CHARACTERISING THE TOXICITY OF FUSARIUM MYCOTOXINS IN NATURALLY CONTAMINATED DIETS ON PERFORMANCE AND HEALTH OF BROILER CHICKENS

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OVERALL ABSTRACT

Fusarium mycotoxins, such as deoxynivalenol (DON), are ubiquitous contaminants of cereal grains. When *Fusarium*-damaged grains are used in feeds, DON and other mycotoxins are a potential health risk to poultry with adverse effects, such as reductions in feed intake, growth, and feed efficiency and increased disease susceptibility. With increasing prevalence of *Fusarium*-contaminated grain worldwide, growing demand for high-quality protein like poultry, and a shift to antibiotic-free poultry production systems, it is important to re-evaluate threshold concentrations for *Fusarium* mycotoxins in poultry diets that are reflected in the current guidance values. Therefore, five animal feeding trials were conducted to better characterize sub-lethal responses to *Fusarium* mycotoxins (primarily DON) in broiler chickens and understand how exposure factors affect broiler responses to contaminated diets. For all trials, experimental diets were prepared with a source of clean wheat (< 0.5 mg/kg DON) or naturally *Fusarium*-contaminated wheat (~ 11.4 mg/kg DON) reflecting diets that would be encountered in broiler production.

In the first study, the effects of timing and duration of exposure to contaminated diets on growth performance and intestinal mucosa structures were examined in a 34-d feeding trial. Starter diets (0.41 and 6.62 mg/kg DON), provided from 1 to 21 day of age (d), and grower diets (0.54 and 7.90 mg/kg DON) provided from 22 to 34 d. Birds fed the DON-contaminated diet over the first 14 d did not exhibit any changes in growth performance; however, growth suppression was observed in the birds fed DON-contaminated diets during the grower phase. Histopathological analysis of the ileal region revealed that birds provided the DON diets throughout the entire trial (1 to 34 d) had shorter villi and shallower crypt than control birds. Based on these results, the grower phase was targeted for the second study where two trials were conducted to determine the effect of short-term exposure (7 days, 21 to 27 d) to DON-contaminated diets on feed preference and feeding behaviour. Three wheat-based experimental diets (control, low and high DON) were prepared and contained 0.14, 2.27 and 5.84 mg/kg DON, respectively. In the preference trial, birds were randomly assigned to one of the three diet pairs (control vs. low, control vs. high and low vs. high DON). In the feeding behaviour trial, birds were randomly assigned to one of the three treatments and received either control, low or high DON diets. The activity of birds was recorded for three 1-h periods daily and birds' behaviour at the feeder was observed. In the preference trial, broilers preferred the control diet over low or

high DON diets. There was no preference indicated between the low vs high DON diets. Behavioural observations revealed that birds fed the DON diet spent more time at the feeder as compared to controls during the observation periods. In the third study, a 28-d pair-feeding trial was conducted to determine whether the performance and gastrointestinal effects of *Fusarium* mycotoxins (primarily DON) are due to a DON-induced reduction in feed intake. Birds were fed either DON-contaminated diets (2.4 to 3.0 mg/kg) or control diets (< 0.1 mg/kg DON). The amount of control diet provided to the pair-fed birds was based on feed consumption of DON treatment at the similar age. During the starter phase, DON-fed birds consumed less feed than control birds while during the grower phase, DON-fed birds had lower feed intake and weight gain than control birds. The pair-fed birds had lower feed intake and weight gain compared to DON-fed birds during the starter period and did not differ during the grower period. Since DON-fed birds and restricted-fed birds with similar (reduced) feed intake had equally suppressed performance, the contribution of dietary DON to growth suppression in broilers is considered independent from other toxic effects of DON. In the final study, a 32-d feeding trial was conducted to determine the efficacy of a yeast cell wall-based feed additive in counteracting the performance effects of mycotoxins in broilers fed a naturally DON-contaminated diet (1.8 to 2.0 mg/kg). The DON-contaminated diet depressed growth performance, while supplementation of the feed additive increased feed intake, weight gain and body weight in birds when included in contaminated diets during the grower stage.

Overall, there was a consistent reduction of feed intake, weight gain and feed efficiency in response to low to moderate concentrations of DON-contaminated diets across the individual feeding trials conducted in this research. Broiler chickens are apparently more sensitive to DON-contaminated diets than previously estimated particularly during later stages of growth. In addition to the performance effects, DON contamination reduces diet palatability and alters broiler feeding behaviour. Although difficult to confirm based on current data, reduced feed intake in broilers is most likely caused by DON-induced sensory aversion and/or anorexia. Based on results of this research, it is recommended to reevaluate the current 5 mg/kg threshold concentrations for DON for poultry to help protect birds from sub-clinical mycotoxin concentrations that can challenge poultry production.

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LIST OF ABBREVIATIONS

3-ADON	3-acetyl deoxynivalenol
15-ADON	3-acetyl deoxynivalenol
ADD	additive
AgRP	agouti-related protein
ANOVA	analysis of variance
AME	apparent metabolic energy
ADG	average daily gain
BW	body weight
CA	California
CCAC	Canadian Council on Animal Care
CFIA	Canadian Food Inspection Agency
CGC	Canadian Grain Commission
cm	centimeter
cm^2	centimeter square
CP	crude protein
cys	cysteine
d	day of age/day post-hatch
DFI	daily feed intake
DON	deoxynivalenol
F:G	feed to gain ratio
FUM	fumonisins
FDK	*Fusarium* damaged kernels
FHB	*Fusarium* head blight
GIT	gastrointestinal tract

g/cm	gram per centimeter
g/d	gram per day
g/kg	gram per kilogram
h	hour
Ig	immunoglobulin
IBV	infectious bronchitis vaccine
IL	interleukin
IU	international unit
kg	kilogram
MB	Manitoba
MA	Massachusetts
MSH	melanocyte stimulating hormone
m	meter
meth	methionine
µg	microgram
µg/kg	microgram per kilogram
µm	micrometer
mg	milligram
mg/kg	milligram per kilogram
NSERC	Natural Sciences and Engineering Research Council
NPY	neuropeptide Y
NDV	Newcastle disease vaccine
ND	not detected
PA	Pennsylvania
PYY	peptide YY

