative Ct method (Livak and Schmittgen 2001). Real-time PCR data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001) to calculate the relative level of each mRNA in each sample and expressed as a ratio relative to β-actin housekeeping gene. The expression levels of both 28 and 104 weeks old birds are indicated as relative values of 28 weeks birds treated with PBS, in order to compare the differences in the expression levels in the SE infected. FB1 treated and

Table 1: Primer sequences of the chicken TLRs genes used for real-time PCR

Gene	Forward	Reverse	Annealing Temp. (°C)
TLR1-2	CCCGTTCAAGTGTTCATGTG	GTTCCGCTCAAGTCTTCTGG	56
TLR2-1	ACATGTGTGAATGGCCTGAA	TTGAGAAATGGCAGTTGCAG	56
TLR3	CCCTGATGGAGTGTTTGCTT	CAGCTGTTGCTGCAATCCTA	59
TLR4	GTTTGACATTGCTCGGTCCT	gctgcctccagaagatatgc	56
TLR5	TTTCAGGAACCTGCCAAATC	TCCAGGATGGAATCTCCAAG	59
TLR7	GATGCAGTGTGGTTTGTTGG	AACCAAGCTCCTCCCTTTGT	59
TLR15	ATCCCGAATACTTCCCATCC	TGGCAGGCAGGTTCTAGTCT	56
TLR21	AGCTGGAGCTGTTGGACCTA	TTCACGTGCCATAGCATCTC	56
β-actin	CTCCCTGATGGTCAGGTCAT	ATGCCAGGGTACATTGTGGT	56

SE and FB1 treated birds, as well as the differences in the expression levels in response to SE infection and FB1 treatment between laying and non-laying birds.

Statistical analysis

The significance of differences among the means of the experimental groups of birds with different ages was examined using one-way ANOVA, followed by Tukey's multiple range test for *post-hoc* analysis. The Levene's test for Homogeneity of Variances was applied in order to test the assumption that groups have approximately equal variance on the dependent variable. The results of the *post-hoc* analysis determine which of the groups are the most statistically different. Furthermore, a t-test was performed to estimate the significance of differences among the SE, FB1, SE+FB1 and PBS infected birds in all experimental groups. Results were expressed as the mean ± SEM. In all applications (one-way ANOVA and t-tests) differences were considered statistically significant at $P<0.05$.

RESULTS

FB1 and SE were introduced to groups of sexually mature (28 weeks old) and aged (104 weeks old) chickens in order to determine the changes in the expression levels of TLRs in the chicken ovary in response to FB1 treatment and SE infection and also to investigate whether reproductive age also affects the response to FB1 treatment and SE infection through TLR synthesis. After 3 weeks of FB1 treatment and 4 days of SE infection, the ovary of the birds was extracted and analyzed for the mRNA expression of the cTLR genes that were previously found to be expressed in the chicken ovary. After normalization to β-actin expression, real-time PCR analysis revealed a significant ($P<0.05$) down regulation in the expression of TLR4, 7 and 15 in the ovaries of SE+FB1 groupin sexually mature 28 weeks old birds compared to the PBS treated birds of the same age (Figures1,2). No significant changes ($P>0.05$) were observed for the rest of cTLRs in the FB1 or SE+FB1 groups 28 weeks old birds. Furthermore, quantitative real-time PCR analysis revealed also a significant down regulation ($P<0.05$) in the expression of TLR4 and 5 in the ovaries of aged 104 weeks old FB1treated birds, compared with the birds of the same age treated with PBS (Figures1,2), and a significant ($P<0.05$) downregulation of TLR3, 4, 5 and 15 in the ovaries of aged 104 weeks old SE+FB1 group, while no significant differences were observed in the ovarian mRNA abundance for the rest of the TLR genes in both groups of 104 weeks old birds.

DISCUSSION

Mycotoxins are secondary products produced by fungi and are frequently found in the livestock industry as contaminants of farm animal feed. Studies analyzing feed mycotoxins have been conducted worldwide and have confirmed the presence of mycotoxins with biological activity, including aflatoxin, ochratoxin A, fumonisin, zearalenone, and deoxynivalenol, in a large proportion of feed samples (Ukwuru et al., 2018; Yang et al., 2020). Exposure to mycotoxins can cause immunotoxicity and impair reproductive function in farm animals, probably due to their endocrine disrupting ability. Endocrine disruptors (EDs) are either natural orartificial substances that may interfere with the body's endocrine system by acting like endogenous hormones and inducing adverse developmental, reproductive, neurological and immune effects (IPCS, 2002; Fernández-Blanco et al., 2016). A few studies suggest that FB1 may act as a potential ED (Collins et al., 1998; Gbore et al., 2012; Fernández-Blanco et al., 2016).

