

## EFFECTS OF NATURALLY PRODUCED DIETARY *FUSARIUM* MYCOTOXINS ON WEANING PIGS

<sup>1</sup>Shin, S.Y., <sup>1</sup>C. Kong, <sup>2</sup>I.H. Kim and <sup>1</sup>B.G. Kim

<sup>1</sup>Department of Animal Science and Technology, Konkuk University, Seoul, Republic of Korea

<sup>2</sup>Department of Animal Resource and Science, Dankook University, Cheonan, Choongnam, Republic of Korea

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### ABSTRACT

Mycotoxins reduce animal productivity and animal health. The influence of *Fusarium* mycotoxins in corn co-products on pig performance is an important issue in swine feed industry. This study was conducted to determine the effects of naturally produced *Fusarium* mycotoxins in Corn Gluten Meal (CGM) on growth performance of nursery pigs. A total 96 weaning pigs comprising 48 gilts and 48 barrows with an initial body weight of 5.08 kg (SD = 1.28) were grouped into 3 blocks in each sex by initial body weight and randomly allotted to 4 treatments in a randomized complete block design. There were 6 replicate pens per treatment and 4 pigs were housed in each pen. The 4 experimental diets mainly based on corn, CGM, dried whey and soybean meal were formulated to contain 4 concentrations of mycotoxins derived from the contaminated CGM. Diet 1 contained 32 µg kg<sup>-1</sup> Deoxynivalenol (DON) and 6 µg kg<sup>-1</sup> Zearalenone (ZON) and diets 2, 3 and 4 contained 532, 1,033 and 1,534 µg kg<sup>-1</sup> DON and 203, 399 and 596 µg kg<sup>-1</sup> ZON, respectively. During the first 14 d of experiment, Average Daily Gain (ADG) was reduced linearly and quadratically ( $p < 0.05$ ) as concentration of dietary mycotoxin increased. Average Daily Feed Intake (ADFI) had a tendency for quadratic decrease ( $p = 0.059$ ) with increasing dietary mycotoxin concentrations. Both ADG and ADFI from d 14 to 28 linearly decreased with increasing concentration of mycotoxins ( $p < 0.05$ ). During the overall experimental period, both ADG and ADFI linearly depressed with increasing concentration of mycotoxins ( $p < 0.05$ ). In conclusion, the current study showed that dietary *Fusarium* mycotoxins derived from contaminated CGM by *Fusarium* fungi resulted in decreased growth performance of nursery pigs. Swine nutritionists may increase nutrient concentrations of diets to partially overcome the negative effects of *Fusarium* mycotoxins in corn co-products on feed intake of pigs.

**Keywords:** Deoxynivalenol, Zearalenone, Growth Performance, Swine

### 1. INTRODUCTION

Mycotoxins are toxic secondary metabolic byproducts of fungal genera and these toxins are known to be detrimental to animal productivity as well as animal health due to their toxicity. Among several genera of mycotoxin-producing fungi such as *Aspergillus*, *Fusarium* and *Penicillium*, *Fusarium* genus fungi commonly grow on corn, wheat and other grains (Pollmann *et al.*, 1985; Munkvold and Desjardins, 1997) and produce several detrimental toxins such as aflatoxins, Deoxynivalenol (DON), Zearalenone (ZON) and fumonisins.

There are a number of studies that have shown the harmful effects of DON (Friend *et al.*, 1983; Smith *et al.*, 1997; Waché *et al.*, 2009) and ZON (Young *et al.*, 1981; Wang *et al.*, 2012) on growth performance of pigs. Deoxynivalenol, also known as vomitoxin, generally damages the digestive system of pigs causing vomiting or feed refusal (Trenholm *et al.*, 1994) while ZON disturbs the reproductive system of female pigs and consequently reduces sow performance (Döll *et al.*, 2003; Papatsiros, 2012). Some studies carried out the experiments using corn (Young *et al.*, 1981; Rotter *et al.*, 1995) or wheat (Friend *et al.*, 1983; Waché *et al.*, 2009)

**Corresponding Author:** B.G. Kim, Department of Animal Science and Technology, Konkuk University, Seoul, Republic of Korea Tel: +82-2-2049-6255

as a natural source of mycotoxin. However, there is limited information on the effects of DON and ZON in corn co-products contaminated by *Fusarium* fungi. Thus, the aim of this study was to determine detrimental effects of dietary *Fusarium* mycotoxins naturally contaminated in Corn Gluten Meal (CGM) on nursery pigs.

