



Original research article

Impacts of low level aflatoxin in feed and the use of modified yeast cell wall extract on growth and health of nursery pigs



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ABSTRACT

This study was to investigate the effect of corn naturally contaminated with aflatoxins (AF) under the regulatory level on the growth performance and health of nursery pigs, and the efficiency of yeast cell wall based feed additive (YC) mainly composed of β -glucans and mannan oligosaccharide (MOS) (Integral A+, Alltech, Lexington, KY) in prevention of mycotoxicosis. Pigs (60 barrows and 60 gilts at 6.02 ± 0.83 kg BW) were randomly allotted to 4 treatments in a randomized complete block design based on a 2×2 factorial arrangement with 10 pens (5 barrow and 5 gilt pens) per treatment and 3 pigs per pen. Pigs were fed experimental diets for 5 wk. First factor was AF (0 or 20 μ g/kg in feed) and the second factor was YC (0 or 2 g/kg in feed). Feed intake and body weight were measured weekly, and blood samples were used to measure blood cell counts, immunoglobulin G (IgG), tumor necrosis factor- α (TNF- α), oxidative damage status, and serological evaluation related to liver health. Aflatoxin decreased ($P < 0.05$) the number of platelet count (247.4 to $193.5 \times 10^3/\mu$ L), and it also tended to increase the level of albumin ($P = 0.055$, 3.46 to 3.63 g/dL), albumin:globulin ratio ($P = 0.050$, 2.09 to 2.37), and Ca ($P = 0.080$, 10.79 to 10.97 mg/dL). Yeast cell wall based feed additive increased ($P < 0.05$) ADG (493 to 524 g/d), and ADFI (796 to 846 g/d) of pigs whereas G:F was not affected, and it also tended to increase ($P = 0.055$) albumin level (3.46 to 3.63 g/dL). Interactions ($P < 0.05$) on hemoglobin, hematocrit, and platelet count indicated that YC further increased their levels when pigs were eating AF contaminated feed. Interactions ($P < 0.05$) on urea nitrogen and blood urea N to creatinine ratio indicated that YC further decreased their levels when feed were contaminated with AF. In conclusion, low level of 20 μ g AF/kg under the regulatory level had minor effects on hematology without affecting growth performance, however the supplementation of 2 g/kg YC as a source of β -glucans and MOS in feed can improve feed intake and therefore the growth of pigs.

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1. Introduction

Corn is the most commonly used energy feed for pigs. However, corn is commonly contaminated with mycotoxins especially when corn is grown under high temperature and drought condition in breeding season and cool and damp condition in harvesting season (Binder et al., 2007; Chaytor et al., 2011a). Among 300 existing mycotoxins, aflatoxin (AF) is the only mycotoxin regulated by the

US Food and Drug Administration (Dersjant-Li et al., 2003; Richard, 2007). Aflatoxin is also one of the most common mycotoxins found in foods processed for human consumption, such as corn, cotton seeds, nuts, peanuts, pistachios, spices, and dry fruits (CAST, 2003). Ingestion of AF by animals can result in many problems, including decreased growth rates, liver damage, immune suppression, and death (CAST, 2003). Pigs are one of the most susceptible commodity species to AF with damages to the gut and the liver (Hussein and Brasel, 2001; Weaver et al., 2013). Most countries limit AF concentration in the corn with different levels. The US is relatively more stringent on AF limitation which is 20 μ g AF/kg in corn. The regulatory level for nursery pigs in US is also 20 μ g AF/kg in corn whereas it is higher for finishing pigs and breeding pigs. Usually multiple mycotoxins are contaminated in corn and grains (Dersjant-Li et al., 2003). Contaminated corn used in this study also contained fumonisin (FUM). At the high level, FUM can damage the brain, lungs, kidneys, and liver (CAST, 2003).

