# The impact of deoxynivalenol, fumonisins, and their combination on performance, nutrient, and energy digestibility in broiler chickens

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**ABSTRACT** This study evaluated the effects of the mycotoxins deoxynivalenol (DON), fumonisins (FUM), and their combination on growth performance, nutrient, and energy digestibility in broilers. A total of 960 Cobb-Cobb male broilers were obtained on the day of hatch and placed 10 birds per cage with 8 cages per treatment. The experiment consisted of 12 treatments: control; DON 1.5 mg/kg; DON 5.0 mg/kg; FUM 20.0 mg/kg; DON 1.5 mg/kg + FUM 20.0 mg/kg; and DON 5.0 mg/kg + FUM 20 mg/kg. The remaining dietary treatments were the correlative nitrogen-free diets (NFD) for determining the endogenous nutrients loss. All birds were fed with a corn−soybean meal diet from days 1 to 15, until birds from latter 6 treatments were switched to their correlative NFD diet from days 15 to 21. Feed and BW were weighed by cage on days 8, 15, and 21. On day 21, ileal digesta was collected for digestibility determination. Both DON 1.5 mg/kg + FUM 20 mg/kg and DON 5.0 mg/kg + FUM 20 mg/kg treatments showed reduced feed intake ($P \leq 0.05$) from days 8 to 15 and days 15 to 21. However, no significant effects were noted for BW gain or mortality-adjusted feed conversion ratio after adding single or combined mycotoxin on days 8 and 15. At day 21, cumulative BW gain was less ($P \leq 0.05$) in birds fed with the mycotoxin combination diets than the control. No significant changes were shown for ileal endogenous amino acids losses. Control treatment had significantly higher ($P \leq 0.05$) apparent ileal energy digestibility than the DON 5.0 mg/kg + FUM 20.0 mg/kg treatment (3,126 vs. 2,895 kcal/kg), representing a 5%-unit loss in apparent DM digestibility. No significant difference was found for standardized crude protein and amino acid digestibility. In conclusion, the combination of DON and FUM (DON 1.5 mg/kg + FUM 20 mg/kg or DON 5.0 mg/kg + FUM 20 mg/kg) reduced DM and ileal energy digestibility, which negatively affected BW gain in broilers.

**Key words:** deoxynivalenol, fumonisins, growth, digestibility, broiler

2020 Poultry Science 99:272–279
http://dx.doi.org/10.3382/ps/pez484

## INTRODUCTION

Deoxynivalenol (**DON** or vomitoxin) and fumonisins (**FUM**), both from the species of *Fusarium*, are 2 frequent mycotoxins in grain feed ingredient (Gelderblom et al., 1988; Rotter et al., 1996; Antonissen et al., 2014a). The *Fusarium graminearum* and *Fusarium culmorum* are the major fungi producing DON (which belongs to the type B trichothecenes) in corn and wheat (Goswami and Kistler, 2004; Audenaet et al., 2014). The *Fusarium verticillioides* and *proliferatum* are the principal FUM producing fungus in moldy grains (Waśkiewicz et al., 2012). Season, geographical location, drought, and time of harvest are several factors that affect the growth and mycotoxin formation in fungus (Murugesan et al., 2015). A recent mycotoxin survey has shown a high prevalence of type B trichothecenes (including DON) and FUM in US corn samples, with 78% (average concentration of 1.042 mg/kg) and 45% (average concentration of 2.365 mg/kg) respectively (Gott et al., 2018). Further, the mycotoxin co-occurrence in grains (samples containing more than 1 mycotoxin) has been frequently reported and reviewed by multiple scientific publications (Streit et al., 2012, 2013; Gonçalves et al., 2018; Franco et al., 2019).

Poultry have been considered relatively resistant to the DON and FUM in the past (Moran et al., 1982; Broomhead et al., 2002). However, with the growth improvements in modern broiler over the past 30 yr, an increasing number of publications have demonstrated a close correlation between low doses of mycotoxin (DON or FUM) and its influence on growth and health status in broiler chickens. Moreover, subclinical mycotoxicosis (when feed mycotoxin concentrations are below the EU limitation) has raised the attention in poultry industry as they more towards the antibiotic-free production.

Received April 15, 2019.
Accepted August 7, 2019.
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Antonissen et al. (2014b) showed the presence of low dose DON (3 to 4 mg/kg) in the diet increased the percentage of subclinical necrotic enteritis and increased intestinal protein availability in lumen which negatively affected the intestinal barrier functions. Other researchers have also demonstrated the subclinical doses of DON and FUM resulted in disrupted metabolic and immunologic effects (Grenier et al., 2016).

However, to the authors acknowledge, no studies have focused on the impact of mycotoxin DON and FUM on endogenous loss and nutrient digestibility in broilers. Moreover, gut integrity is closely related to nutrient digestibility and growth performance of the bird. Birds exposed to aflatoxin B1 have been shown impaired intestinal barrier, reduced standardized nitrogen and amino acid digestibility and increased endogenous nitrogen loss (Chen et al., 2016a). With increasing of co-occurrence of mycotoxins, incidence in the field, and concerns of sub-clinical doses mycotoxin on gut health, the objective of this study was to evaluate the effects of low doses of the mycotoxins, DON and FUM, and their combination on growth performance, endogenous amino acids losses and nutrient digestibility in broiler chickens.