POMC	proopiomelanocortin
SK	Saskatchewan
s	second
s/h	second per hour
SAS	Statistical Analysis Software
SBM	soybean meal
SE	standard error
TNF	tumor necrosis factor
ZEA	zearalenone
α	alpha
β	beta
γ	gamma
°C	degree Celsius

PREFACE

Chapter 1 of this thesis is a general introduction, Chapter 2 is review of the current literature as it pertains to mycotoxins and broiler health, and Chapter 7 is a general discussion with overall major conclusions of the thesis research. Chapters 3 to 6 are organized as manuscripts that are or will be published in peer-reviewed scientific journals; therefore, there may be content that is repeated between the introduction and materials and methods sections across chapters. Chapter 3 has been accepted for publication in *Animal Nutrition*, Chapter 4 under review for publication in *British Poultry Scienc*e whereas Chapters 5 and 6 are being prepared for submission to *Animal Feed Science and Technology*.

CHAPTER 1

GENERAL INTRODUCTION

1. General Introduction

Contamination of cereal crops (such as wheat, rye and barley) with *Fusarium* mycotoxins causes significant economic losses worldwide for both the crop and animal production industries (Grenier and Applegate, 2013). *Fusarium* mycotoxins are fungal secondary metabolites and commonly associated with *Fusarium*-damaged grain products (Kosicki et al., 2016). Cereal crops infected with *Fusarium* fungi are typically down-graded due to mycotoxin contamination and reduced grain quality. Such *Fusarium*-damaged grains cannot be used for human consumption but may be used in animal feed production (Kosicki et al., 2016). Depending on production regions, different sources of cereal grains and corn can be contaminated with wide range of *Fusarium* mycotoxins. Deoxynivalenol (DON) is the most prevalent *Fusarium* mycotoxin present in *Fusarium*-infected grains and dietary exposure in production animals through contaminated feed can result in feed refusal, reduced performance, gut damage and impaired nutrient uptake and immunotoxicity (Grenier and Applegate, 2013).

When *Fusarium*-damaged grains are used in feed processing, DON and other mycotoxins are a potential health risk to poultry. The broiler chicken is an important agriculture animal and has been intensively selected for superior growth performance (Zuidhof et al., 2014). Broilers fed mycotoxin-contaminated diets have demonstrated adverse effects, such as reduction in feed intake, growth or feed efficiency and increased disease susceptibility (Andretta et al., 2011; Awad et al., 2012). Although several studies have evaluated effects of *Fusarium* mycotoxins on broilers, there is significant inconsistency across studies as to the severity of responses and exposure thresholds when negative effects were observed (Awad et al., 2012). Variability in length/timing of exposure, diet formulation and source of wheat, source of *Fusarium* mycotoxins in diet (single spiked or naturally contaminated grain) all attribute to differences in animal responsiveness across studies. Many of the earlier studies actually concluded that poultry were resistant to *Fusarium* mycotoxins, specifically DON (Pestka, 2007b; Grenier and Applegate, 2013). With increasing prevalence of *Fusarium*-contaminated grain worldwide, we will likely see higher inclusion concentrations in animal feed and poultry diets. Understanding the occurrence and prevalence of mycotoxins and their individual as well as combined effects on poultry has become imperative given the rising prevalence of mycotoxins and increased occurrence in animal feed (Canadian Grain Commission (CGC), 2017a). Considering the

industrial aim to raise high-performing broilers under antibiotic-free conditions, we need to focus on optimizing production systems and feed quality while reducing the impact of stressors such as dietary mycotoxins. Therefore, it is important to re-evaluate threshold concentrations for *Fusarium* mycotoxins in chicken feed which are reflected in the current applicable guidance values. Evaluating important factors involved in *Fusarium* mycotoxicosis can provide more in-depth understanding about the *Fusarium* mycotoxins toxicosis in broilers and aid in developing strategies to reduce or prevent negative impacts on broiler performance and health.

CHAPTER 2

LITERATURE REVIEW

2.1 *Fusarium* fungi and associated mycotoxins

Contamination of food and feedstuffs by fungi and associated mycotoxins is a global issue and is of considerable importance for both the human and animal health sectors. *Fusarium* head blight (FHB) is a prominent fungal disease of many cereal-based crops such as wheat and barley. In North America, FHB is responsible for losses exceeding $2 billion within the wheat industry alone (Kelly et al., 2015). In Canada, highly pathogenic *Fusarium* graminearum, *F*. avenaceum and *F*. culmorum are common causes of *Fusarium* infections in cereal grains and corn (Miller and Richardson, 2013; CGC, 2017a; b). The pathogenic *Fusarium* species found in infected grains varies across geographical regions (Miller and Richardson, 2013; CGC 2017a; 2017b). Crops with a significant proportion of *Fusarium*-damaged kernels (FDK) are typically down-graded due to reduced grain quality and the potential for high concentrations of mycotoxins, the toxic secondary metabolites produced by the fungi (Kosicki et al., 2016). The increased presence of FDK is not necessarily correlated with the concentration of specific mycotoxins but measuring occurrence of FDK in grain samples does provide a rapid and economical measurement to evaluate the risk of mycotoxin contamination (Liu et al., 1997; Kautzman et al., 2015b).

