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Effects of dietary fumonisins on nutrients digestibility in weanling pigs

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The aim of the study was to ascertain the potential effects of fumonisins on nutrients' digestibility in the gastrointestinal tract. Eighteen (n= 6 x 3) weanling pigs from the age of 35 days were administered 0, 15 or 30 mg/kg dietary fumonisins (FB_1, FB_2 and FB_3) for a period of 21days, after an acclimatization period of 14 days. Titanium dioxide (0.5%) was added as indigestible marker to the feed and representative fecal samples were taken during a 5-day collection period in order to determine the digestibility of crude protein, crude fat, crude fiber, starch, ash, calcium, phosphorus and energy. The final body weights, cumulative feed intake and relative organ weights of all groups were were not influenced by treatment ($p>0.05$). Digestibility of energy, crude fiber, ash, calcium and phosphorus were significantly higher ($p<0.05$) in the control group relative to either 15 or 30 mg/kg treated pigs. These findings suggest that fumonisins in a dose of 15 or 30 mg/kg potentially distorts total tract nutrient digestibility in weanling pigs and thus, compromise the nutritive value of the mixed feed.

Keywords: fumonisins, pigs, gastrointestinal tract, nutrients digestibility

1 Introduction

The *Fusarium verticillioides* (Sacc.) is the major producer of fumonisins (FUM); a group of water-soluble secondary fungal metabolites of moulds commonly found in maize and maize-based products intended for both human and animal consumption. At least 28 FUM analogues have been characterized (Rheeder et al. 2002) namely the A-, B-, C- and P- series. The B-series FUM which includes FB_1, FB_2, FB_3, and FB_4 are the predominant analogues produced by wild-type isolates of *F. verticillioides* (Nelson et al. 1993). Amongst these, FB_1 is extensively reviewed due to its diverse toxicological characteristics which includes the notorious porcine pulmonary edema (PPE) and equine leukoencephalomalacia (ELEM) (Harrison et al., 1990), both of which are fatal to these animal species. Humans have also had their share on this fungal metabolite through the induction of oesophageal cancer and some neural tube defects (Gelineau-van Waes et al., 2009; Islami et al., 2009). Thus, falling in the group 2B (possibly carcinogenic) category of the International Agency for Research in Cancer (IARC) classifications (IARC, 2002). Structural analogy of FB_1 to the sphingoid bases sphingosine and sphinganine is the key mode for its toxicity. Through this, FB_1 is able to distort the enzyme ceramide synthase (a key enzyme in the *de novo*

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biosynthesis of sphingolipids) activities which subsequently leads to cellular disturbances in cell growth and differentiation, cell survival, and apoptosis (Merrill et al., 2001).

Just like other major existing mycotoxins, FUM cause several clinical disorders in most livestock species. However, the effect of FUM exposure on potential symptoms in gastrointestinal tract (GIT) till now, has not been thoroughly investigated. The pigs' gut nurtures several microbial species, produces several hormones, digests and absorbs a huge majority of nutrients, and is responsible for approximately 20% of body energy expenditure (Ewing, 2008; Choct, 2009). Thus, anything that hampers the normal functioning of the GIT is of utmost significance. The growing interest of intestinal health in the study of mycotoxins in recent years is due to how these ubiquitous substances have found the GIT as a newest functional target (Grenier & Applegate, 2013). Regarding FUM, the poor intestinal absorption of this mycotoxin in monogastric animal species (1 to 6 percentage; Bouhet & Oswald, 2007) suggests that, the gut epithelium is constantly exposed to significantly higher concentrations of FB_1 relative to other tissues (Grenier & Applegate, 2013).

Unfortunately, the amount of knowledge garnered on the biochemical/toxicological actions of FUM barely accentuates the causal mechanisms of FUM toxicity on nutrients digestion and absorption and gut morphology especially in *in vivo* trials. Younger farm animals like weaned piglets are mostly at risk due to the delicate nature of their gut when transitioning from liquid to solid feed i.e. the inescapable stress of weaning. Furthermore, reduced/compromised nutrient absorption at an early age may increase the incidence of intestinal disorders post-weaning and thus an overall reduction in animal performance.

In this study, we sought to evaluate the effects of FUM on total tract nutrients digestibility in weanling pigs exposed to either a diet containing no fungal culture of FUM (FB_1, FB_2 and FB_3), one containing 15 or another containing 30 mg FUM/kg feed for a period of 21 days.