## MATERIALS AND METHODS

This study was conducted at the University of Georgia Poultry Research Center. The protocol used in this study was approved by the University of Georgia Animal Care and Use Committee, and Biosafety Committee.

### *Experimental Design, Dietary Treatments, and Animal Husbandry*

A total of 960 Cobb × Cobb 500 male broilers were obtained on the day of hatch from the Cobb-Vantress hatchery (Cleveland, GA) and placed 10 birds per cage into 8 replicates battery cages per treatment. All birds were weighed individually and sub-grouped into 6 weight categories, with 2 g range for each category. Birds from the lowest and highest weight categories groups were discarded from the study. A total of 10 birds were randomly selected from the middle 4 weight categories (number of birds chosen from each weight category was to be representative of the range and normal distribution of the BW), and allocated into a cage with the same average 440 g initial cage weight. The experiment consisted of 12 treatments: Control; DON 1.5 mg/kg; DON 5.0 mg/kg; FUM 20.0 mg/kg; DON 1.5 mg/kg + FUM 20.0 mg/kg; and DON 5.0 mg/kg + FUM 20 mg/kg. The remaining 6 treatments were the correlative nitrogen free diets (**NFD**) for determining the endogenous nutrient losses: NFD Control; NFD DON 1.5 mg/kg; NFD DON 5.0 mg/kg; NFD FUM 20.0 mg/kg; NFD DON 1.5 mg/kg + FUM 20.0 mg/kg; and NFD DON 5.0 mg/kg + FUM 20 mg/kg. Birds were fed with an unmedicated mash corn−soybean meal broiler starter diet from days 1 to 15 (Table 1), until birds from the latter 6 treatments were switched to the NFD from days 15 to 21. The dietary electrolyte balance (Adedokun and Applegate, 2014) in both the corn−soybean meal diet and the nitrogen-free diet were formulated as 219 mEq/kg of diet = Na + K—Cl mEq/kg of diet, where mEq/kg Na, K, and Cl were determined using the equation [(percentage of Na, K, or Cl in the diet) × 10,000/(molecular weight of Na, K, and Cl)]. The corn starch-to-dextrose ratio was set at 0.30 for the NFD. Chromium oxide served as the ingestible marker and was added at 0.5% into each treatment diet. The mycotoxin concentrations in corn (0.3 mg/kg for DON; 0.5 mg/kg for total FUM B1, B2, and B3) were analyzed before the supplemental mycotoxin premix were added into each treatment for feed mixing. The *Fusarium graminearum* DSM-4528 and *Fusarium verticillioides* M-3125 were used to produce DON and FUM, respectively and the mycotoxin premix was prepared by BIOMIN Research Center (Tulln, Austria). Strains were separately grown on rice and DON and FUM were produced in accordance to the methods described previously (Desjardins et al., 1992; Altpeter

**Table 1.** Ingredient and nutrient composition of the basal and nitrogen-free diets (NFD) (as-fed basis).

| Item | Basal | N-free |
|---|---|---|
| Ingredient, % of diet | | |
| Corn, grain | 54.56 | – |
| Soybean meal, 48% CP | 37.55 | – |
| Corn starch | – | 19.00 |
| Dextrose | – | 64.00 |
| Solka-floc | – | 5.00 |
| Soybean oil | 2.56 | 5.00 |
| Limestone | 1.52 | 1.30 |
| Dicalcium phosphate | 1.52 | 1.90 |
| Sodium choride | 0.44 | – |
| DL-methionine | 0.36 | – |
| L-Lysine · HCl | 0.50 | – |
| Threonine | 0.06 | – |
| Vitamin premix[1] | 0.35 | 0.35 |
| Mineral premix[2] | 0.08 | 0.08 |
| Sodium bicarbonate | – | 1.86 |
| Choline chloride | – | 0.25 |
| Magnesium oxide | – | 0.20 |
| Potassium chloride | – | 0.29 |
| Potassium carbonate | – | 0.27 |
| Chromium oxide | 0.50 | 0.50 |
| Calculated composition | | |
| ME, kcal/kg | 3027 | 3230 |
| CP, % | 22.77 | – |
| Crude fat, % | 4.72 | 4.75 |
| Ca, % | 0.95 | 0.81 |
| Available P, % | 0.48 | 0.41 |
| Lys, % | 1.66 | – |
| Thr, % | 0.97 | – |
| Met, % | 0.72 | – |
| TSAA, % | 1.08 | – |
| Electrolyte balance, mEq/kg of diet | 219 | 219 |

[1]Supplied per kilogram of diet: vitamin A, 5,511 IU; vitamin $D_3$, 1,102 ICU; vitamin E, 11.02 IU; vitamin $B_{12}$, 0.01 mg; biotin, 0.11 mg; menadione, 1.1 mg; thiamine, 2.21 mg; riboflavin, 4.41 mg; d-pantothenic acid, 11.02 mg; vitamin $B_6$, 2.21 mg; niacin, 44.09 mg; folic acid, 0.55 mg; choline, 191.36 mg.