In Canada, *Fusarium* mycotoxins were found in all corn samples and more than 90% of wheat samples during 2010 to 2012 crop years (Tittlemier et al., 2013). The mycotoxins detected in FDK are present in a mixture and varies based on the host plant, growth environment and fungicide application (Miller and Richardson, 2013; Guerre, 2016). The most prevalent mycotoxins in FDK include trichothecenes, zearalenone (ZEA) and fumonisins (FUM) (Miller and Richardson, 2013; Guerre, 2016). Trichothecenes are a group of more than 180 mycotoxins (Wu et al., 2010). Among trichothecenes, T-2, HT-2 Toxin, deoxynivalenol (DON), 3 – acetyl and 15 – acetyl DON (3 – and 15 – ADON) are most frequently detected and present the most serious threat to animal agriculture (Wu et al., 2010; Döll and Dänicke, 2011; Li et al., 2011). Moniliformin, beauvericin and enniatins have also been isolated and identified and are referred to as ‘emerging’ mycotoxins (Meca et al., 2010; Roig et al., 2014). As they are only recently recognized as mycotoxins, there are limited data about the presence of these mycotoxins in animal feed and their effects on animal production.

2.2 Occurrence of trichothecene mycotoxins in animal feed

Deoxynivalenol is the most prevalent *Fusarium* mycotoxin present in Canada and is used as an indicator of the presence of other mycotoxins, such as DON's acetyl derivatives and other trichothecenes (Miller et al., 2014). Deoxynivalenol can be present in animal feed over a range of concentrations and can induce both acute and chronic toxic effects. Acute effects due to high concentrations of DON results in diarrhea, vomiting, leukocytosis and hemorrhage, while the chronic effects of lower concentrations of DON intake include anorexia, neuroendocrine changes, immune modulation, reduced weight gain and feed efficiency (Pestka et al., 2004). The occurrences of acute mycotoxicosis caused by high concentrations of *Fusarium* mycotoxins (primarily DON) are rare; the low dose, chronic dietary exposure to mycotoxins is a more realistic concern in animal agriculture (Grenier and Applegate, 2013).

2.2.1 *Fusarium* mycotoxins and DON in poultry feed

Fusarium-mycotoxin contaminated grains that are not suitable for human consumption may still be used in animal feed production (CGC, 2015). Among agricultural animals, poultry are considered to have a relatively high tolerance to dietary *Fusarium* mycotoxins, especially DON, as compared to swine (Richard, 2007; Grenier and Applegate, 2013). This means that naturally DON-contaminated grains which are not suitable for swine feed production may be still used to produce broiler feed. Therefore, poultry may be more frequently exposed to DON-contaminated diets, especially when compared to swine.

The broiler chicken is an important class of poultry, selected for superior growth performance and require high quality diets to reach their growth potential (Havenstein et al., 2003). However, *Fusarium* mycotoxins (primarily DON) are frequently occurring contaminants in poultry feed (Kosicki et al., 2016). In Canada, the maximum dietary allowance for DON is 5 mg/kg for poultry (Canadian Food Inspection Agency (CFIA), 2015). However, some recent studies report that growth suppression was observed in broilers fed low concentrations of DON naturally-contaminated diets (< 5 mg/kg) (Awad et al., 2011; Yunus et al., 2012b; Lucke et al., 2017).

Moreover, the CFIA allowance is set for individual mycotoxin and *Fusarium*-damaged grains are frequently contaminated with multiple mycotoxins, such as DON, 3–ADON and 15–ADON (Kosicki et al., 2016). Exposure to multiple mycotoxins may induce more severe adverse effects than exposure to a single mycotoxin (Richard, 2007; Grenier and Applegate, 2013).

2.3 Effects of Fusarium mycotoxins on broiler chickens

The most frequently observed effects of feeding broilers *Fusarium* mycotoxin-contaminated (primarily DON) diets include alteration of gastrointestinal tract mucosa structure, immune suppression, reduction in feed consumption and growth (Andretta et al., 2011; Awad et al., 2012). The studies reporting such effects and their implications for broiler health are reviewed in detail in the following sections.

2.3.1 Gastrointestinal tract morphology and function

The gastrointestinal tract (GIT) is the site of nutrient digestion and absorption and a fully functioned GIT is essential for broiler chickens to reach their growth potential (Jin et al., 1998; Denbow, 2015). The gastrointestinal tract is also the first organ exposed to orally-administrated mycotoxins (Maresca, 2013). Feeding broilers DON-contaminated diets, either through spiking with DON or inclusion of naturally-contaminated grain, can alter small intestinal morphology by reducing intestinal villi height, villi surface area and increasing villi to crypt ratio in duodenal and jejunal regions. There is also *in vitro* evidence of DON specially interfering with glucose and amino acid transporter functions in the duodenum and jejunum (Awad et al., 2006b; 2011; Yunus et al., 2012a) and these studies provide a mechanistic basis for the reduction in nutrient absorption in the GIT. Reduced nutrient uptake in the small intestine reduces energy and nutrient availability which can consequently reduce growth (Grenier and Applegate, 2013). Furthermore, exposure to naturally DON-contaminated diets also reduces GIT barrier function by suppressing intestinal mucin production and inducing inflammatory responses, specifically increasing proinflammatory cytokines (Antonissen et al., 2015; Grenier et al., 2016). This reduction of mucosal barrier function subsequently increases broiler susceptibility to feed-borne pathogens like coccidiosis (Grenier et al., 2016). Therefore, growth suppression exhibited by DON-fed broilers may be caused by both reduced nutrient absorption as well as increased susceptibility to

feed-borne pathogens. Feed that is naturally contaminated with DON typically exerts a stronger toxic effect in animals when compared to feeding artificially contaminated (or spiked) with pure DON (Dersjant-Li *et al.*, 2003). In naturally-contaminated grains, DON commonly co-occurs with other mycotoxins including its acetyl derivatives, 3–ADON and 15–ADON (Kosicki et al., 2016). In an *in vitro* study with human colon epithelial (Caco-2) cells, Alassane-Kpembi et al. (2013) observed that binary combinations of DON, 3–ADON and 15–ADON were synergistic at low concentrations and therefore, may be more toxic than predicted from the effects of the mycotoxins alone. Therefore, feeding broilers naturally-contaminated diets may induce more severe adverse effects on GIT as compared to feeding artificially-contaminated or spiked diets.