2 Material and Methods

The research protocol was reviewed and authorized by the Animal Use and Care Administrative Advisory Committee and approved by the Agricultural Administrative Authority (Protocol SOI/31/00308-10/2017).

2.1 Animals, Experimental Diets and Methods

Eighteen male Danbred weanling pigs of 5 weeks of age, after an acclimatization period of 2 weeks (at exactly 7 weeks old) with body weights of 13.5 ± 1.3 kg were randomly assigned, in a completely randomized design to each of 3 diets (n=6). A commercial piglet feed was used as a basal diet complemented with FUM (FB_1, FB_2 and FB_3 in a fungal culture), either 15 or 30 mg/kg whiles the control diet was FUM free. Feed was offered as an amount that covers 2.5 times the maintenance energy requirement and was provided twice a day, in two equal portions. Refused feed was measured back every day. In Table 1 the result of the proximate analysis of the feed is given. Drinking water was available *ad libitum*. Piglets were kept in individual metabolic cages (80x80 cm) and weighed individually with g precision at the beginning of the trial and at weekly intervals. Piglets were monitored every day. The room temperature was adjusted according to the needs of the piglets and diarrhoea score was monitored throughout the trial.

A *Fusarium verticillioides* fungal culture of high FB_1 concentration; a culture named RL 596, according to Bartók et al. (2010) was mixed into the ration of the experimental animals, so as to provide a daily FUM (FB_1+FB_2+FB_3) feed concentration of 15 and 30 mg/kg. The mycotoxin concentration of the control and the experimental feed was determined with LC-MS (Shimadzu, Kyoto, Japan). The limit of detection (LOD) for FB_1 and FB_2 were 0.031 and 0.051 mg/kg respectively; LOD for FB_3 was not measured. The diet fed to the control group did not contain detectable amounts of FUM (complete absence of deoxynivalenol, zearalenone and T-2 toxin was as well controlled and confirmed). Fumonisin B contents were determined with a LC-MS method as described by Bartók et al. (2010).

Table 1 Nutrient content of the basal diet for weanling pigs

Crude protein (%)	17.50
Crude fat (%)	3.30
Crude fiber (%)	3.70
Crude ash (%) Starch (%)	5.00 41.8
Lysine (g/kg)	1.11
Methionine (g/kg)	0.37
Ca (g/kg)	0.65
P (g/kg) Na (g/kg)	0.50 0.18
DE (MJ/kg)	14.70
ME (MJ/kg)	14.10

2.2 Experimental Methods and Feed Analysis

The digestibility trial consisted of the inclusion of titanium dioxide (Ti_2O) at a level of 0.5% to the feed. During a 5-day collection period, representative fecal samples were taken 4 times a day (approximately 200g sample per day). The urine was collected separately in closed plastic containers. Fecal samples were weighed and frozen at -18 °C immediately after collection. At the end of the trial, piglets were euthanized by exsanguination after sedation (Euthanyl-Pentobarbital Sodium, 400 mg/mL, Dechra Veterinary Products, Shrewsbury, UK) in order to evaluate the effect of FUM exposure on visceral organs.

The chemical compositions of the diets and fecal samples collected were used to calculate the apparent total tract digestibility (ATTD) of crude protein, crude fat, crude fibre, starch, ash, calcium and phosphorus and determined according to the methods of AOAC (2000). The gross energy content of the feed and the fecal samples were determined by analysing duplicate samples using IKA-Calorimeter C4000 adiabatic bomb calorimeter with benzoic acid used as a standard.

2.3 Data Calculation and Statistical Analysis

Apparent total tract digestibility of crude protein, crude fat, crude fiber, starch, calcium, phosphorus and ash, as well as dietary energy was calculated using the Ti_2O concentration in feces and feed by using the equation:

$$\mathrm{ATTD} = 100 - (100 * \frac{\mathrm{Marker\_feed} * \mathrm{Nutr\_feces}}{\mathrm{Marker\_feces} * \mathrm{Nutr\_feed}})$$

where *Marker_feed* and *Marker_feces* are % of Ti_2O in the feed and in the feces, and *Nutr_feed* and *Nutr_feces* are nutrients (g/kg) and energy (kJ/g) in the feed and in the feces, respectively on dry matter basis.

Statistical analyses were performed using the SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Results were subjected to a one-way analysis of variance (ANOVA). In case of a significant treatment effect, the intergroup differences were checked by a Tukey post-hoc test. A p value <0.05 was described significant.

