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The effects of feed-borne *Fusarium* mycotoxins and glucomannan in turkey poults based on specific and non-specific parameters

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ABSTRACT

An experiment was conducted to investigate the effects of feeding grains naturally contaminated with *Fusarium* mycotoxins and a yeast derived glucomannan mycotoxin adsorbent (GMA) on selected specific and non-specific parameters in turkey poults. Two hundred and forty 1-day-old male turkey poults were fed the experimental diets for twelve weeks. Experimental diets were formulated with control grains, control grains + 0.2% GMA, naturally-contaminated grains, or naturally-contaminated grains + 0.2% GMA. Deoxynivalenol (DON) was the major contaminant of the contaminated grains and concentrations varied from 4.0 to 6.5 mg/kg in the contaminated diets. Non-specific parameters measured included: performance parameters, plasma biochemistry profiles, morphometry and $CD8^+$ T-lymphocyte counts in the duodenum. Plasma concentrations of DON and de-epoxydeoxynivalenol (DOM-1) were used as specific parameters. Performance parameters and plasma biochemistry were altered by the feeding of contaminated diets and GMA but this was not consistent throughout the trial. The feeding of contaminated diets reduced duodenal villus height and apparent villus surface area. This effect was prevented by GMA supplementation. The feeding of contaminated diets elevated total duodenal $CD8^+$ T-lymphocyte counts but this effect was not prevented by GMA. No significant differences were seen in plasma concentrations of DON and DOM-1 comparing birds fed contaminated and contaminated + GMA diets suggesting that GMA did not prevent DON absorption under these conditions.

1. Introduction

Mycotoxins are secondary metabolites produced by toxigenic fungal species. *Fusarium* fungi frequently infest crops in temperate regions such as Western Europe and North America. The produced mycotoxins (mostly trichothecenes, zearalenone (ZON) and fumonisins (Fig. 1)) can cause deleterious effects on animal health after oral intake of these toxins. Symptoms can vary from vomiting and feed refusal, to estrogenic effects and reduced performance, depending on the toxin and sensitivity of the animal species. In general, feed contamination with mycotoxins leads to important economic losses in animal production (Wu, 2007). A variety of methods to prevent the adverse effects of mycotoxins have been developed. Mycotoxin detoxifying agents (mycotoxin detoxifiers) are the most commonly used preventative method (Jard et al., 2011; Kolosova and Stroka, 2011). These detoxifiers can be classified as mycotoxin binders and mycotoxin modifiers. Mycotoxin binders adsorb the toxin in the gut, resulting in the excretion of toxin-binder complex in faces, whereas mycotoxin modifiers transform the toxin into non-toxic metabolites (Kolosova and Stroka, 2011).

Mycotoxin detoxifiers should be tested for their mycotoxin binding or degrading ability *in vitro* as well as *in vivo*. *In vitro* models are a powerful tool to screen and select a large number of compounds (Devreese et al., 2013). Only *in vivo* trials, however, can fully proof the efficacy of mycotoxin detoxifiers as mycotoxin adsorbents or biotransforming agents as *in vivo* studies are influenced by physiological variables and the composition of feed (Lemke et al., 2001). Mycotoxin detoxifiers are currently evaluated *in vivo* by non-specific parameters. Those include animal performance (e.g. growth rate, feed intake and feed conversion rate), plasma biochemistry (e.g. concentrations of proteins and minerals and enzyme activities), effects on immune function and tissue histological changes. As the criteria are non-specific, differences obtained between treated and untreated animals cannot be solely attributed to the efficacy of the detoxifier. There may be confounding effects such as immuno-modulating activity of β-glucans and antioxidant action

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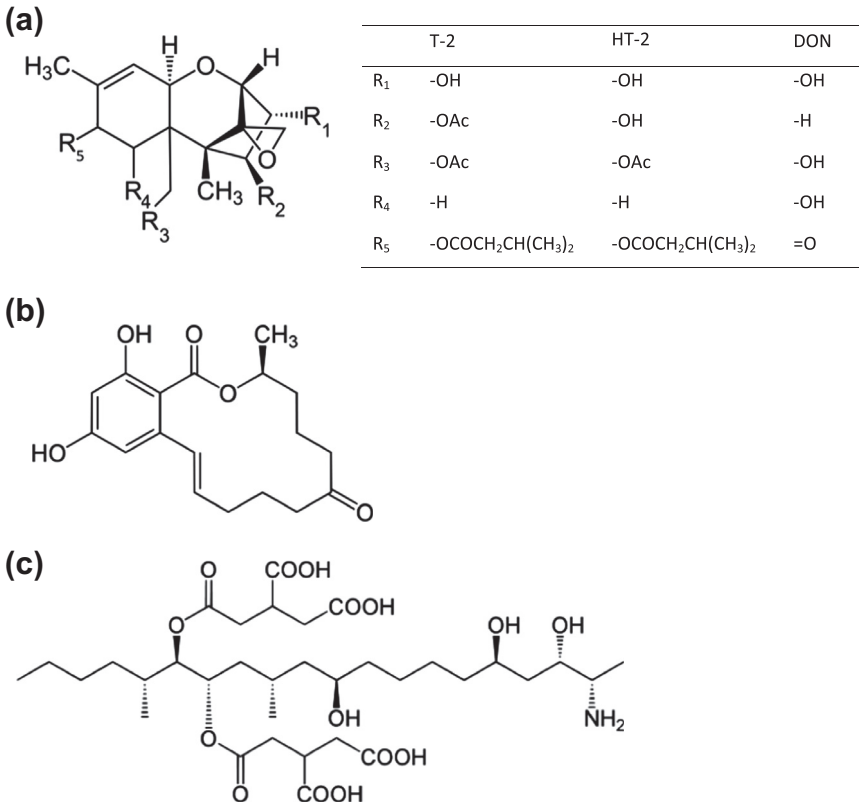