The oral bioavailability of DON and its' acetyl derivatives is less than 20% in broilers (Osselaere et al., 2013; Broekaert et al., 2015). The low oral bioavailability may be because broiler GIT microbes convert toxic DON into less toxic microbial metabolic products (Maresca, 2013). The low DON oral bioavailability also indicates that broiler intestinal epithelial cells are subjected to significantly higher concentrations of orally-administrated DON than other organs, such as spleen and liver (Maresca, 2013; Broekaert et al., 2015). This hypothesis is supported by a study which indicates that alterations of GIT mucosa structures were observed at relatively low concentrations of DON, even before an impairment of growth performance and changes in liver and spleen weights were observed (Awad et al., 2011). Therefore, morphological changes in intestinal structures are frequently used as a sensitive biological endpoint to evaluate dietary DON toxicosis in poultry.

The intestinal mucosal structures are rapidly developing in broiler chickens immediately after hatch. A rapid increase in villi height, crypt depth, villi surface area, absorptive capacity and barrier function occurs during the first 14 days post-hatch (Uni et al., 1999; Iji et al., 2001). During intestinal development, epithelium cells are rapidly proliferating and have high metabolic rates (Iji et al., 2001; Denbow, 2015). It is suggested that cells with high turn-over and metabolic rates are most sensitive to DON (Maresca, 2013; Pinton and Oswald, 2014) so it is likely that intestinal epithelial cells in young broilers (< 14 day (d)) are more susceptible to DON-contaminated diets compared to older broilers (> 14 d). Therefore, feeding young broilers DON-contaminated diets may induce more severe negative effects on GIT and consequently have a greater growth suppressive effect compared to older birds.

2.3.2 The immune system and disease susceptibility

A fully functioning immune system is essential for the modern broiler chicken to maintain adequate health and growth (Korver, 2012). *Fusarium* mycotoxins have well-documented immunomodulatory effects and the immune system is often considered the most sensitive physiological system to DON toxicity (Maresca, 2013). Immune suppressive effects of dietary DON exposure in broilers include increased disease susceptibility, reduced antibody responses and vaccine failure (Awad et al., 2013; Osweiler, 2014). A recent meta-analysis to examine the association of mycotoxins with performance, productive indices, and organ weights in broilers reported that dietary DON exposure can increase flock mortality by 8.8 times (Andretta et al., 2011), possibly because DON-induced immune suppression makes broilers more susceptible to infectious diseases. Similar to DON-induced GIT mucosal alteration, immune suppressive effects are observed at relatively low concentrations of DON, even before impairment of growth performance is observed (Awad et al., 2013).

Mycotoxin-induced immunosuppression may result in decreased host resistance to infectious disease and decrease vaccine efficacy. Reduction of antibody response to infectious bronchitis vaccine (IBV) and Newcastle disease vaccine (NDV) were observed in broilers fed diets naturally contaminated with DON (Dänicke et al., 2003; Ghareeb et al., 2012; Yunus et al., 2012b). The reduction of cell-mediated immune responses were also observed in broilers fed DON-contaminated diets (Girish and Smith, 2008). However, there is also evidence where broiler antibody response to IBV (Swamy et al., 2002), NDV (Yegani et al., 2006), sheep red blood cells (Swamy et al., 2004a) and cell-mediated immune responses (Swamy et al., 2004a) were not affected by feeding DON-contaminated diets. The inconsistency among studies may be due to differences in the source of dietary DON (artificial vs. naturally-contaminated), concentrations of mycotoxins (primarily DON and ranging from 2 – 14 mg/kg) and length of exposure (ranging from 14 – 56 d) (Awad et al., 2013).

The gastrointestinal tract is also an important immune organ and provides the first line of defense against feed-borne pathogens (Smith et al., 2014; Panda et al., 2015). Dietary DON exposure induces apoptosis of gut-associated immune cells, reduction of barrier function and increases GIT permeability to feed-borne pathogens (Grenier and Applegate, 2013; Maresca, 2013; Ghareeb et al., 2015). Coccidiosis is an economically-important disease in broiler production and is caused by the feed-borne pathogens *Eimeria* spp. (Abid et al., 2016). Low

concentration *Fusarium* mycotoxin-contaminated diets (< 5 mg/kg DON) did not affect growth performance in non-challenged broilers, but induced more severe growth suppression and intestinal lesions in the coccidiosis-challenged birds (Girgis et al., 2010a; 2010b; Grenier et al., 2016). These studies concluded that low concentrations of naturally *Fusarium* mycotoxins-contaminated diets (without causing feed refusal and growth suppression) are serious threats to broiler health and should be avoided in broiler production (Girgis et al., 2010a; 2010b; Grenier et al., 2016).

Many countries are moving towards eliminating subclinical dosage of antimicrobials as growth promoters in animal agriculture and this may lead to increased incidence of disease outbreaks (Diarra and Malouin, 2014; Abid et al., 2016). Antimicrobial use reduction emphasizes the importance of maximizing broilers' immune functions to combat pathogen challenges (Korver, 2012). Low concentrations of naturally-occurring trichothecenes (≤ 5 mg/kg DON) may not be sufficient to cause growth suppression in broilers, but can still impair broiler immune functions and increase disease susceptibility (Awad et al., 2013; Grenier and Applegate, 2013; Grenier et al., 2016). In order to maintain adequate flock health and maximize growth performance, low concentrations of mycotoxin-contaminated diets, with particular attention to DON, should be avoided in broiler production.