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The yeast cell wall based feed additive (YC) mainly containing β -glucans and mannan oligosaccharide (MOS) was used in this study and commercially available in the US (Kogan and Kocher, 2007; Ringot et al., 2005; Yiannikouris et al., 2004). Green algae are another component of YC which is a large group of algae that embryophytes are emerged. The green algae are classified into 6 clades, and 4 of them include the composition of β -glucans or MOS (Becker et al., 1991, 1994; Ciancia et al., 2012; Sørensen et al., 2011, 2012). β -glucans and MOS were shown to have binding capabilities to AF, deoxynivalenol (DON), and zearafenone (ZEA) (Huwig et al., 2001; Kogan and Kocher, 2007; Spring et al., 2000) and thus reducing the damaging effects of mycotoxins on pigs. In addition to the possible binding effects of β -glucans and MOS to mycotoxins, it is also shown that β -glucans can alter the balance of interleukin-1 and interleukin-1 receptor antagonist to reduce inflammatory cytokine production (Dritz et al., 1995) and MOS can alter the immune response by binding to mannose receptors on the macrophage cell surface which will enhance macrophage function (Davis et al., 2004).

The main hypothesis of this study was that growth and health of nursery pigs could minimally be affected by AF in the diets

below the regulatory level and YC including β -glucans and MOS would help the growth and health of pigs fed these diets.

2. Materials and methods

The protocol for the use of animals in this study was approved by the North Carolina State University Animal Care and Use Committee.

2.1. Animals

One hundred twenty newly weaned pigs (60 gilts and 60 barrows, Smithfield Premium Genetics, Rose Hill, NC) had an acclimation period with phase 1 diet (Table 1) for 12 d. Pigs were then grouped based on their BW with a same sex and randomly allotted to 4 treatments based on a randomized complete block design with their BW and sex as blocks. Four treatments had 2 × 2 factorial arrangement. The first factor was AF (0 or 20 μ g/kg in feed) and the second factor was YC (0 or 2 g/kg in feed). Each treatment had 10 pens (5 barrow pens and 5 gilt pens) with 3 pigs per pen.

Table 1
Composition of experimental diets (as-fed basis).¹

Item	Phase 1		Phase 2		Phase 3					
	No AF		No AF		AF		No AF		AF	
	No YC	No YC	YC	No YC	YC	No YC	YC	No YC	YC	
Ingredient, %										
Yellow corn	42.1	53.8	53.6	52.8	52.6	65.42	65.22	64.42	64.22	
Soybean meal	25	30	30	30	30	30	30	30	30	
DairyLac 80	20	9	9	9	9					
Blood plasma	8	2.5	2.5	2.5	2.5					
L-Lys HCl	0.05	0.15	0.15	0.15	0.15	0.1	0.1	0.1	0.1	
DL-Met	0.05	0.05	0.05	0.05	0.05					
Salt	2	0.22	0.22	0.22	0.22	0.3	0.3	0.3	0.3	
Vitamin premix ²	0.22	0.03	0.03	0.03	0.03	0.15	0.15	0.15	0.15	
Mineral premix ³	0.03	0.15	0.15	0.15	0.15	0.03	0.03	0.03	0.03	
Dicalcium P	0.15	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	
Limestone, ground	1.8	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6	
poultry fat	0.6	2	2	2	2	2	2	2	2	
YC			0.2		0.2		0.2		0.2	
Corn AF ⁴				1	1			1	1	
Calculated composition										
DM, %	91.3	90.5	90.5	90.5	90.5	89.8	89.8	89.8	89.8	
ME, Mcal/kg	3.45	3.42	3.42	3.42	3.42	3.42	3.42	3.42	3.42	
CP, %	22.4	21.1	21.1	21.1	21.1	19.7	19.7	19.7	19.7	
Lys, %	1.45	1.34	1.34	1.34	1.34	1.15	1.15	1.15	1.15	
Cys + Met, %	0.84	0.76	0.76	0.76	0.76	0.66	0.66	0.66	0.66	
Trp, %	0.3	0.26	0.26	0.26	0.26	0.23	0.23	0.23	0.23	
Thr, %	0.98	0.83	0.83	0.83	0.83	0.74	0.74	0.74	0.74	
Lactose sugar	16	7.2	7.2	7.2	7.2					
Ca, %	0.91	0.8	0.8	0.8	0.8	0.72	0.72	0.72	0.72	
Available P, %	0.56	0.4	0.4	0.4	0.4	0.33	0.33	0.33	0.33	
Total P, %	0.89	0.72	0.72	0.72	0.72	0.64	0.64	0.64	0.64	
AF, μ g/kg				20	20			20	20	
YC, %			0.2		0.2		0.2		0.2	
Analyzed composition, %										
DM	92.7	91.6	91.4	91.6	91.8	90.5	91.1	90.4	90.5	
CP	21.5	20.2	19.6	21.8	19.5	19	20.3	18.6	18.6	
ADF	3.15	2.75	2.21	3.48	2.92	2.91	3.35	3.7	3.47	
Ca	0.79	0.74	0.83	0.66	0.83	0.73	0.69	0.66	0.69	
P	0.86	0.68	0.74	0.67	0.73	0.64	0.64	0.64	0.63	
AF ⁵ , μ g/kg				27	39			36	29	

