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Feed exposure to FB1 can aggravate pneumonic damages in pigs provoked by *P. multocida*

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ABSTRACT

The possible interaction between *Pasteurella multocida* and the mycotoxin fumonisin B1 (FB1), recognised as one of the most often food/feed contaminant, was studied with the aim to evaluate whether and how FB1 can influence and/or complicate the development and severity of various pathological damages provoked by *Pasteurella multocida* in some internal organs of pigs. Heavier lung pathology was seen in pigs experimentally infected with *Pasteurella multocida*, when the same were exposed to 20 ppm dietary levels of fumonisin B_1 (FB_1) as was assessed by gross pathology, pathomorphological examinations, clinical biochemistry and some immunological investigations. The most typical damages in FB_1 treated pigs were the strong oedema in the lung and the slight oedema in the other internal organs and mild degenerative changes in the kidneys, whereas the typical pathomorphological findings in pigs infected with *Pasteurella multocida* was broncho-interstitial pneumonia. FB1 was found to aggravate pneumonic changes provoked by *P. multocida* in the cranial lobes of the lung and to complicate pneumonic damages with interstitial oedema in the lung. No macroscopic damages were observed in the pigs infected only with *Pasteurella multocida.* It can be concluded that the feed intake of FB1 in pigs may complicate or exacerbate the course of P. multocida serotype A infection.

1. Introduction

Mycotoxins are toxic secondary metabolites produced by certain fungi that grow in target agricultural products susceptible to mold infestation. The most mycotoxins have strong immunosuppressive effects and often compromise animals and human health provoking development of secondary bacterial infections (Stoev et al., 2000) or deteriorating some existing diseases (Pósa et al., 2011, 2013). It is well known, that Fumonisin B_1 (FB_1) is one of the most frequent contaminant of the feeds for pigs in all over the world (Dutton, 2009). In Hungary, FB_1 was found in relatively high percentage of maize used as animal feed (Fazekas et al., 1998).

FB_1 was found to be the cause of a number of outbreaks of equine leukoencephalomalacia (Conkova et al., 2003) and porcine pulmonary oedema killing many horses and pigs in the U.S. during 1989 and 1990 (Marasas, 2001). These lesions appeared to be a consequence of impairment of vascular permeability provoked by disruption of sphingolipid metabolism by FB_1 (Ramasamy et al., 1995). FB_1 is also suspected to be responsible for the pulmonary fibrosis in pigs, which develops in the case of chronic exposure and follows oedematous changes in the lung (Zomborszky-Kovács et al., 2002).

Some studies clearly confirmed that immunosuppression may be the first expressed toxic effect of some mycotoxins, such as ochratoxin A (Stoev et al., 2000). It was also found that FB_1 given at diet levels of 10 ppm, has immunosuppressive effect on humoral immune response in pigs (Stoev et al., 2012) and therefore can provoke some secondary bacterial infections, which arise in immunocompromised animals. In this regard, Oswald and collaborators observed a significant increase of intestinal colonization of pathogenic *Escherichia coli* in FB_1-treated pigs (Oswald et al., 2003). On the other hand, a heavy progression of *Pasteurella multocida* (Halloy et al., 2005) or porcine reproductive and respiratory syndrome (PRRS) virus infections in swine (Ramos et al., 2010) were seen when the same animals were compromised by feed exposure to FB_1. There are also some recent reports about aggravations of pathological infections in pigs, when fed on FB1 contaminated diet (Pósa et al.,

Abbreviations: BAL, Bronchoalveolar lavage; FB_1, fumonisin B1; PA, phagocytic activity; PBS, phosphate buffered saline; SA, sphinganine; SO, sphingosine; TNF, tumor necrosis factor.

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2011, 2013). Nevertheless, the data available about the possible interaction between FB_1 and some frequent infectious pathogens in pigs can be evaluated as insuficient and scarce. As FB1 is a frequent contaminant of pigs' diet, and *P. multocida* is the most frequent secondary pathogen which can generate a respiratory disorder in predisposed or immunocompromised pigs (Halloy et al., 2005), such studies could have significant scientific and/or practical importance. In this regard, the data on the predisposing effect of FB_1 on the appearance and/or progression of the lesions provoked by toxigenic strain of *P. multocida,* serotype A and the interaction between the toxin and the bacterium are scarce (Halloy et al., 2005), independently of the circumstance that both agents (toxic and infectious) have the same target organ localization (lung), and the same can be encountered simultaneously in the pig production farms.

It is also implied the problem of recognising the pathological damages provoked by FB_1 and/or the respective infectious agents, especially in the case when such damages are very similar or localized in the same internal organ such as lung. In our former experiment we found that feeding a diet containing 10 ppm FB_1, in combination with *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida* serotype D infection, increased the incidence and the extension of pulmonary lesions in pigs as well as aggravated the severity of the same lesions (Pósa et al., 2011). This experiment is the next step of our framework programme designed to reveal what can be the real adverse effects of FB_1 on various contagious or non-contagious diseases having similar pathology and whether the same mycotoxin is able to facilitate or complicate the typical pathological finding of these diseases. In the current experiment, we also studied the possible interaction of *Pasteurella multocida* with the mycotoxin FB_1, recognised respectively as one of the most often food/feed contaminant, with the aim to evaluate whether and how FB_1 can influence and/or complicate the development and/or severity of various pathological changes provoked by *Pasteurella multocida* in some internal organs of pigs.