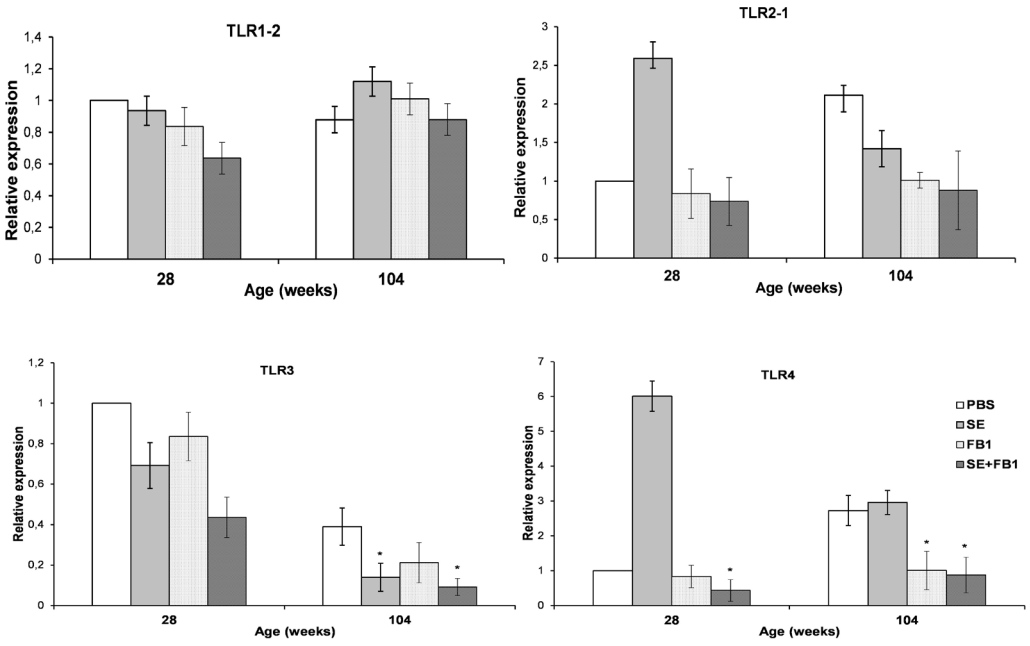


Fig. 1. Changes in the expression of TLR1-2, 2-1, 3 and 4 in the chicken ovary following SE infection and FB1 treatment. The mRNA expression levels were examined using quantitative real-time PCR analysis. Values represent the mean ± SEM (n=6). Asterisk indicates that the difference in the expression levels are significantly different (P<0.05).