2.3.3 Regulation of feed intake and behaviour

2.3.3.1 Broiler feed intake regulation

Chickens regulate feed intake through complex neurochemical pathways, involving multiple organ systems such as the central nervous immune and digestive systems (Richards and Proszkowiec-Weglarz, 2007; Scanes, 2015). The hypothalamus, a part of the central nervous system, is considered critical for poultry feed intake regulation (Richards and Proszkowiec-Weglarz, 2007; Classen, 2016). The hypothalamus not only produces various feed intake regulatory neuro-endocrine chemicals, but also integrates the feed intake regulatory signals/biological active compounds produced by the peripheral organs such as immune organs and digestive system (Richards and Proszkowiec-Weglarz, 2007; Song et al., 2013).

Various orexigenic neuro-endocrine chemicals such as neuropeptide Y (NPY), agouti-related protein (AgRP), peptide YY (PYY), anorexic neuro-endocrine chemicals such as

serotonin, proopiomelanocortin (POMC), α/β- melanocyte stimulating hormone (MSH), and cholecystokinin (CCK) play important roles in feed intake regulation in poultry (Richards and Proszkowiec-Weglarz, 2007; Song et al., 2013). Proinflammatory cytokines such as IL-1β, IL-6 and tumor necrosis factor-α (TNF-α) concentrations are also involved in mediating appetite (Tachibana et al., 2017). In general, elevating orexigenic neuro-endocrine chemicals increase chicken appetite and initiate eating behaviours, while increasing anorexigenic neuro-endocrine chemicals and/or proinflammatory cytokines suppress chicken appetite and terminate the eating behaviours (Richards and Proszkowiec-Weglarz, 2007; Tachibana et al., 2017).

Broilers and laying hens are two important poultry types. Compared to laying hens, broiler chickens have significantly higher feed intake (Bokkers and Koene, 2003). The higher feed intake in broilers is not achieved by increasing hypothalamic expression of orexigenic neuropeptides or increasing sensitivity to orexigenic neuropeptides (Yuan et al., 2009; Saneyasu et al., 2011) but is achieved by reducing birds' hypothalamic expression and sensitivities to anorexia neuropeptides (Honda et al., 2012). Other studies also indicate that suppression of immune responses, such as inflammatory responses also contribute to increased feed intake in broilers (Van Der Most et al., 2011; Korver, 2012). Broiler feed intake is also affected by multiple dietary factors, such as different levels of energy, proteins, amino acids, dietary form and the presence of antinutritional factors, such as *Fusarium* mycotoxins (Korver, 2012; Classen, 2016).

2.3.3.2 Modulation of feed intake by mycotoxins

Reduction of feed intake is one the most well-known responses observed in animals that are fed DON-contaminated feed and is frequently referred to as feed refusal (Vesonder et al., 1973; Osweiler, 2014). In studies with rodents, high concentrations of DON (2 – 25 mg/kg BW) were administrated through oral gavage to look at effect of trichothecenes on feed refusal (Flannery et al., 2011; Girardet et al., 2011; Wu et al., 2013; 2014). After oral gavage, upregulation in the expression of the hypothalamic anorexia neurochemical POMC was observed, whereas orexigenic NPY and AgRP mRNA expression remained unchanged (Girardet et al., 2011). The elevated plasma concentrations of gut-origin anorexia neurochemical PYY and CCK were also observed following oral gavage (Flannery et al., 2012; Wu et al., 2014). These elevated concentrations of anorexia neurochemicals corresponded with reduction of feed intake

observed in the same studies (Girardet et al., 2011; Flannery et al., 2012; Wu et al., 2014). Increased expression of IL-1β, IL-6, and TNF- α in the hypothalamus, spleen and the liver as well as increased plasma concentrations of IL-1β, IL-6, and TNF-α were also reported in rodents after DON oral gavage (Wu and Zhang, 2014). The cytokines, IL-1β, IL-6, and TNF-α have well-documented anorexigenic effects and their elevation corresponded with a reduction in feed intake observed in the same study (Wu and Zhang, 2014). Taken together, these studies suggested that DON-induced feed refusal are the result of elevated anorexigenic neurochemicals and/or inflammatory responses.

The DON oral gavage studies with rodents provide insight into the mechanisms of DON-induced feed refusal. However, oral gavage with a single trichothecene does not completely reflect the challenges faced in animal production where animals are more frequently exposed to a mixture of mycotoxins for long periods (days to weeks) (Grenier and Applegate, 2013). The second concern is that the trichothecenes were directly delivered at relatively high doses (mg/kg BW) in the oral gavage studies. Under a production setting, animals would be more typically exposed to low concentrations of DON-contaminated diets (μg/kg BW) (Grenier and Applegate, 2013; Lebrun et al., 2015). The third consideration is that although feed regulation in poultry is very similar to mammals, there are some significant differences. Peptide YY is a good example of a neurochemical that has opposite effects in mammals and poultry. In mammals, PPY has potent anorexigenic effects, but it is orexigenic in poultry (Richards and Proszkowiec-Weglarz, 2007). Therefore, specific studies into the effects of naturally DON-contaminated diets on feed intake regulation in poultry is warranted. In the few studies that focus on poultry, elevated serotonin concentrations (Swamy et al., 2004b) and upregulated IL-1β, IL-6, and TNF-α expression (Awad et al., 2013; Grenier et al., 2016) were observed in broilers fed DON-contaminated diets and corresponded with the reduction in feed intake and growth suppression observed in related studies (Swamy et al., 2002; Awad et al., 2011; Grenier et al., 2016). The reduction of feed intake observed in the DON-fed broilers could be attributed to anorexia effects and/or inflammatory responses.

