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Short communication

Effects of the *Fusarium* mycotoxin deoxynivalenol on *in vitro* rumen fermentation

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ABSTRACT

The objective of this study was to determine the effect of deoxynivalenol (DON) on *in vitro* rumen fermentation by assessing pH, ammonia-N, total gas, volatile fatty acid (VFA) production, and DON degradation under the context of two different carbon sources (corn starch or cellulose). Fifty millilitre of ruminal fluid:buffer (1:1) was incubated for up to 6 h with four possible treatments: corn starch 1.5 g, corn starch 1.5 g + DON at 40 mg/kg dry matter (DM), cellulose 1.5 g, and cellulose 1.5 g + DON at 40 mg/kg DM. Our results indicate that carbon source appears to markedly influence all rumen fermentative parameters ($P<0.05$), whereas DON negatively impacted certain aspects of rumen fermentative capacity, such as ammonia-N and total gas production, especially acetate and propionate production having been reduced ($P<0.01$). Therefore, it may be possible for carbon source to influence and limit the degree upon which DON's effects are exerted. DON degradation rate was especially influenced by carbon source context ($P=0.0105$) with cellulose leading to a higher rate of DON degradation than that of corn starch. This would indicate that concentrate/forage ratio, in the diet, may have an effect on DON degradation.

1. Introduction

Fusarium comprises of a large genus of filamentous fungi widely distributed in soil, and found in association with plants (May et al., 2000). Although most species are harmless, some produce mycotoxins found on cereal crops, affecting animal and human health, if allowed to enter into the food chain. As such, *Fusarium* is considered to be the most important toxigenic fungi, since they produce the most prevalent mycotoxins, including deoxynivalenol (DON) and zearalenone (Seeling et al., 2006). Ruminants appear to be relatively insensitive to the toxic effects of DON, due to the detoxifying effects of rumen microorganisms, which have been shown to metabolize DON to the less toxic metabolite, de-epoxy DON (Shima et al., 1997). Hence, rumen microbial metabolism has been considered to play a major role in DON detoxification (Volkl et al., 2004; Sasanya et al., 2008). Indeed, ruminants can tolerate 10–20 times more DON than pigs (Pestka, 2007), and it has been reported that the majority of ingested DON (up to 89 mg/100 mg DON) is converted into de-epoxy DON by the time it reaches the duodenum (Dänicke et al., 2005). Consequently, feeding DON-contaminated grain to ruminants is considered to be a useful means of utilizing grain that is unsuitable for humans or other livestock species. However, as mycotoxins

Abbreviations: A/P, acetate/propionate; DM, dry matter; DON, deoxynivalenol; VFA, volatile fatty acid.

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have antimicrobial properties, it is possible that they may alter microbial fermentation and nutrient utilization in ruminants (Dänicke et al., 2005; Seeling et al., 2006). Although there have been recent reports documenting the effect of *Fusarium* toxin-contaminated feed on performance, metabolism and immunity of dairy cows (Keese et al., 2008; Korosteleva et al., 2007, 2009), only a handful of publications have reported the effects of *Fusarium* toxin-contaminated feed on rumen fermentation to our knowledge. The range of DON previously used in experiments determining the effect of DON on performance and metabolism of cows has been as low as 3.5 mg/kg dry matter (DM) and as high as 66 mg/kg DM (Côté et al., 1986; Dänicke et al., 2005; Diaz, 2005; Seeling et al., 2006; Korosteleva et al., 2007, 2009).

The objective of this study was to determine the effect of DON at 40 mg/kg DM on *in vitro* fermentation by assessing rumen fermentation and DON degradation with either of two carbon sources, corn starch or cellulose.

2. Materials and methods

2.1. Chemicals

The DON used in our *in vitro* fermentation experiments was purified from cultured rice medium of *Fusarium graminearum* according to Clifford et al. (2003). The DON standard used for quantitative analysis, and all other chemicals, were purchased from Wako Pure Chemical Industries (Osaka, Japan). Milli-Q purified deionized water was used throughout our study.