AF = aflatoxin; YC = yeast cell wall based feed additive; ADF = acid detergent fiber.

¹ Factor AF with the presence of 20 μ g AF/kg. Factor YC with the supplementation of 2 g Integral A +/kg (Integral A + was a product of Alltech, Lexington, KY).

² Vitamin premix provided the following per kilogram of complete diet: 22,045,000 IU of vitamin A; 3,306,900 IU of vitamin D₃; 66,138 IU of vitamin K; 88 mg of vitamin B₁₂; 15,432 mg of riboflavin; 88,184 mg of niacin; 61,729 mg of d-pantothenic acid; 8,818 mg of menadione; 220 mg of biotin.

³ Mineral premix provided the following composition: 1.10% of Cu; 198.0 mg/kg of I; 11.02% of Fe; 2.64% of Mn; 198.4 mg/kg of Se; 11.02% of Zn.

⁴ Corn AF was corn naturally contaminated with 20 μ g AF/kg and 1.6 mg fumonisin/kg.

⁵ Aflatoxin in diets were analyzed by a commercial laboratory (Food and Drug Protection Division Laboratory, NC).

Pigs were fed experimental diets for 5 wk based on 2 phases (2 wk for phase 2 and 3 wk for phase 3; Table 1). Body weight and feed intake were measured weekly on d 0, 7, 14, 21, 28, and 35 for computation of growth performance.

2.2. Experimental diets

Corn contaminated with AF and FUM (Snow Hill, NC) was used to achieve targeted concentrations of each mycotoxin. Feeding period was separated into 3 phases. Phase 1 was the acclimation period. Corn contaminated with AF and FUM was used in phase 2 and 3 diets. Sampling of corn was completed by obtaining subsamples from 10 different locations to obtain accurate mycotoxin concentrations (Chaytor et al., 2011a; Munkvold et al., 2005; Whitaker et al., 2005). Yeast cell wall based feed additive (Integral A+, Alltech Inc., Nicholasville, KY) was added in phase 2 and 3 diets. Aflatoxin in corn was measured by North Carolina Department of Agriculture and Consumer Service Food and Drug Protection Division Laboratory. A detection limit was 0.1 µg/kg. The calculated value and analyzed value were showed in Table 1. During the 5 wk feeding period, all pigs had free access to feed and water. Concentrations of nutrients met the requirements suggested by NRC (1998).

2.3. Blood sampling

Blood samples were collected via jugular vein from one pig representing the average BW of each pen on d 31. For each pig, the blood sample was collected in 2 vacutainers (BD, Franklin Lakes, NJ): one containing EDTA (7 mL) to obtain whole blood samples for hematological analysis, the other one without anticoagulant (10 mL) to collect serum for liver function test, immunoglobulin subset, cytokine, and oxidative damage status measurements. Plasma and serum samples were obtained after centrifugation ($3,000 \times g$ for 15 min at 4°C) and stored at –80°C until further analysis.

2.4. Hematological measurements and liver function test

Eosinophils, hematocrit, hemoglobin, lymphocytes, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), monocytes, neutrophils, platelet count, red blood cell (RBC), and white blood cell (WBC) in plasma were determined using an automated hematology analyzer by a commercial laboratory (Antech Diagnostics, Cary, NC). Concentration of albumin to globulin ratio, albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen to creatinine ratio, Ca, Cl, cholesterol, creatine phosphokinase, creatinine, globulin, glucose, P, K, Na, Na to K ratio, total bilirubin, total protein, and urea nitrogen in serum were measured for determination of liver function by the commercial laboratory (Antech Diagnostics, Cary, NC) using a chemistry-immuno analyzer (AU640e, Olympus America Inc., Center Valley, PA).