[2]Supplied per kilogram of diet: Mn, 107.2 mg; Zn, 85.6 mg; Mg, 21.44 mg; Fe, 21.04; Cu, 3.2 mg; I, 0.8 mg; Se, 0.32 mg.

**Table 2.** Analyzed mycotoxin concentration (mg/kg) and amino acid composition of the basal and nitrogen-free diets (NFD) (as-dry matter basis).

| Item | Basal diet | | | | | | NFD | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | DON1.5 | DON5.0 | FUM20.0 | DON1.5+ FUM20.0 | DON5.0+ FUM20.0 | Control | DON1.5 | DON5.0 | FUM20.0 | DON1.5+ FUM20.0 | DON5.0+ FUM20.0 |
| Mycotoxin, mg/kg[1] | | | | | | | | | | | | |
| DON | 0.2 | 1.3 | 4.3 | 0.3 | 1.3 | 4.3 | 0.0 | 1.4 | 3.7 | 0.0 | 1.3 | 4.1 |
| FUM | 0.9 | 1.0 | 0.9 | 16.3 | 17.7 | 17.4 | 0.0 | 0.0 | 0.0 | 21.9 | 20.6 | 20.1 |
| Indispensable amino acids, % of DM | | | | | | | | | | | | |
| Arginine | 1.65 | 1.67 | 1.54 | 1.65 | 1.71 | 1.72 | – | – | – | – | – | – |
| Histidine | 0.64 | 0.64 | 0.61 | 0.63 | 0.65 | 0.65 | – | – | – | – | – | – |
| Leucine | 2.08 | 2.03 | 1.99 | 2.03 | 2.06 | 2.11 | – | – | – | – | – | – |
| Isoleucine | 1.16 | 1.14 | 1.08 | 1.13 | 1.16 | 1.17 | – | – | – | – | – | – |
| Lysine | 1.84 | 1.92 | 1.78 | 1.82 | 1.85 | 1.86 | – | – | – | – | – | – |
| Methionine | 0.70 | 0.71 | 0.82 | 0.75 | 0.75 | 0.79 | – | – | – | – | – | – |
| Phenylalanine | 1.25 | 1.23 | 1.18 | 1.23 | 1.26 | 1.28 | – | – | – | – | – | – |
| Threonine | 0.95 | 0.95 | 0.96 | 0.96 | 0.99 | 1.00 | – | – | – | – | – | – |
| Tryptophan | 0.29 | 0.29 | 0.29 | 0.29 | 0.30 | 0.29 | – | – | – | – | – | – |
| Valine | 1.22 | 1.21 | 1.13 | 1.17 | 1.21 | 1.22 | – | – | – | – | – | – |
| Dispensable amino acids, % of DM | | | | | | | | | | | | |
| Alanine | 1.18 | 1.19 | 1.15 | 1.17 | 1.19 | 1.20 | – | – | – | – | – | – |
| Aspartic acid | 2.54 | 2.55 | 2.46 | 2.51 | 2.63 | 2.60 | – | – | – | – | – | – |
| Cysteine | 0.37 | 0.39 | 0.39 | 0.36 | 0.41 | 0.39 | – | – | – | – | – | – |
| Glutamic acid | 4.42 | 4.32 | 4.19 | 4.33 | 4.44 | 4.52 | – | – | – | – | – | – |
| Glycine | 1.04 | 1.05 | 0.98 | 1.03 | 1.05 | 1.06 | – | – | – | – | – | – |
| Proline | 1.42 | 1.41 | 1.35 | 1.39 | 1.42 | 1.45 | – | – | – | – | – | – |
| Serine | 1.00 | 0.98 | 1.05 | 1.06 | 1.08 | 1.08 | – | – | – | – | – | – |
| Tyrosine | 0.84 | 0.84 | 0.76 | 0.85 | 0.87 | 0.88 | – | – | – | – | – | – |

[1]Diets samples were analyzed in duplicates for the DON and FUM (B1, B2 and B3) concentration.

and Posselt, 1994). After the feed mixing, each treatment feed sample was collected from 8 different locations in the batch and pooled. The mycotoxins concentrations in each treatment diet were analyzed at Romer Labs (Union, MO) by LC-MS/MS methods and the results are shown in Table 2. All birds were allowed ad libitum access to feed and water with the 24 h light program from days 0 to 15. During the last week, all birds received a 2 h dark period per day in the morning so that enough ileal digesta samples were collected per cage on day 21. Birds were observed twice daily with regards to general flock condition, unanticipated events for the rearing room, and mortality for each cage.

## Growth Performance Data and Sample Collection

Feed and BW were measured by cage on days 0, 8, 15, and 21 for BW gain and mortality-adjusted feed conversion ratio calculation. On day 21, all birds from each cage were euthanized by $CO_2$ inhalation for the sampling of digesta from the lower 2/3 of ileal intestine. The digesta samples were collected by flushing with distilled, deionized water and stored at −20°C before freeze-drying. The dried digesta samples were then ground with a coffee grinder for subsequent DM, gross energy, nitrogen, amino acids, and chromium analyses.