## 2. MATERIALS AND METHODS

### 2.1. Feedstuffs and Diets

Two different sources of CGM were prepared for the experiment; a CGM source that was naturally contaminated by *Fusarium* genus fungi and a CGM source that was not contaminated. The concentrations of DON and ZON were 6,390 and 2,483  $\mu\text{g kg}^{-1}$ , or 132 and 24  $\mu\text{g kg}^{-1}$  in the contaminated or the control CGM, respectively.

The experimental diets were provided in a 2-phase feeding program with phase I and II diets being fed for 2 wk for each period. The diet 1 was formulated to meet or exceed nutritional requirement of weaning pigs (NRC, 2012) and was mainly based on corn, the control CGM, dried whey and soybean meal (**Table 1**). The mycotoxin-

containing diet 2, 3 and 4 were prepared to contain 80, 160 and 240  $\text{g kg}^{-1}$  of the contaminated CGM at the expense of the control CGM, respectively.

### 2.2. Animals and Feeding

The experimental procedure was approved by the Institutional Animal Care and Use Committee at Konkuk University. A total of 96 weaning pigs comprising 48 gilts and 48 barrows with an initial Body Weight (BW) of 5.08 kg (SD = 1.28) was used in the experiment. Pigs were grouped into 3 blocks in each gender based on initial BW and randomly allotted to 4 treatments in a randomized complete block design. There were 6 replicate pens per treatment and 4 pigs were housed in each pen. The pens were equipped with a two-hole stainless steel feeder, a nipple drinker and fully slatted plastic floor. Diets and water were freely available for 28 d of the entire experimental period.

Individual BW and feed consumption were measured on a pen basis on d 14 and 28. Based on these records, the growth performance including Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and gain to feed ratio (G:F) were calculated.

**Table 1.** Ingredient composition and amount of mycotoxins of experimental diets (as-fed basis)

Ingredient <sup>a</sup> (g/kg)	Phase I				Phase II			
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 3	Diet 4
Corn	387.6	387.6	387.6	387.6	499.5	499.5	499.5	499.5
CGM, contaminated	0.0	80.0	160.0	240.0	0.0	80.0	160.0	240.0
CGM, control	240.0	160.0	80.0	0.0	240.0	160.0	80.0	0.0
Dried whey	200.0	200.0	200.0	200.0	100.0	100.0	100.0	100.0
Soybean meal	100.0	100.0	100.0	100.0	90.0	90.0	90.0	90.0
SDPP	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Limestone	13.5	13.5	13.5	13.5	13.0	13.0	13.0	13.0
Dicalcium phosphate	6.5	6.5	6.5	6.5	5.5	5.5	5.5	5.5
L-Lysine-HCl	5.4	5.4	5.4	5.4	5.0	5.0	5.0	5.0
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Mineral premix <sup>b</sup>	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix <sup>c</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
<b>Mycotoxin<sup>d</sup> (<math>\mu\text{g/kg}</math>)</b>								
DON	32.0	532.0	1,033.0	1,534.0	32.0	532.0	1,033.0	1,534.0
ZON	6.0	203.0	399.0	596.0	6.0	203.0	399.0	596.0

<sup>a</sup>CGM = Corn Gluten Meal; SDPP = Spray Dried Plasma Protein

<sup>b</sup>Provided as milligrams per kilogram of diet: 4.16 of Zn from zinc sulfate; 10.95 of Fe from iron sulfate; 6.29 of Mn from manganese sulfate; 6.31 of Cu from copper sulfate; 0.08 of I from calcium iodide; 0.02 of Se from sodium selenite; 0.04 of Co from cobalt sulfate

<sup>c</sup>Provided per kilogram of diet: 25,000 IU of vitamin A; 40 IU of vitamin E; 85 mg of vitamin C; 40 mg of niacin

<sup>d</sup>Calculated value in micrograms per kilogram of diet based on analyzed mycotoxin concentration in CGM

### 2.3. Mycotoxin Analysis

Analyses of AFLatoxin (AFL), DON, Fumonisin B1 (FB1), Ochratoxin A (OTA) and ZON were carried out by HPLC at Romer Labs (Romer Labs Singapore Pte Ltd, Singapore). Two major mycotoxins, DON and ZON, were analyzed by using enzyme-linked immunosorbent assay (ELISA) kits (AgraQuant®, Romer Labs Inc. Singapore) which had a detection limits at 200 and 20 µg kg<sup>-1</sup> for DON and ZON, respectively.