	T-2	HT-2	DON
R_1	-OH	-OH	-OH
R_2	-OAc	-OH	-H
R_3	-OAc	-OAc	-OH
R_4	-H	-H	-OH
R_5	$-OCOCH_2CH(CH_3)_2$	$-OCOCH_2CH(CH_3)_2$	=O

Fig. 1. Chemical structure of the most important *Fusarium* mycotoxins: trichothecenes (deoxynivalenol (DON), T-2 toxin (T-2) and HT-2 toxin (HT-2)) (a), zearalenone (ZON) (b) and fumonisin B1 (FB1) (c).

of other feed components. Due to the lack of specificity of these parameters, the European Food Safety Authority (EFSA) recently proposed other end-points based on specific toxicokinetic parameters (EFSA, 2010). As mycotoxin binders are deemed to adsorb mycotoxins in the gut, a lowered intestinal absorption is expected. According to the EFSA, the most relevant parameter to evaluate the efficacy of these products against mycotoxins is the plasma concentration of these toxins or their main metabolites (Devreese et al., 2012).

The goal of the present study was: (1) to determine the effects of diets naturally contaminated with *Fusarium* mycotoxins, mainly DON, on specific and non-specific parameters on turkey poults, and (2) to evaluate the efficacy of a yeast derived glucomannan mycotoxin binder (GMA). The selection of non-specific parameters for this trial was based on previous research (Girish and Smith, 2008; Girish et al., 2008; Girish et al., 2010; Yunus et al., 2012a; Yunus et al., 2012b). These included performance parameters, plasma biochemistry profile, intestinal morphometry and $CD8^+$ cell population in the duodenum. Specific parameters, plasma concentrations of DON and its main metabolite de-epoxydeoxynivalenol (DOM-1), were selected as advised by the EFSA (EFSA, 2010).

2. Materials and methods

2.1. Experimental birds and diets

Two hundred and forty-one-day-old male Hybrid turkey poults (Hybrid Turkeys, Kitchener, ON, Canada) were individually weighed and randomly distributed in 12 pens at the Arkell Poultry Research Station of the University of Guelph (Guelph, ON, Canada). Three pens were randomly assigned to each of the four different diets. The temperature and lighting programs were followed according to standard recommendations of the supplier. Birds were managed as has been prescribed by the Canadian Council on Animal Care with the Animal Utilization Protocols approved by the Animal Care Committee of the University of Guelph (CCAC, 2009). Four different corn-, wheat- and fish meal-based diets were formulated for each rearing phase including starter (0–3 w), grower (4–6 w), developer (7–9 w) and finisher (10–12 w). The diets met the standard nutritional specifications for turkey poults. The mycotoxin contaminated diets were formulated to the nutrient specifications of the control diets by replacing control corn with corn naturally contaminated with *Fusarium* mycotoxins. GMA-supplemented diets contained 0.2% GMA (Mycosorb®, Alltech Inc., Lexington, KY, USA).

2.2. Analysis of dietary mycotoxin concentrations

Dietary concentrations of DON, 3-acetyl-DON (3-aDON), 15-acetyl-DON (15-aDON), nivalenol (NIV), fusarenon-X (FUS-X), neosolaniol (NEO), diacetoxyscirpenol (DAS), T-2 toxin (T-2) and HT-2 toxin (HT-2) were analyzed using gas chromatography-mass spectrometry (GC–MS) according to Raymond et al. (2003). The limits of detection (LODs) were 0.06, 0.05, 0.05, 0.12, 0.11, 0.07, 0.06, 0.04 and 0.06 μg/g, respectively. Ochratoxin A (OTA) and zearalenone (ZON) concentrations were analyzed by high performance liquid chromatography with ultraviolet detection (HPLC-UV) (Wang et al., 2008). The LODs were respectively 0.2 and 20 ng/g. The sum of aflatoxin (AF) B1, B2, G1 and G2 concentrations and the sum of fumonisin B1 (FB1) and B2 (FB2) concentrations were analyzed by enzyme-linked immunosorbent assay (ELISA) and the LODs were 1.0 and 25.0 ng/g, respectively.

2.3. Body weight, feed intake and feed conversion ratios

Poults were individually weighed at placement and at the end of each growth phase. Feed consumption was measured for each pen at the end of each growth phase. Weight gain, feed intake, and feed conversion ratio (FCR) were calculated. Feed intake and FCR were adjusted for mortalities when necessary.

2.4. Plasma and tissue collection

At week 1 and at the end of starter phase (week 3), 4 birds per pen (12 birds/diet) were euthanized by cervical dislocation. Blood was sampled from the jugular vein prior to euthanasia. Blood was centrifugated (2851g, 4 °C, 10 min) and plasma was collected and frozen (⩽−15 °C) until analysis. Proximal and intermediary sections of the *duodenum descendens* were collected and flushed with 0.9% saline for immunohistochemistry and histology, respectively. For immunohistochemistry, tissue samples were embedded in Optimum Cutting Temperature (OCT) compound (Tissue-Tek, Sakura Finetek, Torrance, CA, USA) and snap frozen in liquid nitrogen. Frozen tissue samples were sectioned at 5 μm thickness, placed on Superfrost® Excell® glass slides (Fisher Scientific, Ottawa, ON, Canada) and stored at −20 °C until further staining. For histology, tissues were fixed in 10% neutral-buffered formalin