Reduced diet palatability and induced taste aversion is another proposed mechanism by which DON-contamination causes feed refusal (Osweiler, 2014). In two early studies, both laying hens and broilers that were provided a choice between uncontaminated feed and naturally DON-contaminated feed, demonstrated a clear preference towards the uncontaminated feed

(Hamilton et al., 1986; Mannion and Blaney, 1988). In the broiler study, Mannion and Blaney (1988) only measured broiler feed preference on a weekly basis so it remains unclear how rapidly broilers develop preference towards uncontaminated feed (immediate vs. after prolonged exposure). The contaminated feed contained 11 mg/kg DON which is higher than recommended limits for DON in poultry feed. Overall, the predominant action of DON to elicit feed refusal in broilers (through taste aversion, anorexia or immune modulation) remains largely unknown.

2.3.3.3 Feeding behaviour

Feeding behaviours, such as meal length, meal size and meal frequency are considered a reflection of internal feed intake regulation mechanisms (Reddingius, 1980). Bokkers and Koene (2003) examined broiler feed intake regulation (hunger vs. satiety mechanism) by examining broiler feeding behaviours with *ad libitum* feeding and with feed deprivation. They concluded that broiler feed intake regulation is dominated by satiety mechanisms and broilers tend to eat until their maximal physical GIT capacity is reached. Honda et al. (2012) compared concentrations of feed intake regulatory neurochemicals in the hypothalamus of both broilers and laying hens. Broilers' hypothalamic expression of and sensitivity to anorexigenic neurochemicals, such as POMC and α/β-MSH, were suppressed compared to laying hens (Honda et al., 2012). These findings support the results of Bokkers and Koene (2003) and Honda et al. (2012) who also demonstrated that observing feeding behaviours can provide insight to feed intake regulation in broiler chickens.

Observing changes in behaviour can provide a rapid and sensitive measure of sub-lethal toxin exposure (Melvin and Wilson, 2013). However, feeding behaviours have never been fully examined in poultry given DON-contaminated diets. In previous long-term feeding studies, broiler growth performance and feed intake is often evaluated in a weekly basis (Awad et al., 2011; Yunus et al., 2012b; Lucke et al., 2017). These growth performance parameters have significant economic importance but do not capture dynamic information such as changes in feeding behaviours and how rapidly birds exhibit feed refusal (immediately or after prolonged exposure). How birds modify their feeding behaviour with mycotoxin-contaminated diets and the mechanisms by which DON exerts its action on feed intake remain largely unknown. Behavioural observations would provide valuable information to better understand how such a commonly encountered mycotoxin can modify feed intake in poultry.

To date, most of the studies to evaluate the effects of oral trichothecenes exposure on feeding behaviours have been conducted with rodents. Changes in feeding behaviours, such as reduction in meal size and meal frequency, were observed in rodents after acute oral exposure to T-2 (Gaigé et al., 2014) and DON (Girardet et al., 2011). Low concentrations of T-2 (0.5 mg/kg BW) suppressed meal size but not meal frequency (Gaigé et al., 2014), while high concentrations of T-2 (2-5 mg/kg BW) and DON (3-12.5 mg/kg BW) suppressed both meal size and frequency (Girardet et al., 2011; Gaigé et al., 2014), suggesting that alteration in behaviour is somewhat dependent on trichothecene dose. Oral gavage of T-2 and DON also elevated the anorexigenic neurochemical CCK (Flannery et al., 2011; Wu et al., 2014) and cytokines (Gaigé et al., 2014; Wu and Zhang, 2014) in rodents. Subcutaneous administration of CCK induced satiation in rodents and reduced meal size but did not affect meal frequency (Hsiao and Wang, 1983), while injections of lipopolysaccharide and proinflammatory cytokines induced inflammation in rodents and reduced both meal size and meal frequency (Langhans et al., 1993; Langhans, 2000). Based on studies with rodents, trichothecene exposure appears to modulate feed intake through changes in brain neurochemistry and specifically orexigenic and/or inflammatory responses (Lebrun et al., 2015). It has been suggested that *Fusarium* mycotoxin-induced feed refusal in poultry is due to changes in brain neurochemistry (Swamy et al., 2004b; Girish et al., 2008a); however, there have been no studies conducted that actually evaluate whether basic feeding behaviours in poultry are altered when birds are provided *Fusarium*-mycotoxin contaminated diets.

3.3.5 Nutrient utilization and performance

The effects of *Fusarium* mycotoxins-contaminated feed and specifically DON on broiler performance were examined in several studies; however, results across studies were inconsistent. Overall, meta-analytical data suggest a reduction of weight gain of 1.2% per mg DON per kg diet (Andretta et al., 2011) and a review by Awad et al. (2012) concluded that feeding broiler chickens *Fusarium* mycotoxin-contaminated feed (primarily DON) negatively affects growth performance by suppressing feed intake, weight gain and reducing birds' body weight. However, effects of DON-contaminated diets (both spiked and naturally contaminated) on broiler growth performance are inconsistent among the individual studies. The reduction of feed intake and weight gain were observed in broilers fed 2 – 12 mg/kg DON in naturally-contaminated diets (Ghareeb et al., 2012; Yunus et al., 2012b; Lucke et al., 2017). Conversely, other studies report

that feeding broilers around 5 mg/kg DON in naturally-contaminated diets did not affect feed intake and weight gain (Awad et al., 2006b; Antonissen et al., 2015). The inconsistent effects of contaminated diets on broiler growth performance may be because broilers are considered relative resistant to DON when compared to other monogastrics, especially swine (Richard, 2007; Grenier and Applegate, 2013). The inconsistency among studies may also be caused by various factors, such as different sources of contamination (spiked vs naturally contaminated), duration of experimental period, different feed composition and genetic background of birds. As stated previously, a reduction in growth performance may be caused by DON inhibiting the function of amino acid and glucose transporters in the intestinal mucosa and thus reduces nutrient absorption (Awad et al., 2004; 2014). This concept is supported by a meta-analytical study which indicates that supplementation of extra nutrients (crude protein or amino acids such as methionine) to DON-contaminated diets improved broiler weight gain (Andretta et al., 2011).