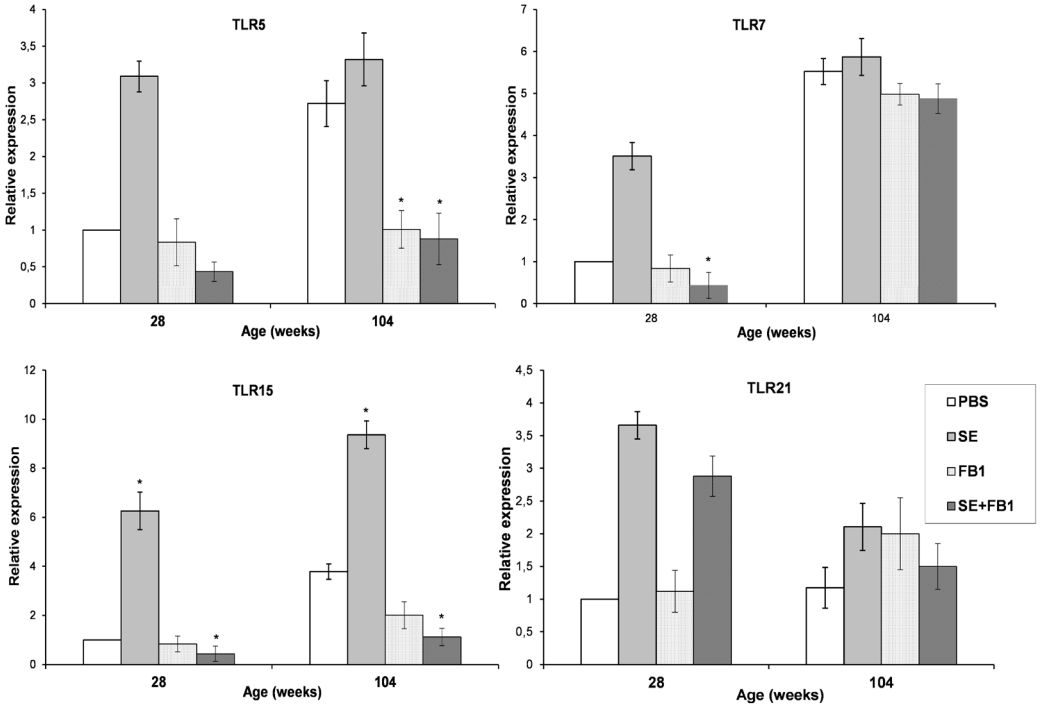


Fig. 2. Changes in the expression of TLR5, 7, 15 and 21 in the chicken ovary following SE infection and FB1 treatment. The mRNA expression levels were examined using quantitative real-time PCR analysis. Values represent the mean ± SEM (n=6). Asterisk indicates that the difference in the expression levels are significantly different (P<0.05).

While there is not enough data to confirm that FB1 is a developmental or reproductive toxicant in animals or humans, Collins et al., (1998)reported that FB1 was toxic to maternal rats and the foetus at the rate of 15 mg/kg of feed. In addition, Gbore (2009) reported that FB1 affected fertility in pigs by causing a delay in sexual maturity and poor sperm production and quality. Fumonisin B1 has also been associated with various diseases in animals, but cause different species-specific adverse effects in domestic animals, such as leukoencephalomalacia in horses, nephropathy in rabbits, immunosuppression, porcine pulmonary edema and hydrothorax, hepatocarcinoma, liver and kidney toxicity, hepatotoxic carcinogenic effects and liver cancer (Glenn et al., 2007; Albonico et al., 2016; Corrêa et al., 2018; Buszewska-Forajta 2020).

Furthermore, various studies have reported that animal feed contaminated with FB1can cause an array of metabolic, physiologic, and immunologic disturbances, resulting in decrease in humoral and cellular immunity response in farm animals (Bryden et al., 2012; Bondy and Pestka, 2000).Exposure to FB1, even at low doses, is related to sex-specific decrease of antibody following vaccination and increased susceptibility to pathogens (Marin et al., 2006; Pierron et al., 2016b).

The effects of FB1 in the ovary of domestic animals has not been extensively studied, nevertheless it seems that exposure of FB1,*in vitro*, have direct effects on ovarian cells, altering oocyte maturation, inhibiting granulosa cell proliferation, and changing the gonadotropin-supported steroid production (Gboreet al., 2012). In addition, *in vivo*, exposure of FB1 may alter follicular growth and atresia, ovulation, and puberty onset. In particular, ingestion of dietary FB1 at the rate of 10mg/kg or higher in rats was found to significantly reduce serum gonadotropin levels and lower fertility without inducing histopathological changes in the ovaries (Gbore 2012). Furthermore,*in vitro* studies demonstrated that FB1is able to directly influence GC proliferation and steroid production in different species, such as pigs (Ranzenigo et al., 2008; Caloni et al., 2009; Cortinovis et al., 2014) and cows (Pizzo et al., 2016).

Despite the plethora of studies that explained in details the effects of fumonisins in reproductive and immune parameters in farm animals, relevant information for poultry is rather scarce. Previous studies have reported that lymphocyte cell proliferation in chicks was lower when cells were exposed to FB1, while fumonisin induced apoptosis in turkey peripheral blood lymphocytes (Li et al., 1999; Dombrink-Kurtzman 2003). Although these studies indicate that FB1 has immunosuppressive activities further experiments are necessary to elucidate the effects of FB1 in chicken innate immune mechanisms.

It is now well established that the vertebrate innate immune system is the first line of defense against potential pathogens. One of the most critical components of the innate immune response is the Toll-like receptor (TLR) pathway. The Toll-like receptors are a group of membrane bound proteins characterized by a leucine-rich repeat extracellular domain and a Toll/IL-1R cytoplasmic domain.TLRs can recognize Gram-positive and Gram-negative bacteria, viruses, fungi, and parasites, and are activated by a variety of ligands derived from various microorganisms (Akira and Takeda 2004).