2.5. Immunoglobulin subset

Total concentration of the immunoglobulin subsets immunoglobulin G (IgG) in serum, as an indicator of general status of humoral immune system, was measured via commercial ELISA kits (Bethyl Laboratories, Montgomery, TX) as described (Chaytor et al., 2011b). Serum samples were diluted to 1:20,000 with 0.05% Tween 20. Further procedures were conducted following the manufacturer's instruction. Absorbance was read at 450 nm using an ELISA plate reader (Synergy HT Multi-Mode Microplate Reader, BioTek Instruments, Inc., Winooski, VT) and software (KC4 Data Analysis Software, BioTek Instruments, Inc., Winooski, VT).

Samples were quantified against the standard curve constructed with known amounts of pig immunoglobulin subset. Detection limits were 7.8 to 500 ng/mL for IgG.

2.6. Cytokine measurement

Concentration of tumor necrosis factor- α (TNF- α) in serum was measured using a Porcine TNF- α Colorimetric ELISA Kit (Pierce Biotechnology, Inc., Rockford, IL) as an indicator of systemic inflammation and acute phase reaction (Chaytor et al., 2011b). Briefly, 50 µL of standard plus dilute or 100 µL of sample was added to microplate wells which were already coated with capture antibody in conjunction with biotinylated antibody reagent. Detection occurred by the use of horseradish peroxidase, TMB substrate, and a stop solution of 0.18 M H₂SO₄. Absorbance was read at 450 and 540 nm by an ELISA plate reader and the KC4 data analysis software. Detection limit for TNF- α was 5 pg/mL.

2.7. Oxidative damage status measurement

Concentration of malondialdehyde (MDA) in serum was measured using an OxiSelect TBARS Assay Kit (Cell Biolabs, Inc., San Diego, CA) as an indicator of lipid peroxidation (Vahiquist and Duvic, 2007). Absorbance was read at 532 nm using the Synergy HT ELISA plate reader and KC4 data analysis.

2.8. Statistical analysis

This study used a randomized complete block design based on 2×2 factorial arrangements of treatments. Initial BW and sex were blocks. The first factor was AF (0 or 20 µg/kg) and the second factor was YC (0 or 2 g/kg). Pen was the experimental unit as pigs in a pen were group fed. Data were analyzed using the Mixed Model (PROC MIXED) of SAS (SAS Inst. Inc., Cary, NC). Probability values less than 0.05 were considered statistically significant and probability between 0.05 and 0.10 were considered as trends.

3. Results

3.1. Growth performance

Initial BW of pigs did not differ among treatments. At the end of wk 1, BW of pigs did not differ among treatments (Table 2). From wk 2 to 5, the presence of AF did not affect the BW of pigs whereas the supplementation of YC increased ($P < 0.05$) BW.

Average daily gain was not affected by the presence of AF during the entire 5 wk feeding period. The supplementation of YC did not affect ADG at wk 1, 3, and 4. At wk 2 and 5, the supplementation of YC increased ($P < 0.05$) ADG. From wk 1 to 2, wk 3 to 5, and wk 1 to 5, the supplementation of YC increased ($P < 0.05$) ADG whereas the presence of AF did not affect ADG.

Average daily feed intake was not affected by the presence of AF during the entire 5 wk feeding period. The supplementation of YC did not affect ADFI at wk 2 and 4. The supplementation of YC tended to increase ($P = 0.060$) ADFI at wk 1. At wk 3 and 5, the supplementation of YC increased ($P < 0.05$) ADFI. From wk 1 to 2, wk 3 to 5, and wk 1 to 5, the supplementation of YC increased ($P < 0.05$) ADFI. Interaction in wk 1 ADFI between AF and YC existed ($P < 0.05$). The presence of YC decreased ADFI when feed were not contaminated with AF but increased ADFI when feed were contaminated with AF. The G:F did not differ among treatments during the entire 5 wk period.