2.2. In vitro fermentation experimental design

In vitro rumen fermentation was according to O'hara and Sugihashi (1972) at the Nippon Veterinary and Life Science University farm in Yamanashi. The four treatments were: corn starch alone, corn starch + DON at 40 mg/kg DM, cellulose alone, and cellulose + DON at 40 mg/kg DM. A Holstein dairy cow, used to obtain ruminal fluids, was fed with a diet of 12 kg timothy hay and 1 kg of a concentrate mixture twice daily (*i.e.*, morning and evening). The concentrate mixture contained 480 g/kg DM of corn, 190 g/kg DM of brans (corn gluten feed, wheat and rice bran), 180 g/kg DM of oil meals (soybean and rapeseed meal), and 150 g/kg DM of others (alfalfa meal, molasses, calcium carbonate, sodium chloride). Rumen fluid, 1.5 L, was obtained from the cow using a flexible stainless steel stomach tube before morning feeding, and passed through four layers of gauze to collect 1000 mL of filtrate, followed by diluting (1:1) with artificial saliva (McDougall, 1948). The diluted rumen fluid was anaerobically maintained by CO_2 gas for 10 min. The two carbon sources (1.5 g each), corn starch representing concentrate or cellulose representing forage, were added into the flasks (100 mL). Additionally, 1 mL of working DON solution (2 mg/mL in distilled water) was added to the flasks and adjusted to DON at 40 mg/kg DM as a starting concentration. After adding 50 mL of the dilute rumen fluid, flasks were sealed with a rubber stopper and incubated at 39.5 °C for 6 h.

2.3. Fermentation parameter analysis

Total gas was measured every 60 min by insertion of a glass syringe needle through the butyl rubber stopper, and the volume of gas exceeding 1 atmospheric pressure was measured through displacement of the syringe plunger. After 1, 3, and 6 h incubation, the bottles were uncapped and the resultant solution for each sample was centrifuged at 4000 × *g* for 5 min at 4 °C, and then supernatants were used for measurement of pH, ammonia-N, volatile fatty acid (VFA) and DON. The pH was measured with a pH meter (ATAGO digital pH meter, Tokyo, Japan). For VFA measurement, the sample solution was acidified with 5 N-H_2SO_4 containing 250 mg/mL metaphosphoric acid, and the VFA concentration was determined by gas chromatography (GC-2010; Shimadzu, Kyoto, Japan) using a Stabilwax-DA column (0.25 μm × 0.53 mm × 30 m) and flame-ionization detector (column temp. program; 93 °C (2 min)–0.5 °C/min–96 °C, injector temp. 230 °C and detector temp. 140 °C). The carrier gas (He) flow rate was 29.1 mL/min. Ammonia-N concentration was measured using a UV/VIS spectrophotometer (Beckman coulter DU530, Fullerton, CA, USA) after sample preparation using a kit of a modified Fujii-Okuda method (Wako Pure Chemical Industries).

For DON measurements, SPE cartridges were conditioned with 4 mL of methanol and 4 mL of Milli-Q water (Millipore, Billerica, MA, USA) prior to extraction of DON with Bond elute C18 SPE cartridges (Varian, Palo Alto, CA, USA). A 10 mL of third fractions were drawn through the cartridges at a flow rate of 5–10 mL/min. Cartridges were subsequently washed with 2.5 mL of Milli-Q water and dried. Finally, the DON was eluted with 1 mL acetonitrile and was kept at 4 °C until analysis. The liquid chromatographic system (LC-Prominence, Shimadzu, Kyoto, Japan) consisted of a binary pump, solvent degasser, auto-sampler and column oven. DON separation used an Inertsil ODS-SP column (5 μm, 75 mm × 4.6 mm ID, GL Science, Tokyo, Japan). The column flow rate was 1 mL/min, mobile phase was isocratic mode (acetonitrile:Milli-Q v/v 50:50), and the column temperature was 40 °C. A 20 μl sample volume was injected and detected at 220 nm.

2.4. Statistical analysis

All statistical analyses were performed using the PROC MIXED procedure with REPEATED statement of SAS (SAS v 9.1; SAS Inst. Inc., Cary, NC, USA). Fixed effects in the model were different carbon source, incubation time, DON, and the interaction