## Nutrient Digestibility

The feed and ileal digesta samples were analyzed for DM at 105°C for 16 h (method 934.01 AOAC International, 2006). The gross energy was determined using the adiabatic bomb calorimeter. The amino acids were determined at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) following AOAC methods [method 982.30 E (a, b, c); AOAC International, 2006]. Nitrogen (crude protein equals nitrogen multiplied by 6.25) was determined using a Fisions 2000 model combustion analyzer (method 990.03; AOAC International, 2000). Chromium was determined using the inductively coupled plasma atomic emission spectroscopy (modified method developed by Perkin Elmer) at the University of Arkansas.

The following equation was used for ileal digestible energy (IDE) calculation (Scott et al., 1982):

$$\mathrm{IDE, kcal/kg} = \mathrm{GE_d} - \mathrm{GE_i}\left(\mathrm{Cr_d}/\mathrm{Cr_i}\right)$$

The following equations were used for apparent ileal digestibility (AID), endogenous losses (EL), and standardized ileal digestibility (SID) calculation (Adedokun et al., 2016):

$$\mathrm{AID, \%} = \left[1 - \left(\mathrm{Cr_d}/\mathrm{Cr_i}\right)\left(\mathrm{N_i}/\mathrm{N_d}\right)\right]100$$

$$\mathrm{EL,\ mg/kg\ of\ DM\ intake} = \left(\mathrm{Cr_d}/\mathrm{Cr_i}\right)\left(\mathrm{N_i}\right)$$

$$\mathrm{SID,\ \%} = \mathrm{AID} + \left[100\ \mathrm{EL}/\mathrm{N_d}\right],$$

where $Cr_d$ represents the concentration of chromium in the diet in milligrams per kilogram; $Cr_i$ represents the

**Table 3.** Growth performance of birds fed diets varying in deoxynivalenol (DON), fumonisins (FUM), and their combination until day 21.

| Item[1] | Control | DON 1.5 | DON 5.0 | FUM 20.0 | DON 1.5 + FUM 20.0 | DON 5.0 + FUM 20.0 | SEM | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Days 1 to 8 | | | | | | | | |
| BW gain, g/bird | 148.4 | 145.6 | 151.1 | 149.3 | 148.1 | 145.7 | 0.86 | 0.44 |
| Feed intake, g/bird | 165.4 | 164.0 | 167.9 | 166.7 | 166.5 | 164.4 | 0.94 | 0.86 |
| Mortality-adjusted FCR | 1.11 | 1.13 | 1.11 | 1.12 | 1.13 | 1.13 | 0.004 | 0.86 |
| Days 8 to 15 | | | | | | | | |
| BW gain, g/bird | 269.5 | 260.0 | 260.5 | 257.3 | 257.2 | 257.5 | 1.50 | 0.13 |
| Feed intake, g/bird | 362.6[a] | 353.4[a,b] | 353.1[a,b] | 351.1[a,b] | 348.2[b] | 350.8[b] | 1.24 | < 0.05 |
| Mortality-adjusted FCR | 1.35 | 1.36 | 1.36 | 1.37 | 1.35 | 1.37 | 0.006 | 0.93 |
| Days 15 to 21 | | | | | | | | |
| BW gain, g/bird | 289.6 | 271.0 | 278.9 | 264.6 | 261.9 | 262.2 | 3.66 | 0.15 |
| Feed intake, g/bird | 419.3[a] | 408.0[a,b] | 414.3[a] | 390.1[b] | 389.8[b] | 391.0[b] | 3.35 | < 0.05 |
| Mortality-adjusted FCR | 1.45 | 1.51 | 1.49 | 1.48 | 1.51 | 1.49 | 0.01 | 0.77 |
| Days 1 to 21 | | | | | | | | |
| BW gain, g/bird | 707.4[a] | 676.6[a,b] | 690.5[a,b] | 671.2[a,b] | 667.2[b] | 666.1[b] | 4.23 | < 0.05 |
| Feed intake, g/bird | 940.6 | 918.6 | 926.8 | 907.9 | 903.2 | 906.0 | 3.90 | 0.05 |
| Mortality-adjusted FCR | 1.33 | 1.36 | 1.34 | 1.35 | 1.36 | 1.36 | 0.005 | 0.30 |
| Mortality, % | 1.25 | 0.00 | 1.25 | 0.00 | 2.50 | 1.25 | 0.23 | 0.34 |

[a,b]Means within a row with no common superscripts differ significantly ($P \leq 0.05$).
[1]Mean represent 8 cages of 10 birds/cage.

concentration of chromium in the ileal content in milligrams per kilogram; $N_d$ represents the concentration of DM, nitrogen, and amino acids in diet in milligrams per kilogram; and $N_i$ represents the concentration of DM, nitrogen, and amino acids in the ileal content in milligrams per kilogram.

### *Statistical Analysis*

All data were analyzed as the one-way ANOVA using General Linear Model via the SPSS Version 23.0 (IBM Corp; Armonk, NY). The cage was used as the experimental unit for growth performance, nutrient and energy digestibility. Means were deemed significant at $P \leq 0.05$ and were separated by Tukey's Multiple Range Tests. *P* value for the mortality was via the Kruskal-Wallis Test. The standard error of the mean was adopted as the measure of error.