2. Materials and methods

2.1. Experimental design

The piglets used in this study were obtained from a Seghers hybrid herd (Hungaro-Seghers Ltd., Mohács) in which the incidence of respiratory diseases was low and were free from five major infectious diseases (Brucellosis, Leptospirosis, Pasteurellosis, Aujeszky and porcine reproductive and respiratory syndrome – PRRS). The sows ($n = 10$) delivering the piglets were also serologically free from *Pasteurella multocida* and serologically negative for *M. hyopneumoniae*. Twenty eight 3-days old female piglets of 2.25 ± 0.3 kg average body weight, obtained from the same sows and received colostrum to get maternal (colostral) immunity were divided in four groups (7 piglets in each) using the principle of equality, and housed in two separate rooms in elevated-level battery cages of identical size as follow: (1) the two non-infected groups (Group A — controls and Group B — FB_1 exposed) were kept in one of the rooms in two separated cages, and (2) the infected with *Pasteurella multocida* groups (Group C and Group D — FB_1 exposed) were housed in the other room in a similar way. Both rooms were adjusted to identical air temperature (27 °C) using thermostat-regulated central heating, while the required air exchange was ensured by the use of exhaust fans. The battery cages, the drinkers and the rooms were cleaned twice a day, and the piglet-rearing installations were dismantled and washed off every second day. The respective hygienic and/or protective measures as wearing of protective clothes and foot & hand disinfection with the aqueous solution of Virkon S (KRKA d. d./Antec International Ltd., Novo Mesto, Croatia) were strictly respected at the time of entering or exiting the rooms.

2.2. Feeding and water supply

Up to day 16 from the birth all experimental piglets were fed on milk replacer consisting of skimmed milk powder, vegetable fats and whey powder, containing 23% crude protein, 23% crude fat and 1.6% lysine (Salvana Ferkel Ammen Milch, Salvana Tiernahrung GmbH, Klein-Offenseth Sparrieshoop, Germany) from an automatic feeder (Sloten B.V., Deventer, The Netherlands).

From day 7, a dry creep feed of coarse meal form, containing 16 MJ/kg energy, 18.5% crude protein, 9% crude fat and 1.65% lysine (Salvana Pre-meal, Salvana Tiernahrung GmbH, Klein-Offenseth Sparrieshoop, Germany) was also given *ad libitum* to the piglets, and then, from day 16 until the end of the experiment (day 40) only the latter was available to them.

Fresh drinking water was available *ad libitum* from nipple drinkers. At the beginning of the experiment (between day 0 and 7), drinking water was also provided by the free water surface of the plastics drinking bowls.

2.3. Mycotoxin exposure

The necessary quantity of FB_1 was produced by the fungus *Fusarium verticillioides* as described previously (Fodor et al., 2006) as the final fungal culture typically contained 3–4 mg/g FB_1, and small quantities of less toxic compounds FB_2 and FB_3 (0.3–0.6 mg/g). Starting from day 14, a defined quantity of the same fungal culture was thoroughly homogenised into the piglets' ration of groups B and D to give the required concentration of 20 ppm (mg/kg feed) FB_1 in the diet and these groups were exposed to the same feed levels of FB_1 until the end of the experiment (day 40), i.e. over a period of 26 days.

FB1 and FB2 concentration in the fungal culture and in the diet was checked using LC-MS system (LC-MS 2020 Single Quadrupole Mass Spectrometer, LC-20AD pumps with DGU-20A degasser, SIL-20ACHT autosampler, CTO-20-AC Column Owen and CBM-20A Interface, SHIMADZU, Kyoto, Japan), and the diet did not contain other mycotoxins (such as T-2, zearalenone, deoxynivalenol, ochratoxin A, aflatoxins, etc.) in detectable quantities. Concentration of T-2 and total aflatoxin was measured by ELISA kits, AgraQuant® T-2 Toxin Assay and AgraQuant® Total Aflatoxin Assay (RomerLabs, Singapur), respectively, following the instructions of the producer. The basic diet was also free from detectable quantities of the mycotoxins assayed.

2.4. Experimental infection

On day 14, the pigs of groups C and D were infected with toxigenic strain of *Pasteurella multocida* serotype A (strain DE3011, 4.4×10^8 CFU/mL). The bacterial suspensions were prepared as described previously (Magyar et al., 2002). A volume of 1.0 mL was inoculated through an endotracheal tube in all cases.

Microbiological investigations revealed no presence of other respiratory pathogens in the inoculum as well as in the lungs of the control and infected pigs at the end of the study except the main pathogen *Pasteurella multocida* found only in experimentally infected pigs.

2.5. Immunisation with ovalbumin

All piglets were injected intraperitoneally with 100 μg ovalbumin/animal on days 19 and 27 in order to provoke immune response. 100 μg ovalbumin was solved in 400 μL phosphate buffered saline (PBS, Sigma-Aldrich, Hungary) and thereafter 400 μL incomplete Freund adjuvant (Sigma-Aldrich, Hungary) was added.

2.6. Blood clinico-biochemical and immunological investigations

Blood samples were taken from the *v. cava cranialis* on days 14 (the beginning of the experiment), 27 and 39 after the respective narcosis and premedication as described below.

Serum clinical/biochemical parameters were determined in a professional veterinary laboratory (Vet-med Laboratory, Budapest, Hungary), using Roche Hitachi 912 Chemistry Analyzer (Hitachi, Tokyo, Japan)