We have previously reported the expression of 8 members of the chicken TLR family in the chicken ovary and the changes in the expression levels of TLRs in the chicken ovary following SE infection (Michailidis et al., 2010). Quantitative real-time PCR analysis revealed that the ovarian mRNA abundance of TLRs differ with respect to sexual maturation. SE infection resulted in a significant induction of TLR4, and 15 in the ovary of sexual mature birds, and in a significant induction of TLR15 in the ovary of aged birds, while a significant down-regulation was observed for TLR3 in the ovary of aged birds. These findings suggest that a TLR-mediated immune response mechanism exists in the chicken ovary.In the present study we investigated the changes in the expression levels of TLR genes in the chicken ovary, following FB1 treatment and SE+FB1 treatment in an attempt to understand whether FB1 has immunosuppressive activity in the chicken reproductive organs. The data presented in this study reports the down regulation in the expression of 5 TLR genes expressed in the ovary *in vivo*,-following FB1 treatment and SE+FB1 treatment. To our knowledge, this is the first comprehensive study on the *in vivo* characterization of the effects of FB1 in the innate immune mechanism of the chicken reproductive tract, and demonstrates that FB1 has the ability to suppress innate immune responses in the chicken ovary, through down regulation in the expression levels of the certain TLR genes in the chicken ovary.

CONCLUSIONS

Collectively, the data provided in this study reveals that certain members of the chicken TLR family are down regulated in the chicken ovary *in vivo*, following FB1 and SE+FB1 treatment. These novel findings provide strong evidence to suggest that FB1 has an immunosuppressive activity in the chicken reproductive tract, through the alteration in the expression levels of key genes involved in the innate immune mechanism. Further experiments will be necessary in order to determine TLR activation and signalling patterns following treatmentswith mycotoxins in the chicken reproductive organs *in vivo*. A clear understanding of the effects of mycotoxins in poultry will contribute in the reduction of farm household economic loss and address the health concerns of people who consume poultry meat and egg products.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Akira S, Takeda K (2004) Toll-like receptor signalling. Nat Rev Immunol 4:499-511.

Albonico M, Schütz LF, Caloni F, Cortinovis C, Spicer LJ (2016). Toxicological effects of fumonisin B_1 alone and in combination with other fusariotoxins on bovine granulosa cells. Toxicon 118:47-53.

Bondy GS, Pestka JJ, (2000) Immunomodulation by fungal toxins. J Toxicol Environ Health Part B Critical Reviews 3:109-143.

Boutigny AL, Beukes I, Small I, Zühlke A, Spiteller M, Van Rensburg BJ, Flett B, Viljoen A (2012) Quantitative detection of *Fusarium* pathogens and their mycotoxins in South African maize. 61(3):522-531.

Bryden WL, (2012) Mycotoxin contamination of the feed supply chain: Implication of animal productivity and feed security. Anim Feed Sci and Tech 173:134-158.

Buszewska-Forajta M (2020) Mycotoxins, invisible danger of feedstuff with toxic effect on animals. Toxicon 182:34-53.

Caloni F, Ranzenigo G, Cremonesi F, Spicer LJ (2009) Effects of a trichothecene, T-2 toxin, on proliferation and steroid production by porcine granulosa cells. Toxicon 54:337-344.

Collins TF, Shackelford ME, Sprando RL, Black TN, Láborde JB, Hansen DK, Eppley M, Trucksess MW, Howard PC, Bryant MA, Ruggles DI, Olejnik N, Rorie JI (1998) Effects of Fumonisin B1 in Pregnant Rats. Food ChemToxicol 36:397-408.

Corrêa JAF, Orsoa PB, Bordinb K, VazHaraa R, Luciano FB (2018) Toxicological effects of fumonisin B_1 in combination with other Fusarium toxins. Food ChemToxicol 121:483-494.

Cortinovis C, Caloni F, Schreiber NB, Spicer LJ (2014) Effects of fumonisin B1 alone and combined with deoxynivalenol or zearalenone on porcine granulosa cell proliferation and steroid production. Theriogenology 81:1042-1049.

Dombrink-Kurtzman MA (2003) Fumonisin and beauvericin induce apoptosis in turkey peripheral blood lymphocytes. Mycopathologia 4:357-364.

Fernández-Blanco C, Frizzell C, Shannon M, Ruiz MJ, Connolly L (2016) An in vitro investigation on the cytotoxic and nuclear receptor transcriptional activity of the mycotoxins fumonisin B1 and beauvericin. Toxicol Lett 257:1-10.

Fink-Gremmels J (2008) The role of mycotoxins in the health and performance of dairy cows. Vet J 176(1):84-92.

Fung F, Richard C (2004) Health effects of mycotoxins: A toxicological overview. J Toxicol 42:217-234.