Table 2
Growth performance of pigs fed diets contaminated with aflatoxin (0 or 20 µg/kg) and supplemented with amodified yeast cell wall extract (0 or 2 g/kg).¹

Item	No AF		AF		SEM	P-value		
	No YC	YC	No YC	YC		AF	YC	Interaction
BW, kg								
Initial BW	6.0	6.0	6.0	6.0	0.3	0.788	0.993	0.979
Wk 1 BW	7.6	7.6	7.4	7.8	0.3	0.731	0.189	0.189
Wk 2 BW	10.6	10.9	10.7	11.2	0.5	0.306	0.036	0.509
Wk 3 BW	13.8	14.5	14.1	14.7	0.6	0.401	0.025	0.977
Wk 4 BW	18.2	19.0	18.5	19.1	0.7	0.615	0.039	0.714
Wk 5 BW	23.1	24.3	23.5	24.4	0.9	0.563	0.007	0.668
ADG, g								
Wk 1	221	221	199	260	26	0.721	0.184	0.185
Wk 2	430	473	464	481	21	0.134	0.034	0.374
Wk 1 to 2	326	347	331	371	20	0.298	0.034	0.506
Wk 3	464	511	486	495	21	0.847	0.085	0.220
Wk 4	621	650	629	626	27	0.620	0.430	0.318
Wk 5	700	762	714	763	25	0.664	0.004	0.736
Wk 3 to 5	595	641	610	628	21	0.935	0.009	0.232
Overall	487	523	498	525	19	0.559	0.007	0.667
ADFI, g								
Wk 1	425	411	402	491	27	0.146	0.060	0.012
Wk 2	591	618	604	632	27	0.491	0.159	0.995
Wk 1 to 2	508	515	503	561	24	0.177	0.040	0.100
Wk 3	717	806	762	800	35	0.373	0.007	0.250
Wk 4	985	1,051	1,018	1,023	49	0.939	0.216	0.273
Wk 5	1,225	1,315	1,226	1,316	47	0.966	0.005	0.989
Wk 3 to 5	976	1,057	1,002	1,046	40	0.715	0.006	0.370
Overall	789	840	803	852	32	0.417	0.003	0.940
G:F								
Wk 1	0.544	0.534	0.485	0.537	0.076	0.598	0.682	0.560
Wk 2	0.725	0.771	0.771	0.762	0.021	0.383	0.401	0.206
Wk 1 to 2	0.645	0.676	0.658	0.659	0.030	0.931	0.481	0.509
Wk 3	0.648	0.636	0.640	0.621	0.013	0.307	0.200	0.755
Wk 4	0.631	0.620	0.620	0.617	0.013	0.560	0.565	0.743
Wk 5	0.573	0.582	0.585	0.581	0.011	0.585	0.791	0.490
Wk 3 to 5	0.610	0.618	0.610	0.602	0.007	0.568	0.344	0.568
Overall	0.619	0.625	0.622	0.615	0.008	0.666	0.966	0.376

AF = aflatoxin; YC = yeast cell wall based feed additive.

¹ Factor AF with the presence of 20 µg AF/kg. Factor YC with the supplementation of 2 g Integral A+/kg.

3.2. Hematological measurements

The presence of AF decreased ($P < 0.05$) the number of platelet count. The interactions in hemoglobin, hematocrit, and platelet count between AF and YC existed ($P < 0.05$). The presence of YC decreased hemoglobin, hematocrit, and platelet count with no presence of AF but increased their levels with the presence of AF (Table 3).

3.3. Liver function

The albumin to globulin ratio, albumin level, and concentration of Ca tended to be increased ($P = 0.050$, $P = 0.055$, and $P = 0.080$, respectively) by the presence of AF. The supplementation of YC tended to increase ($P = 0.055$) albumin level. There were interaction ($P < 0.05$) in urea nitrogen and blood urea nitrogen to creatinine ratio between AF and YC. The presence of YC decreased urea nitrogen and blood urea nitrogen to creatinine ratio when feed were not contaminated with AF but increased their levels when feed were contaminated with AF. The interaction in albumin level between AF and YC tended to exist ($P = 0.089$). The increase in level of albumin with presence of YC was greater when feed were contaminated with AF compared with those without AF (Table 4).