## RESULTS

The average mycotoxin concentration of corn used in this study was 0.3 mg/kg for DON and 0.5 mg/kg for FUM B1, B2, and B3. The basal corn−soybean meal starter diet included 54.56% of corn (Table 1), which meant the corn contributed 0.15 mg/kg DON and 0.25 mg/kg FUM for each treatment diet. Based on the results, additional mycotoxin was added separately via premix into each treatment diet to reach the targeted 1.5 mg/kg or 5.0 mg/kg for DON, and 20.0 mg/kg for FUM (Table 2). In addition, the amino acids concentration of the basal and NFDs were also analyzed and showed in Table 2.

### *Growth Performance*

Growth performance results from days 1 to 21 are presented in Table 3. Results showed no significant effect of mycotoxin on growth performance to day 8. From days 8 to 15, both DON and FUM combination treatments (DON 1.5 mg/kg + FUM 20 mg/kg; DON 5.0 mg/kg + FUM 20 mg/kg) impaired feed intake ($P \leq 0.05$) compared with the control treatment. Similar results continued from days 15 to 21 ($P \leq 0.05$), with the control treatment having the highest phase feed intake (419.3 g/bird). However, no significant effects were noted for BW gain or mortality-adjusted feed conversion ratio after adding single or combined mycotoxin in the diets on days 8 and 15. At day 21, cumulative BW gain was less ($P \leq 0.05$) in birds fed diets with DON 1.5 mg/kg + FUM 20 mg/kg (667.2 g/bird) and DON 5.0 mg/kg + FUM 20 mg/kg (666.1 g/bird) compared with the control (707.4 g/bird), with a trend for feed intake ($P = 0.05$) also affected by the combination mycotoxin from days 1 to 21. Mortality was unaffected by treatment, which was low throughout the study and ranged from 0 to 2.50%. From days 15 to 21, among the 6 N-free diet treatments, there were no significant differences on BW gain, feed intake, or mortality-adjusted feed conversion ratio. No mortality was found in birds during the N-free diet period from days 15 to 21.

### *Endogenous Nutrient Loss, Apparent Ileal Digestibility, and Standardized Ileal Digestibility*

The endogenous nitrogen and amino acids losses results are shown in Table 4. Single and combination of mycotoxins had no significant effect on the ileal endogenous amino acids losses. For the apparent ileal digestibility results (Table 5), the control treatment had significantly higher ($P \leq 0.05$) apparent ileal energy digestibility than the DON 5.0 mg/kg + FUM 20.0 mg/kg treatment (3,126 vs. 2,895 kcal/kg). The FUM 20.0 mg/kg, DON 1.5 mg/kg + FUM 20 mg/kg,

**Table 4.** Endogenous nitrogen and amino acid losses (mg/kg of dry matter intake) of the birds fed nitrogen-free diets varying in deoxynivalenol (DON), fumonisins (FUM), and their combination from days 15 to 21.

| Item[1] | Control | DON 1.5 | DON 5.0 | FUM 20.0 | DON 1.5 + FUM 20.0 | DON 5.0 + FUM 20.0 | SEM | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Nitrogen | 2,593 | 2,737 | 2,812 | 2,603 | 2,795 | 2,805 | 126.33 | 0.992 |
| Indispensable amino acids, mg/kg of DM intake | | | | | | | | |
| Arginine | 612 | 703 | 640 | 637 | 676 | 654 | 37.50 | 0.991 |
| Histidine | 261 | 292 | 274 | 262 | 290 | 296 | 14.73 | 0.974 |
| Leucine | 933 | 1044 | 1000 | 963 | 1036 | 1044 | 50.59 | 0.985 |
| Isoleucine | 584 | 644 | 621 | 611 | 651 | 630 | 30.63 | 0.993 |
| Lysine | 796 | 892 | 848 | 799 | 867 | 889 | 53.20 | 0.993 |
| Methionine | 194 | 225 | 201 | 217 | 216 | 223 | 13.03 | 0.982 |
| Phenylalanine | 566 | 629 | 605 | 566 | 616 | 623 | 28.12 | 0.979 |
| Threonine | 851 | 910 | 921 | 847 | 915 | 903 | 34.26 | 0.981 |
| Tryptophan | 102 | 115 | 120 | 98 | 110 | 124 | 6.78 | 0.876 |
| Valine | 849 | 907 | 908 | 855 | 941 | 930 | 40.04 | 0.982 |
| Dispensable amino acids, mg/kg of DM intake | | | | | | | | |
| Alanine | 632 | 695 | 682 | 652 | 694 | 706 | 33.96 | 0.990 |
| Aspartate | 1104 | 1249 | 1127 | 1161 | 1202 | 1178 | 53.75 | 0.984 |
| Cysteine | 355 | 392 | 379 | 405 | 412 | 375 | 12.13 | 0.792 |
| Glutamine | 1480 | 1652 | 1588 | 1514 | 1628 | 1630 | 81.33 | 0.990 |
| Glycine | 696 | 766 | 741 | 735 | 766 | 744 | 34.34 | 0.994 |
| Proline | 746 | 801 | 796 | 772 | 815 | 791 | 30.37 | 0.992 |
| Serine | 681 | 745 | 685 | 686 | 743 | 751 | 30.71 | 0.965 |
| Tyrosine | 471 | 515 | 498 | 484 | 510 | 513 | 20.41 | 0.988 |
| Total amino acids | 13,274 | 14,584 | 14,111 | 13,524 | 14,543 | 14,553 | 620.20 | 0.985 |

[1]Mean represent 7 cages of 10 birds/cage.