Garrido CE, Pezzani CH, Pacin A (2012) Mycotoxins occurrence in Argentina's maize (*Zea mays* L.), from 1999 to 2010. Food Control 25(2):660-665.

Gbore FA (2009) Growth performance and puberty attainment in growing pigs fed dietary fumonisin B1. J AnimPhysiolAnimNutr 93:761-767.

Gbore FA, Owolawi TJ, Erhunwunsee M, Akele O, Gabriel-Ajobiewe RAO (2012) Evaluation of the reproductive toxicity of dietary fumonisin B1 in rats. Jordan J. Biol. Sci. 5:183-190.

Glenn AE (2007) Mycotoxigenic Fusarium species in animal feed. Anim Feed SciTechnol 137:213-240.

Guard-Petter J, (2001) The chicken, the egg and *Salmonella enteritidis*. Enviromental Microbiology, 3(7):421-430.

Haque MA, Wang Y, Shen Z, Li X, Saleemi MK, Cheng He (2020) Mycotoxin contamination and control strategy in human, domestic animal and poultry: A review. MicrobPathog 142:104095.

International Programme on Chemical Safety (2002) Global Assessment of the State-of the-Science of Endocrine Disruptors. World Health Organization, Geneva.

Li YC, Ledoux DR, Bermudez AJ, Fritshe KL, Rottinghaus GE (1999) Effects of Fumonisin B1 on Selected Immune Responses in Broiler Chicks. Poult Sci 78:1275-1282.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-ΔΔC[T]) method. Methods 25:402-408.

Marin DE, Taranu I, Pascale F, Lionide A, Burlacu R, Bailly JD, Oswald IP (2006) Sex-related differences in the immune response of weanling piglets exposed to low doses of fumonisin extract. Br J Nutr 95:1185-1192.

Martins FA, Ferreira FMD, Ferreira FD, Bando E, Nerilo SB, Hirooka EY, MachinskiMJr (2012) Daily intake estimates of fumonisins in corn-based food products in the population of Parana, Brazil. Food Control 23:614-618.

Michailidis G, Theodoridis A, Avdi M (2010) Transcriptional profiling of Toll-like receptorsin chicken embryos and in the ovary during sexual maturation and in response to *Salmonella enteritidis* infection. AnimReprodSci122:294-302.

Moretti A, Pascale M, Logrieco AF (2019) Mycotoxin risks under a climate change scenario in Europe. Trends Food Sci Technol 84:38-40.

National Research Council (1985) Guide for the Care and Use of Laboratory Animals. Publication No. 85-23 (Rev.). Natl Institute of Health, Bethesda, MD.

National Research Council (1994) Nutrient Requirements of Poultry. 8th rev ed National Academy Press Washington DC.

Oswald IP, Marin DE, Bouhet S, Pinton P, Taranu I, Accensi F (2005) Immunotoxicological risk of mycotoxins for domestic animals. Food AdditContam 22(4):354-360.

Pierron A, Alassane-Kpembi I, Oswald IP (2016a) Impact of mycotoxin on immune response and consequences for pig health. AnimNutr 2:63-68.

Pierron A, Alassane-Kpembi I, Oswald IP (2016b) Impact of two myco-

toxins deoxynivalenol and fumonisin on pig intestinal health.Porc Health Manag 2:21.

Pizzo F, Caloni F, Schreiber NB, Cortinovis C, Spicer LJ (2016) In vitro effects of deoxynivalenol and zearalenone major metabolites alone and combined, on cell proliferation, steroid production and gene expression in bovine small-follicle granulosa cells. Toxicon 109:70-83.

Ranzenigo G, Caloni F, Cremonesi F, Aad PY, Spicer LJ (2008) Effects of Fusarium mycotoxins on steroid production by porcine granulosa cells. AnimReprodSci 107:115-130.

Rottinghaus GE, Olsen B, Osweiler GD (1982) Rapid screening method for aflatoxin B1, zearalenone, ochratoxin A, T-2 toxin, diacetoxyscirpenol and vomitoxin. In: Proceedings of the 25th Annual American Association of Veterinary Laboratory Diagnosticians, Nashville, TN: pp 477-484.

UkwuruMU, Ohaegbu CG, Muritala A (2018) An overview of mycotoxin contamination of foods and feeds. J BiochemMicrobToxicol 1:101

Yang C, Song G, Lim W (2020) Effects of mycotoxin-contaminated feed on farm animals. J Hazard Mater 389:122087.