3.4. Cytokines

The levels of IgG, MDA, and TNF- α in serum did not differ among treatments (Table 5).

4. Discussion

Aflatoxin is one of the most common mycotoxins found in feedstuffs such as corn, barley, and wheat (Chaytor et al., 2011a). Feed contaminated with AF can cause liver damage and immune suppression, decrease feed intake and weight gain, and eventually cause significant economic losses (Chaytor et al., 2011b; Weaver et al., 2013).

Swine are highly susceptible to AF compared with other species. The regulatory level for nursery pigs in complete feedstuffs is 20 µg AF/kg in US (FAO Food and Nutrition Paper No. 81, 2004). Numerous studies in swine observed decreased BW when diets contained 60 to 3,000 µg AF/kg (Chaytor et al., 2011b; Harvey et al., 1989; Marin et al., 2002; Thieu et al., 2008). The effects of AF on pigs are still questioned when reducing the concentration below 20 µg AF/kg. The other objective was to determine the effects of mycotoxin in naturally contaminated corn instead of purified form of mycotoxins contaminated corn on growth and health of pigs. Studying on a purified form of mycotoxin can be an accurate way to evaluate the effects of individual mycotoxin on performance of animals. However, grains are usually contaminated with more than one kind of mycotoxin. Corn used in this study contained AF and FUM. The concentration of FUM in the diet was 1.6 mg FUM/kg. Previous studies showed that less than 5 mg FUM/kg did not cause liver damage in swine (Zomborszky et al., 2000), suggesting FUM as low as 1.6 mg FUM/kg would not have negative effects on growth performance, gut health, and liver function in current study.

Table 3Hematological measurement of pigs fed diets contaminated with aflatoxin (0 or 20 µg/kg) and supplemented with modified yeast cell wall extract (0 or 2 g/kg).¹

Item	No AF		AF		SEM	P-value		
	No YC	YC	No YC	YC		AF	YC	Interaction
Eosinophils, cell/µL	402	476	416	491	96	0.843	0.321	0.996
Hematocrit, %	39.9	38.1	37.7	40.5	1.2	0.880	0.642	0.045
Hemoglobin, g/dL	12.2	11.6	11.6	12.4	0.3	0.837	0.685	0.045
Lymphocytes, cell/mL	7,194	6,032	7,647	6,709	1,446	0.636	0.393	0.924
MCH, pg	18.8	19.1	19.1	19.9	0.5	0.205	0.208	0.587
MCHC, g/dL	30.5	30.5	30.7	30.5	0.3	0.714	0.639	0.733
MCV, fL	61.6	62.7	62.7	65.2	1.7	0.252	0.246	0.652
Monocytes, cell/mL	782	705	832	852	161	0.488	0.840	0.727
Neutrophils, cell/mL	5,289	4,860	4,895	6,550	767	0.366	0.391	0.155
Platelet count, 10 ³ /µL	268	227	172	215	32	0.011	0.957	0.035
RBC, 10 ⁶ /µL	6.49	6.08	6.07	6.28	0.19	0.395	0.448	0.127
WBC, 10 ³ /µL	13.8	12.0	14.1	14.7	2.1	0.434	0.756	0.518

AF = aflatoxin; YC = yeast cell wall based feed additive; WBC = white blood cells; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

¹ Factor AF with the presence of 20 µg AF/kg. Factor YC with the supplementation of 2 g Integral A+/kg.

Table 4Biochemical blood assays of pigs fed diets contaminated with aflatoxin (0 or 20 µg/kg) and supplemented with modified yeast cell wall extract (0 or 2 g/kg).¹