**Table 5.** Apparent DM, ileal energy digestibility (kcal/kg) and apparent crude protein (nitrogen multiplied by 6.25), amino acid digestibility (%) of birds fed diets varying in deoxynivalenol (DON), fumonisins (FUM), and their combination on day 21.

| Item[1] | Control | DON 1.5 | DON 5.0 | FUM 20.0 | DON 1.5 + FUM 20.0 | DON 5.0 + FUM 20.0 | SEM | *P*-value |
|---|---|---|---|---|---|---|---|---|
| DM, % | 70.7[a] | 68.5[a,b,c] | 68.6[a,b] | 66.5[b,c] | 65.6[b,c] | 65.0[c] | 0.44 | 0.001 |
| ADE, kcal/kg | 3126[a] | 3050[a] | 3080[a] | 3037[a,b] | 2981[a,b] | 2895[b] | 17.89 | 0.001 |
| Crude protein, % | 80.6 | 79.1 | 79.2 | 79.9 | 79.1 | 78.7 | 0.26 | 0.351 |
| Indispensable amino acids, % | | | | | | | | |
| Arginine | 87.3 | 87.2 | 85.3 | 87.1 | 87.4 | 87.5 | 0.23 | 0.052 |
| Histidine | 83.7 | 83.4 | 82.1 | 82.8 | 83.0 | 82.8 | 0.24 | 0.532 |
| Leucine | 82.9 | 82.1 | 81.1 | 82.1 | 81.9 | 82.3 | 0.25 | 0.475 |
| Isoleucine | 82.3 | 81.7 | 80.0 | 81.4 | 81.3 | 81.6 | 0.29 | 0.330 |
| Lysine | 86.6 | 86.9 | 85.3 | 86.6 | 86.7 | 86.9 | 0.26 | 0.491 |
| Methionine | 92.0 | 92.2 | 92.8 | 93.2 | 92.9 | 93.0 | 0.15 | 0.139 |
| Phenylalanine | 83.1 | 82.4 | 80.9 | 82.2 | 82.2 | 82.4 | 0.26 | 0.294 |
| Threonine | 76.9 | 75.1 | 74.6 | 74.8 | 74.9 | 74.9 | 0.31 | 0.281 |
| Tryptophan | 82.8 | 82.3 | 81.9 | 82.3 | 82.0 | 81.5 | 0.27 | 0.832 |
| Valine | 79.8 | 79.5 | 76.9 | 78.4 | 78.6 | 78.8 | 0.35 | 0.218 |
| Dispensable amino acids, % | | | | | | | | |
| Alanine | 81.5 | 81.4 | 80.0 | 81.1 | 80.9 | 81.0 | 0.27 | 0.719 |
| Aspartate | 80.6 | 80.3 | 80.0 | 78.8 | 79.5 | 79.5 | 0.26 | 0.315 |
| Cysteine | 68.4 | 68.9 | 69.5 | 65.7 | 67.9 | 65.4 | 0.47 | 0.057 |
| Glutamine | 86.6 | 85.9 | 85.0 | 85.5 | 85.8 | 86.1 | 0.22 | 0.424 |
| Glycine | 76.9 | 76.6 | 74.6 | 75.3 | 75.4 | 75.4 | 0.33 | 0.329 |
| Proline | 82.5 | 82.2 | 81.1 | 80.7 | 80.9 | 81.2 | 0.26 | 0.230 |
| Serine | 78.7 | 77.9 | 78.5 | 78.6 | 78.9 | 78.9 | 0.27 | 0.928 |
| Tyrosine | 82.3[a] | 82.1[a,b] | 79.5[b] | 81.9[a,b] | 82.0[a,b] | 82.0[a,b] | 0.27 | 0.020 |
| Total amino acids | 82.6 | 82.1 | 80.9 | 81.7 | 81.8 | 81.9 | 0.24 | 0.536 |

[a-c]Means within a row with no common superscripts differ significantly ($P \leq 0.05$).
[1]Mean represent 8 cages of 10 birds/cage.

and DON 5.0 mg/kg + FUM 20 mg/kg treatments all had significantly lower apparent DM digestibility (66.5, 65.6, and 65.0%, respectively) compared with the control (70.7%). For the apparent amino acid digestibility, no significant difference was found for the indispensable amino acids and dispensable amino acids, except that the control treatment had a higher apparent digestibility of tyrosine than the DON 5.0 mg/kg treatment ($P \leq 0.05$). The standardized crude protein and amino acids digestibility of birds fed with DON and FUM contaminated feed on day 21 are shown in Table 6. No significant difference was found for the standardized crude protein and amino acid digestibility in birds in the study.