Broiler feed efficiency is an important production parameter and can be used to estimate the broiler nutrient utilization (maintenance vs. growth). However, effects of naturally DON-contaminated diets on broiler feed efficiency are also inconsistent among studies (Grenier and Applegate, 2013). Many studies report no change in feed efficiency after broilers were fed trichothecene-contaminated diets (Swamy et al., 2002; 2004a; Awad et al., 2011), while other studies conclude that feeding broilers DON-contaminated diets reduce feed efficiency in broilers (Dänicke et al., 2002; Mohaghegh et al., 2017). The wide range of DON concentrations in diets across studies (2 – 14 mg/kg), varying feed composition, different duration of exposure as well as the source of contamination (spiked vs naturally contaminated) may account for some of the variation in effects on feed efficiency. Specifically, DON-induced effects on feed intake versus weight gain (the two parameters in feed efficiency) may differ depending on DON concentration. At lower concentrations, DON suppresses broiler growth through inducing feed refusal which is thought to be a protective mechanism that limits the total mycotoxin intake (Osweiler, 2014), while at higher concentrations DON may induce additional direct systemic toxicity, such as metabolic alteration and intestinal damage.

Improved protein digestion, feed efficiency and even enhanced growth have been observed in broilers fed naturally DON-contaminated diets (Dänicke et al., 2003; 2007; Yunus et al., 2012b). Similar improvements of dietary crude protein and starch digestibility were also observed in the pigs fed naturally DON-contaminated diets and is suggested to be a

compensatory mechanism which the animal develops to adjust for the reduction in feed intake (Goyarts et al., 2005). *Fusarium* fungi infections also cause changes in grain starch granule structures and damage storage proteins (Jackowiak et al., 2005; Schmidt et al., 2016) and it is hypothesized that this can increase starch availability for animals, increase nutrient digestion and improve feed efficiency (Grenier and Applegate, 2013).

It can be concluded that changes in feed intake and performance following intake of diets naturally-contaminated with DON are inconsistent between different studies. This may be caused by multiple factors, such as differences in diet formulation, timing and duration of exposure, levels of feed intake, and co-contaminations of other contaminants/pathogens (Grenier et al., 2016). Examining the relative importance of these factors in DON-induced growth suppression can assist to refine experimental design and reduce inconsistency for future research. Moreover, this information may help target mycotoxin mitigation strategies to improve animal health and reduce economic loss in the production setting. Some examples of such strategies include avoiding mycotoxin exposure during the critical sensitive periods of broiler growth, providing extra nutrients to overcome DON-induced deficiency, and enhancing birds' appetite to lessen the impact of feed refusal.

2.4 Factors influencing *Fusarium*-mycotoxin toxicity in broilers

2.4.1 Timing of dietary trichothecene exposure

Deoxynivalenol and other *Fusarium* mycotoxins are not evenly distributed in naturally-contaminated grains and thus, during formulation, a specific proportion of completed feed can be contaminated with significantly higher concentrations of mycotoxins (Whitaker et al., 2010; Turner et al., 2015). Multiple diets (e.g. starter, grower, finisher) are commonly used in broiler feeding program and birds are fed different diets corresponding to their ages (Aviagen, 2014c). This means that broilers can be subjected to dietary mycotoxins during a specific part or throughout the entire of production cycle. In addition, duration of exposure and exposure concentration have important implications on severity of effects observed with dietary mycotoxin exposure. For example, chronic exposure is most associated with decreased performance and nutritional efficiency and in swine, intake of 1 to 2 mg/kg vs. 12 mg/kg DON

can mean the difference between decreased feed intake and total feed refusal (Pierron et al., 2016).

Naturally DON-contaminated diets were frequently provided to broiler chickens through the entire trial period in the previous broiler feeding studies [e.g. 1 – 35 days (Yunus et al., 2012b) and 1 – 56 days (Swamy et al., 2002; 2004a)] and therefore, may not completely reflect the mycotoxins challenges faced by industry. Reduction of feed intake and growth suppression were observed during mid (21 – 42 d, Swamy et al., 2004a) and later (43 – 56 d, Swamy et al., 2002) growth stages in the previous studies. The growth suppression observed by Swamy et al. (2002, 2004a) suggest that mycotoxins (primarily DON) may have an accumulative toxic effect on broiler feed intake and growth performance. The occurrence of mycotoxin-induced broiler growth suppression during the later stages of growth may be also because the older broilers (22 – 56 d) consume significantly more feed than younger birds (1 – 21 d) (Aviagen, 2014c; Zuidhof et al., 2014). However, these hypotheses cannot be fully answered based on these previous studies as the birds were fed the contaminated diets through the entire experimental period. Identifying the critical period of sensitivity for growth performance effects of DON-contaminated feed in broilers is necessary in order to better understand the mycotoxin challenges faced in commercial production.

2.4.2 Reduction of feed intake and growth suppression

Broiler chickens are selected for superior growth performance and require *ad libitum* access to a nutrient-balanced diet to reach their growth potential (Havenstein et al., 2003). As reviewed previously, broilers respond to DON-contaminated diets by reducing their feed intake, which is thought to contribute to the subsequent growth suppression (Awad et al., 2012). Feed refusal is one of the most well-known responses to trichothecene exposure in animals (Vesonder et al., 1973; Osweiler, 2014). The basis of mycotoxin-induced feed refusal has not been fully elucidated but is thought to be a protective mechanism that prevents animals from developing further trichothecene toxicosis, such as alteration of GIT functions and disruption of metabolism (Osweiler, 2014). However, feed refusal also reduces total dietary nutrient intake and prevents broilers from achieving their growth potential leading to decreased growth performance. In previous studies reporting DON-induced reduction in feed intake and growth suppression, broilers were fed DON-contaminated diets *ad libitum* (Awad et al., 2011; Yunus et al., 2012b)

and this makes it difficult to ascertain whether performance effects of DON are attributed to decreased feed intake or other aspects of DON toxicity such as alteration of intestinal function and disruption of metabolism.