Item	No AF		AF		SEM	P-value		
	No YC	YC	No YC	YC		AF	YC	Interaction
A:G ratio	2.10	2.07	2.23	2.51	0.23	0.050	0.377	0.275
Albumin, g/dL	3.45	3.47	3.47	3.79	0.10	0.055	0.055	0.089
Alk phosphatase, U/L	281	272	288	263	15	0.255	0.924	0.601
ALT, U/L	26.3	28.7	26.1	28.6	2.2	0.944	0.261	0.982
AST, U/L	30.7	30.3	27.5	28.9	2.2	0.277	0.811	0.668
BUN/creatinine ratio	18.5	14.7	14.2	15.6	1.2	0.146	0.300	0.030
Ca, mg/dL	10.7	10.9	11.0	11.0	0.1	0.080	0.485	0.425
Cl, mEq/L	102	102	102	102	1.	0.762	0.762	0.952
Cholesterol, mg/dL	75.9	79.3	80.2	77.2	4.2	0.958	0.772	0.402
CPK, U/L	1,112	1,177	943	1,311	245	0.944	0.383	0.539
Creatinine, mg/dL	0.69	0.66	0.70	0.69	0.03	0.459	0.459	0.710
Globulin, g/dL	1.69	1.71	1.67	1.55	0.92	0.292	0.364	0.302
Glucose, mg/dL	133	124	124	124	6	0.407	0.407	0.447
K, mEq/L	5.71	5.68	5.50	5.59	0.63	0.304	0.836	0.679
Na, mEq/L	143	144	144	143	1	0.741	0.741	0.324
Na:K ratio	25.7	26.0	26.8	26.2	3.2	0.263	0.794	0.436
P, mg/dL	11.0	11.0	10.7	10.8	0.5	0.716	0.790	0.484
Total bilirubin, mg/dL	0.13	0.10	0.10	0.10	0.02	0.326	0.326	0.326
Total protein, g/dL	5.14	5.18	5.14	5.34	0.09	0.380	0.192	0.380
Urea nitrogen, mg/dL	12.6	9.5	9.9	10.6	0.8	0.288	0.116	0.016

AF = aflatoxin; YC = yeast cell wall based feed additive; A/G ratio = albumin to globulin ratio; AST = aspartate aminotransferase; ALT = alanine aminotransferase; Alk phosphatase = alkaline phosphatase; BUN/creatinine ratio = blood urea N to creatinine ratio; CPK = creatine phosphokinase.

¹ Factor AF with the presence of 20 µg AF/kg. Factor YC with the supplementation of 2 g Integral A+/kg.

Table 5TNF-α, IgG, and MDA of pigs fed diets contaminated with AF (0 or 20 µg/kg) and supplemented with modified yeast cell wall extract (0 or 2 g/kg).¹

Item	No AF		AF		SEM	P-value		
	No YC	YC	No YC	Yes YC		AF	YC	Interaction
TNF-α, pg/mL	59.1	61.6	64.2	57.8	7.0	0.896	0.664	0.324
IgG, mg/mL	8.60	10.74	11.47	9.64	2.04	0.621	0.928	0.271
MDA, µmol/L	15.43	14.60	16.81	16.02	1.75	0.394	0.621	0.992

AF = aflatoxin; YC = yeast cell wall based feed additive; TNF-α = tumor necrosis factor-α; IgG = immunoglobulin G; MDA = malondialdehyde.

¹ Factor AF with the presence of 20 µg AF/kg. Factor YC with the supplementation of 2 g Integral A+/kg.

Modified yeast cell wall extract may have effects on preventing minor health concerns in nursery pigs fed diet containing 20 µg AF/kg. The 3 major components of YC are β-glucans, MOS, and green algae. In previous studies, nursery pigs fed diets

supplemented with 0.025% β-glucans had increased growth performance, which was due to the increased ADFI (Dritz et al., 1995). Supplementation of 0.03% β-glucans tended to increase feed intake but did not affect feed efficiency (Hiss and Sauerwein, 2003). Schoenherr et al. (1994) suggested that the optimal inclusion level of β-glucans was between 0.025 and 0.05% throughout the nursery period. The supplementation of MOS enhanced growth performance and feed efficiency of nursery pigs (Davis et al., 2002; LeMieux et al., 2001; Rozeboom et al., 2005). Another study found that α-D-mannan could suppress toxic activity of mycotoxins probably by interacting with their toxic radical metabolites (Madrigal-Bujaidar et al., 2002).