## DISCUSSION

The occurrence and risk of mycotoxin contamination is continuously high in livestock industry (Streit et al., 2012, 2013), especially the co-occurrence of

**Table 6.** Standardized crude protein (nitrogen multiplied by 6.25) and amino acid digestibility (%) of birds fed diets varying in deoxynivalenol (DON), fumonisins (FUM), and their combination on day 21.

| Item[1] | Control | DON 1.5 | DON 5.0 | FUM 20.0 | DON 1.5 + FUM 20.0 | DON 5.0 + FUM 20.0 | SEM | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Crude protein, % | 87.0 | 86.0 | 86.2 | 86.3 | 85.9 | 85.2 | 0.44 | 0.929 |
| Indispensable amino acids, % | | | | | | | | |
| Arginine | 91.0 | 91.3 | 89.4 | 91.0 | 91.1 | 90.7 | 0.32 | 0.583 |
| Histidine | 87.8 | 87.8 | 86.6 | 87.0 | 87.2 | 86.8 | 0.36 | 0.915 |
| Leucine | 87.4 | 87.1 | 86.1 | 86.9 | 86.6 | 86.5 | 0.38 | 0.943 |
| Isoleucine | 87.3 | 87.2 | 85.7 | 86.8 | 86.6 | 86.2 | 0.43 | 0.901 |
| Lysine | 90.9 | 91.4 | 90.0 | 91.0 | 91.0 | 91.0 | 0.44 | 0.978 |
| Methionine | 94.8 | 95.4 | 95.2 | 96.1 | 95.7 | 95.7 | 0.24 | 0.708 |
| Phenylalanine | 87.6 | 87.5 | 86.0 | 86.8 | 86.7 | 86.6 | 0.36 | 0.848 |
| Threonine | 85.9 | 84.6 | 84.2 | 83.6 | 84.0 | 83.7 | 0.54 | 0.856 |
| Tryptophan | 86.3 | 86.4 | 86.0 | 85.7 | 85.9 | 85.6 | 0.38 | 0.992 |
| Valine | 86.7 | 86.7 | 85.0 | 85.7 | 86.0 | 85.6 | 0.56 | 0.941 |
| Dispensable amino acids, % | | | | | | | | |
| Alanine | 86.8 | 87.1 | 86.0 | 86.7 | 86.4 | 86.2 | 0.43 | 0.984 |
| Aspartate | 85.0 | 85.0 | 83.5 | 83.5 | 83.7 | 83.4 | 0.38 | 0.682 |
| Cysteine | 78.2 | 78.8 | 79.2 | 76.8 | 78.0 | 74.9 | 0.61 | 0.349 |
| Glutamine | 90.0 | 89.6 | 88.8 | 89.0 | 89.2 | 89.1 | 0.29 | 0.882 |
| Glycine | 83.6 | 83.7 | 82.1 | 82.4 | 82.4 | 81.7 | 0.52 | 0.876 |
| Proline | 87.8 | 87.6 | 87.0 | 86.2 | 86.3 | 86.0 | 0.37 | 0.646 |
| Serine | 85.5 | 85.4 | 85.0 | 85.0 | 85.5 | 85.4 | 0.42 | 0.999 |
| Tyrosine | 87.9 | 88.1 | 86.0 | 87.8 | 87.5 | 87.2 | 0.39 | 0.684 |
| Total amino acids | 87.9 | 87.9 | 86.8 | 87.2 | 87.3 | 87.0 | 0.38 | 0.951 |

[1]Mean represent 7 cages of 10 birds/cage.

mycotoxins being common and frequent in recent years (Murugesan et al., 2015). The DON and FUM, together with aflatoxin, zearalenone, ochratoxin A and T-2 toxins are considered to have significant impact on poultry productivity and health (Murugesan et al., 2015). Previous research has shown that mycotoxin contamination can alter the performance, metabolic enzyme activity, enterocyte integrity and immune response in poultry (Applegate et al., 2009; Antonissen et al., 2014b; Chen et al., 2016a, b; Grenier et al., 2016). However, little research has been conducted to evaluate the combination effect of DON and FUM at sub-clinical concentrations on endogenous nutrient loss and nutrient digestibility in broiler chickens.

Inconsistent results were shown on growth performance after adding DON and FUM in the diet from previous publications. The source of the mycotoxin used in the study ("naturally occurring" vs. "purified" form) may also impact the results. In the past, chickens are considered to have a higher tolerance to DON and FUM than other animal species due input to low digestibility (Murugesan et al., 2015). Reports showed no toxic effect for DON until the dietary concentration of 116 mg/kg in the diet (Morgan et al., 1982). Later publications also mentioned that DON at less than 16 mg/kg and FUM less than 80 mg/kg did not affect the performance in birds (Henry et al., 2000; Eriksen and Pettersson, 2004). In a study that determined the effects of FUM on bird's performance, the performance was not affected until the 7 wk of age (Broomhead et al., 2002). The results from the current study agree with the previously descriptions in the literature, that DON (1.5 mg/kg and 5.0 mg/kg) and FUM (20 mg/kg) in the diet alone did not negatively affect the growth performance in broilers. Dänicke (2002) reviewed that DON may adversely affect the performance and immune response when the concentration is higher than 5.0 mg/kg in poultry. Other researches have reported the BW and DM intake were negatively affected by DON at 5.0 mg/kg under the experimental condition (Lucke et al., 2017). The contrary results on performance results may be related to the year of the study was conducted, strain of the birds used, and actual effective mycotoxin concentration in diet for animal trial. Especially the LC-MS/MS analytical method has rapidly developed, which allows more accurate mycotoxin analytical inclusion concentration results for the animal trial (Murugesan et al., 2015). On the other hand, DON and FUM showed a synergistic effect on phase feed intake and cumulative BW gain by day 21. The DON 1.5 mg/kg + FUM 20 mg/kg and DON 5.0 mg/kg + FUM 20 mg/kg both showed lower BW gain compared with the non-contaminated control treatment.