### 2.4. Statistical Analysis

Data were analyzed by analysis of variance using generalized linear model procedure (SAS Inst. Inc., Cary, NC). The model included dietary treatment, sex and block as independent variable. Least squares means of each treatment were calculated for each variable. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of increasing dietary mycotoxin concentrations on the growth performance of pigs. A pen was the experimental unit and the statistical significance was declared at an alpha-level of 0.05. Probability values between 0.05 and 0.10 were considered tendency towards a difference.

### 3. RESULTS

The mycotoxin concentrations in the control CGM and the contaminated CGM are presented in **Table 2**. The concentrations of AFL in both CGM samples were below 1 µg kg<sup>-1</sup> whereas the other toxin contents were greater in the contaminated CGM than in the control CGM. The concentration of DON and ZON in the contaminated CGM determined by ELISA method fairly corresponded to values derived from HPLC method.

**Table 2.** Analyzed concentration of mycotoxins from 2 sources of corn gluten meal (as-is basis)

Item <sup>a</sup>	CGM, control		CGM, contaminated	
	HPLC <sup>b</sup>	ELISA	HPLC	ELISA
DON	132	105	6,390	4,441
ZON	24	6	2,483	2,998
FB1	450	- <sup>c</sup>	2,911	-
OTA	14	-	24	-
AFL	<1	-	<1	-

<sup>a</sup>µg kg<sup>-1</sup>; DON = deoxynivalenol; ZON = zearalenone; FB1 = fumonisin B1; OTA = ochratoxin; AFL = aflatoxin.

<sup>b</sup>HPLC = high performance liquid chromatography; ELISA = enzyme-linked immunosorbent assay.

<sup>c</sup>Not analyzed.

**Table 3.** Growth performance of pigs fed diets containing 4 concentrations of mycotoxins

Item	Dietary number				SEM <sup>b</sup>	P-value	
	1	2	3	4		Linear	Quadratic
<b>Mycotoxin<sup>c</sup> (µg kg<sup>-1</sup>)</b>							
Deoxynivalenol	32.000	532.000	1,033.000	1,534.000			
Zearalenone	6.000	203.000	399.000	596.000			
<b>Body weight (kg)</b>							
D 0	5.120	5.040	5.130	4.970	0.09	0.391	0.689
D 14	11.610	11.280	11.200	11.080	0.08	0.006	0.198
D 28	15.130	14.660	14.460	14.320	0.11	<0.001	0.154
<b>Day 0 to 14</b>							
ADG, g d <sup>-1</sup>	273.000	259.000	250.000	257.000	3.96	0.005	0.018
ADFI, g d <sup>-1</sup>	336.000	319.000	321.000	324.000	4.86	0.134	0.059
Gain:feed, g g <sup>-1</sup>	0.814	0.814	0.782	0.794	0.02	0.225	0.701
<b>Day 14 to 28</b>							
ADG, g d <sup>-1</sup>	442.000	428.000	416.000	411.000	4.03	<0.001	0.310
ADFI, g d <sup>-1</sup>	546.000	539.000	531.000	524.000	5.37	0.007	0.923
Gain:feed, g g <sup>-1</sup>	0.809	0.794	0.784	0.784	0.01	0.032	0.377
<b>Day 0 to 28</b>							
ADG, g d <sup>-1</sup>	357.000	3440.000	333.000	334.000	2.66	<0.001	0.014
ADFI, g d <sup>-1</sup>	441.000	429.000	426.000	424.000	3.48	0.003	0.154
Gain:feed, g g <sup>-1</sup>	0.810	0.801	0.783	0.788	0.01	0.021	0.356

<sup>a</sup>Each least squares of means represent 6 pens of 4 pigs per pen.