During the entire experimental period, pigs fed 20 µg AF/kg did not have significant difference in ADG and ADFI compared with pigs fed no AF. These results are different from what previous studies found, which is mainly because current study used much lower concentration of AF (20 µg AF/kg). Chaytor et al. (2011b) found that weight gain and feed intake decreased by 17.3 and 11.5%, respectively, when pigs fed 124 µg AF/kg diet. However, the

diet with 60 to 130 μg AF/kg did not affect the feed efficiency (Chaytor et al., 2011b; Doll et al., 2003), which is consistent with our finding. Therefore, 20 μg AF/kg of diet was not high enough to affect growth of nursery pigs.

Supplementation of YC increased pigs BW from wk 2 to 5 and at the end of this study by 5.2 and 3.8%, respectively. At the same periods, ADG increased with the supplementation of YC by 7.3 and 5.4%, respectively. Increases in BW and ADG are mainly caused by the increased ADFI. These results were supported by previous studies (Dvorak and Jacques, 1998; Li et al., 2006; Miguel et al., 2002; Zhao et al., 2012). Li et al. (2006) observed that β -glucans from *Saccharomyces cerevisiae* improved ADG of nursery pigs with the concentration of 0.005%, which is much lower than inclusion levels of 0.025 to 0.05% used in study of Schoenherr et al. (1994) and Shen et al. (2009). The difference in inclusion levels may indicate that the effective range of β -glucans varies with different sources. Miguel et al. (2002) claimed that the application of MOS as a growth promoter in early nursery period has been demonstrated. During the entire experimental period, G:F was not affected by the supplementation of YC. According to the previous studies, MOS could improve feed efficiency whereas β -glucans did not affect feed efficiency (Hiss and Sauerwein, 2003; Miguel et al., 2002; Rozeboom et al., 2005). Therefore, further study is required to investigate the contradictory effects of MOS and β -glucans on feed efficiency.

Even concentration of 20 μg AF/kg was not high enough to affect growth of nursery pigs, hematological, serological variables, inflammatory status, and oxidative status were determined to evaluate the effect of AF on health of pigs. Platelet count was the only one hematological parameter decreased with the presence of AF. With limited information available concerning the hematological and biochemical effects of low level AF exposure, few information about platelet count were mentioned in previous study. Chaytor et al. (2011a,b) reported that there was a numerical decrease in platelet count when nursery pigs were fed diets contaminated with combination of AF and DON. Mycotoxin T-2 toxin was able to inhibit platelet activity with an additive inhibitory effect on platelet aggregation response (Gentry et al., 1987). The decreased platelet number in this study may indicate the negative effect of AF on platelet activity.

Serological variables were slightly affected by AF and YC, which was similar to the hematological parameters. Consistent with current result, albumin to globulin ratio increased with supplementation of low level of FUM (Rotter et al., 1994). The increase of albumin level can be explained by inadequate water intake which may cause dehydration of blood and subsequently increases albumin concentration (Kaneko et al., 1997). Khera et al. (1984) reported that there was a decrease in water intake when mice were exposed to feed containing DON. However, considering these variables were within the normal ranges, the alterations in liver function caused by low level of AF was too slight to generate change in growth performance of nursery pigs, as the previous results indicated.

The supplementation of YC and presence of AF did not affect TNF- α , IgG, and MDA in this study. These results were similar to some previous studies with low concentration of mycotoxins. Marin et al. (2002) concluded that concentration of TNF- α did not change when providing dietary AF at 140 or 280 $\mu\text{g}/\text{kg}$. Accensi et al. (2006) found that DON at level from 280 to 840 $\mu\text{g}/\text{kg}$ did not affect IgG concentration. T-2 toxin at levels from 540 to 2,102 $\mu\text{g}/\text{kg}$ and AF at level of 500 $\mu\text{g}/\text{kg}$ did not change MDA concentration of nursery pigs (Harper et al., 2010; Meissonnier et al., 2008).

5. Conclusion

Collectively, based on the results of this study, the supplementation of 20 μg AF/kg did not affect pigs' growth performance. However, it had negative effects on platelet count, serum albumin, and calcium homeostasis. Supplementation of modified yeast cell wall extract mainly composed of β -glucans and MOS increased ADG of pigs by increasing ADFI, but did not affect the feed efficiency. Mechanisms on increased feed intake by β -glucans and MOS combination or individually warrant further research.

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