The impaired growth performance results may be related to the gut physiological changes caused by mycotoxin. Previous work found DON at 1 mg/kg or 5 mg/kg both suppressed the villus growth in jejunum (Awad et al., 2011), which decreased the nutrient absorption surface area and negatively affected the overall nutrient digestibility and BW. The digestive enzyme activities, such as maltase and sucrase, were also affected when the birds ingested with the mycotoxin (aflatoxin) contained feed (Applegate et al., 2009; Chen et al., 2016a). In addition, a review by Grenier and Oswald (2011) indicate most of the mycotoxin studies in poultry showed a synergistic or additive interaction, which when individually fed may not cause negative effects on the performance. The mechanism at the cellular and metabolic level of these synergistic effects was not elucidated in the current study.

Quantification of nutrient and energy utilization of the presence of mycotoxin contamination is greatly lacking. Studies have shown aflatoxin could reduce the nutrient digestibility and energy utilization: Applegate et al. (2009) reported that addition of aflatoxin at 0.6 and 1.2 mg/kg reduced the apparent digestible energy and AMEn in a 2-week laying hen trial, with the reduction through a 6% increase in the maintenance energy requirement of the hen. In the current study, the DON 5.0 mg/kg + FUM 20.0 mg/kg treatment showed a 7% lower apparent ileal energy digestibility. Additionally, both FUM 20.0 mg/kg, DON 1.5 mg/kg + FUM 20 mg/kg, and DON 5.0 mg/kg + FUM 20 mg/kg treatments had significantly lower apparent DM digestibility. It is commonly known that intestine is the major place for nutrient digestion and absorption. Researchers have shown DON and FUM are lowly absorbed in the intestinal tract, which increases the concentration of as it passes distal in the intestinal lumen (Grenier and Applegate, 2013). In the current study, no significant effects on endogenous nutrient loss and standardized digestibility for nitrogen and amino acids were observed. Chen et al. (2016a) noted that aflatoxin, however, increased endogenous nitrogen loss, where the increase may come from the sloughed mucosal layer. The increased mucin and proenzymes contribute adequate substances for the high protein turnover rate of the intestinal epithelium cells, especially during the aflatoxicosis (Cant et al., 1996; Chen et al., 2016a). In a review paper, the author summarized several mycotoxins (aflatoxin, OTA, FUM B1, DON, and T-2 toxin) may affect the trans-epthelial electrical resistance in the intestinal mucosa (Akbari et al., 2016). Besides the effects of DON and FUM on growth and nutrient utilization and digestion, the indirect effects of DON and FUM that are related to the intestinal barrier and immune response should also be taken into consideration. Tight junction proteins are closely related to the function of the intestinal barrier in birds. Reports have shown DON could reduce the gene expression related to tight junction constituent claudin (Van de Walle et al., 2010) and negatively influence the epithelial barrier (Antonissen et al., 2014b). The damage of the gut integrity increases gut permeability and leads to nutrient leakage (such as plasma amino acids) into the intestinal lumen (Antonissen et al., 2014b). Under antibiotic free rearing programs in poultry industry, birds may be predisposed to development of the infectious pathogens, such as the *Clostridium perfringens* induced necrotic enteritis (Grenier et al., 2016). Furthermore, studies have shown *Fusarium* contaminated feed (contained DON, 15-acetyl DON, and zearalenone) involves the alteration of immunoglobulin A and G concentration, as well as the cecal interferon-$\gamma$ gene expression within the coccidial challenge model (Girgia et al., 2008). Other researchers have also demonstrated that purified FUM B1 (98.1% pure) could alter the serum glutamate oxaloacetate aminotransaminase: aspartate aminotransferase ratios and levels of free sphinganine in the serum (Henry et al., 2000).

In conclusion, results from the current study showed that the combination of DON and FUM (DON 1.5 mg/kg + FUM 20 mg/kg or DON 5.0 mg/kg + FUM 20 mg/kg) had negative effects on BW gain from days 0 to 21; whereas the individual additions of these mycotoxins did not impact BW gain in broilers. Adding the combination of mycotoxins (DON 5.0 mg/kg + FUM 20.0 mg/kg) in the diet led to a significant decrease in DM and ileal energy digestibility. However, DON, FUM or their combination had no influence on endogenous amino acid loss and standardized crude protein and amino acid digestibility.

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