3 Results

3.1 Clinical Signs and Growth Rate

No mortality or diseases of any sort was observed during the 21-day long exposure of weanling pigs to either a control, 15 or 30 mg/kg FUM contaminated diet. There was diarrhea during the 14- day adaptation period but the piglets recovered before the start of the trial.

There were no significant differences ($p>0.05$) in the final body weights of pigs but numeric increments were observed in the 15 and 30 mg/kg dosed groups (data not shown). Except for one, all animals ate the daily ration, so generally, there was no feed refusal. A pig in treatment 30 mg/kg left some orts and therefore, its feed consumption was 95.5% of the others. In addition, there were no significant differences ($p>0.05$) in the relative kidney, liver and pancreas weights at the end of the trial (data not shown).

3.2 Nutrients Digestibility

The apparent total tract nutrient digestibility (ATTD) values in weanling pigs were higher for the control group as compared to those on the 15 and 30 mg/kg diets for each variable except for starch which remained the same (0.99) in all treatments (Table 2). Crude fiber, phosphorus, calcium and ash digestibility were significantly higher ($p<0.05$) in the control group relative to either the 15 or 30 mg/kg groups. The crude fat digestibility albeit did not differ significantly ($p>0.05$) in different treatments, but appeared numerically higher in the control group as compared to the two FUM treated groups. As a result of the difference in fiber and the shift in fat digestibility, the total tract digestibility of dietary energy was also compromised by the FUM exposure.

Table 2 Apparent total tract digestibility (ATTD, g/g) of weanling pigs fed with different levels of dietary fumonisins (data are means ± standard deviation (SD) of 6 piglets/group)

ATTD, g/g	Control	15 mg/kg	30 mg/kg
Crude Protein	0.84 ± 0.02	0.83 ± 0.01	0.82±0.02
Crude fat	0.64 ± 0.07	0.56 ± 0.09	0.57±0.10
Crude fiber	0.46 ± 0.03^{b}	0.40 ± 0.03^{a}	0.41±0.03^{a}
Ash	0.59 ± 0.03^{b}	0.53 ± 0.02^{a}	0.54±0.02^{a}
Calcium	0.65 ± 0.07^{b}	0.56 ± 0.03^{a}	0.54±0.07^{a}
Phosphorus	0.68 ± 0.03^{b}	0.63 ± 0.01^{a}	0.60±0.04^{a}
Starch	0.99 ± 0.00	0.99 ± 0.00	0.99 ± 0.00
Digestible energy (DE)	0.83 ± 0.011^{b}	0.82 ± 0.007^{a}	0.82 ± 0.009^{ab}

^{ab}: Means on same row with different superscripts differ significantly. $P<0.05$ is described significant.

4 Discussion

The present study was designed to evaluate the effects of FUM on the fecal digestibility of nutrients in weanling pigs. According to the scientific literature, mycotoxins challenge typically and frequently impairs growth and productive performance in most examined animal species. Yet, our results revealed no such physiological disorders on the weanling pigs fed either a 15 or 30 mg FUM/kg feed. Corroborating to our results is that of Tóth and co-workers (2000) who reported no remarkable effect of an even much higher dosage of dietary fumonisin B1 (FB_1; 40 ppm) on feed intake and body weight gain of pigs, although pigs developed strong porcine pulmonary edema.

Regardless, it seems that the FUM managed to interfere with the nutrients digestion of the pigs. In line with this, the establishment that mycotoxins, including FUM are capable of inducing non-specific GIT hypo-functionality in animals is attested (Gbore et al., 2010). Although the rate of digestibility of crude protein, crude fat and starch was unperturbed by FUM intoxication, the decline in digestibility values of calcium, phosphorus and ash suggests a potentially distorted absorptive functions along the gut of the pigs. Concurring to this outturn, a sub-chronic study by Gbore et al. (2010) revealed a remarkable

reduction in digestibility values of wistar rats exposed to increasing levels of FB_1 (0.2, 10 or 20 mg/kg feed) albeit along with this outcome, the authors observed a negative impact on growth performance. Moreover, the decline in crude fiber digestibility signifies a possible shift in the composition of the residential microbes that harbor the gut of the pigs since dietary fiber is a key player in the state of existence of intestinal microbes.