<sup>b</sup>Standard error of the means

<sup>c</sup>Calculated value in micrograms per kilogram of diet based on analyzed mycotoxin concentration in corn germ meal

Dietary *Fusarium* mycotoxins negatively affected growth performance of weaning pigs (**Table 3**). During the first 14 d of the experiment, ADG decreased linearly and quadratically ( $p < 0.05$ ) as mycotoxin concentration increased. The ADFI tended to be affected by dietary treatments (quadratic,  $p = 0.059$ ). During d 14 to 28, ADG ( $p < 0.001$ ), ADFI ( $p = 0.007$ ) and gain: Feed ( $p = 0.032$ ) decreased linearly as mycotoxin concentrations increased, showing that there were reductions of ADG, ADFI and gain: Feed by 7.0, 4.0 and 3.1%, respectively, in the highest concentration compared with the lowest concentration of mycotoxins. During the overall period, ADG, ADFI and gain: Feed decreased linearly ( $p < 0.05$ ) as mycotoxin concentration increased.

#### 4. DISCUSSION

In the current study, DON and ZON were major mycotoxins in the contaminated CGM. European Union commission suggests the guidance values of DON, ZON, OTA and FB1 for typical swine diets at 0.9, 0.25, 0.05 and 5 mg kg<sup>-1</sup>, respectively (EC, 2006). This indicates that the concentrations of both DON and ZON in diet 3 and 4 used in the present experiment were above the guidance values of (EC, 2006).

Fumonisin B1 in the ingredients used in the current study also existed at the concentration of 2,911 and 450 µg kg<sup>-1</sup> for the contaminated and control CGM, respectively and concentration was diluted at 108, 305, 502 and 699 µg kg<sup>-1</sup> in diets 1, 2, 3 and 4, respectively. According to Zomborszky-Kovács *et al.* (2002), 1,000 µg kg<sup>-1</sup> of FB1 was considered a tolerable concentration for pigs, thus the concentration of FB1 in the current study would not be the major cause for the reduction in growth performance of pigs.

The overall reductions in ADG and G: F of pigs fed diet 4 compared with diet 1 were 6.4 and 2.7%, respectively. The respective values were within ranges that were predicted with collective data from the literature (Mok *et al.*, 2013). Decreased ADG observed in the present experiment appears to be mainly affected by the reduced feed intake but not directly by mycotoxins. Andretta *et al.* (2012) also suggested that the degeneration of weaning pigs' growth was due to reduced feed intake, but not by presence of mycotoxin *per se*. In agreement, several reports have been shown that dietary DON adversely affected growth performance of pigs by reducing feed intake (Young *et al.*, 1983; Smith *et al.*, 1997; Waché *et al.*, 2009). Reduced feed intake caused by DON would be attributed to reduced hepatic protein synthesis which causes raised blood tryptophan, a precursor of serotonin and subsequently

elevated brain serotonin concentrations (Díaz-Llano and Smith, 2006). The elevated concentration of serotonin consequently would be responsible for anorexic effects of DON (Leathwood, 1987).

In contrast, the effects of ZON on ADFI of pigs were not always significant. James and Smith (1982) reported that ZON up to 40 mg kg<sup>-1</sup> did not affect feed consumption of pigs and this result was in agreement with other reports that suggested no adverse effect of ZON on feed intake when gilts or weaning pigs were fed 1 to 2 mg kg<sup>-1</sup> of ZON (Rainey *et al.*, 1990; Jiang *et al.*, 2012). In addition, dietary ZON may negatively affect feed intake when combined with dietary DON, but not ZON solely (Young *et al.*, 1981; Williams and Blaney, 1994).

#### 5. CONCLUSION

In conclusion, the present study showed that dietary *Fusarium* mycotoxins derived from contaminated CGM by *Fusarium* fungi resulted in decreased the growth performance of pigs which could be mainly attributed to the negative effects of DON on feed consumption of pigs. The mechanisms contributing the main action in DON are still unclear. Further research is warranted to elucidate the mode of actions by which dietary DON reduces feed intake.

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