Furthermore, even though digestibility of crude fat did not differ statistically, the observed numeric decline in its digestibility values in both treatment groups suggests a possible interference in the lipid metabolic pathways of the pigs; the major toxicity pathway of FUM widely established. In slight attestation, Gbore & Egbunike (2007) observed, in a dose-response fashion, a decrease in crude fat and crude protein digestibility when an increasing dose of dietary FB_1 (0.2, 5.0, 10 and 15 mg/kg) was fed to growing pigs in a 6-month feeding trial. In this regard, an appropriate suggestion could be that our 21-day long dietary FUM exposure period was not enough to provide reliable difference in fat digestibility even in a dosage of 30mg/kg feed.

5 Conclusion

This study has proven a detrimental effect of dietary FUM on the nutritive value of the feed for weanling pigs. In reference to the above findings, it is proper to conclude that, increasing levels of dietary FUM up to 30 mg/kg for a period of 21 days potentially distorts nutrients, particularly fiber and minerals as well as energy digestion and absorption in the GIT of weanling pigs without necessarily depressing growth performance.

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References

Association of Official Analytical Chemists. (2000). *AOAC International, Gaithersburg*, 17. ed MD, USA.

Bartók, T. et al. (2010). Detection and characterization of twenty-eight isomers of fumonisin B1 (FB_1) mycotoxin in a solid rice culture infected with *Fusarium verticillioides* by reversed-phase high-performance liquid chromatography/electrospray ionization time-of-flight and ion trap mass spectrometry. *Rapid Communications in Mass Spectrometry*, 24(1), 35–42.

Bouhet, S. and Oswald, I.P. (2007). The intestine as a possible target for fumonisin toxicity. *Molecular Nutrition and Food Research,* 51(8), 925–931.

Choct M. (2009). Managing gut health through nutrition. *British Poultry Science,* 50(1), 9–15.

Ewing, W.N. (2008). *The Living Gut*. 2. ed. Nottingham, UK: Nottingham University Press.

Gbore, F.A., Yinusa, R.I. and Salleh, B. (2010). Evaluation of sub-chronic dietary fumonisin B1 on nutrient digestibility and growth performance of rats. *African Journal of Biotechnology*, 9(38), 6442–6447.

Gbore, F.A. (2007). Effect of dietary fumonisin B1 on histomorphology and histopathology of organs of pubertal boars. *American-Eurasian Journal of Scientific Research,* 2, 75–79.

Gbore, F.A. and Egbunike, G.N. (2007). Influence of dietary fumonisin B_1 on nutrient utilization by growing pigs. *Livestock Research for Rural Development, Volume19, Article #93.* Retrieved June 5, 2020 from http://www.lrrd.org/lrrd19/7/gbor19093.htm

Gelineau-van Waes, J. et al. (2009). Maternal fumonisin exposure as a risk factor for neural tube defects. *Advances in Food and Nutrition Research,* 56, 145–181.

Grenier, B. and Applegate, T. (2013). Modulation of intestinal functions following mycotoxin ingestion: Meta-analysis of published experiments in animals. *Toxins,* 5(2), 396–430.

Harrison, L.R. et al. (1990). Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of *Fusarium moniliforme*. *Journal of Veterinary Diagnostic Investigation,* 2(3), 217–221.

International Agency for Research on Cancer (IARC) (2002). Working group on the evaluation of carcinogenic risks to humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monographs on the evaluation of carcinogenic risks to humans,* 82, 1–556.

Islami, F. et al. (2009). Oesophageal cancer in Golestan Province, a high-incidence area in northern Iran - a review. *European Journal of Cancer,* 45(18), 3156–3165.

Merrill, A. H. et al. (2001). Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. *Environmental Health Perspect*ives, 109(2), 283–289.

Nelson, P.E., Desjardins, A.E. and Plattner, R.D. (1993). Fumonisins, mycotoxins produced by Fusarium species: biology, chemistry, and significance. *Annual Review of Phytopathology,* 31, 233–252.

Rheeder, J.P., Marasas, W.F. and Vismer, H.F. (2002). Production of fumonisin analogs by Fusarium species. *Applied and Environmental Microbiology,* 68(5), 2101–2105.

SPSS. (2012). SPSS for Windows version 20, SPSS: Chicago, IL, USA.

Tóth, Á. et al. (2000). Effect of low doses of the mycotoxin fumonisin B1 on the body weight gain, feed intake and feed conversion rate of pigs. *Agriculture,* 6, 149–151.