Separating the effects of DON on feed intake from the other DON toxicities could also provide potential methods to mitigate these adverse effects (increase birds' feed intake vs. reduce dietary mycotoxin toxicities). Consumption of the same amount of feed (i.e. a paired-feeding regimen) is necessary to differentiate the direct effect of DON from the consequence of reduced feed intake. A paired-feeding approach was previously used in a swine feeding experiment and the study concluded that DON-induced growth suppression was the result of reduction in feed intake (Goyarts et al., 2005). Conversely, paired-feeding with rainbow trout demonstrated that DON-induced growth suppression was the result of both a reduction in feed intake and nutrient utilization (Hooft et al., 2011). Similar studies in poultry have yet to be conducted and could provide further understanding about DON-induced growth suppression and direct potential methods to mitigate these adverse effects (increase birds' feed intake vs. reduce dietary mycotoxin toxicities).

2.5 Strategies to counteract *Fusarium* mycotoxins

Economic losses and health concerns caused by increased occurrence of mycotoxin contamination have spurred research to explore various methods as mitigation strategies. The adverse effects in livestock and poultry associated with ingestion of mycotoxin-contaminated feed can be reduced through the use of physical, chemical, nutritional, or biological approaches. In general, methods used to reduce *Fusarium* mycotoxins can be classified into two main categories: pre-harvest and post-harvest methods. The pre-harvest methods focus on limiting and preventing *Fusarium* fungi infections before cereal gains are harvested (Luo et al., 2018; Peng et al., 2018). The post-harvest mycotoxin control methods include removing *Fusarium* damaged kernels, incorporating mycotoxin absorbents/binders into feed or applying biological treatments to feed to convert mycotoxins to less toxic compounds (Zhu et al., 2016; Alberts et al., 2017). In the following section, some post-harvest methods to reduce or detoxify *Fusarium* mycotoxins are discussed.

2.5.1 Removing *Fusarium*-damaged kernels

Ideally, contaminated grains would be avoided completely when formulating diets for livestock. Removing *Fusarium*-damaged grains, which are typically associated with having higher trichothecene mycotoxin concentrations, through various sorting methods is a rapid method to reduce mycotoxin contamination. Current sorting methods can be separate into two major categories: (1) sorting grains in bulk using centrifugation force and flotation in air flow, and (2) removing the individual FDK from healthy kernels by using an optical sorter or near infrared sorter (Karlovsky et al., 2016). It should be noted that the presence of FDK may not indicate the actual concentration of mycotoxin contamination – *Fusarium* mycotoxins may not be evenly distributed among damaged kernels with some less damaged kernels being contaminated with higher concentrations of mycotoxins (Liu et al., 1997; Beyer et al., 2010). However, sorting and removing damaged grains is still considered a cost-effective method to reduce *Fusarium* mycotoxin contamination under production settings (Peiris et al., 2010; Karlovsky et al., 2016).

Two single-seed sorting methods, optical seed sorting and near infrared single kernel sorting, can be used to separate FDK from uninfected kernels and reduce associated mycotoxin contamination in feed production. Single seed sorting technologies can significantly improve grain quality and reduce mycotoxins contamination by removing a smaller proportion of damaged grains (specifically the FDKs) compared to removing damaged grain by bulk (Karlovsky et al., 2016). The operational theory of the optical sorter is to direct streams of grain along an array of optical sensors which analyses the color of individual grains (Karlovsky et al., 2016). The FDK have chalky to pink colour compared with undamaged kernels (CGC, 2015). Near-infrared single kernel sorting separates FDK from uninfected grain kernels by analyzing various chemical compositions (Pettersson and Åberg, 2003). Using near infrared single kernel sorting technology has been demonstrated to remove *Fusarium* damaged kernels from healthy kernels, therefore improving grain quality and reducing mycotoxin contamination (Kautzman et al., 2015b). In future, seed sorting technologies (both optical and near infrared technologies) will be more widely applied in feed production.

Different sources of wheat can have varying nutrient compositions which, when incorporated into the diet, can affect broiler growth performance (Scott et al., 1998; Smeets et al., 2015). In the previous studies where poultry were fed naturally DON-contaminated diets,

different sources of wheat (clean and contaminated) were used in different proportions to produce diets with graded concentrations of mycotoxins (Swamy et al., 2004a; Awad et al., 2006b; Yunus et al., 2012b). Therefore, the effects of the mycotoxins and effects of using different wheat sources on broiler growth performance could not be completely separated. Using seed sorting technology allows the production of a wide range of *Fusarium* mycotoxin-contaminated diets from a single source of FDK grain and reduces experimental variables (Kautzman et al., 2015a).

2.5.2 Feed additives and bio-transformation treatments

Many feed additives, such as absorbents and binders as well as microbial mycotoxin degrading enzymes have been tested for their ability to reduce the effects of dietary *Fusarium* mycotoxins in animals. Absorbents and binders, such as yeast cell wall compounds can reduce bioavailability of DON by more than 40% (Zhu et al., 2016). Yeast glucomannan, a water-soluble polysaccharide derived from the cell wall of yeast, has demonstrated potential to partially prevent some of the characteristic adverse effects of dietary DON exposure, such as feed refusal, reduced vaccine responses and increased inflammatory responses in turkey (Devreese et al., 2014), broilers (Girgis et al., 2010b; Li et al., 2012) and laying hens (Chowdhury and Smith, 2004). Other absorbents, such as celluloses and hemicelluloses (plant cell wall compounds) are also reported to absorb *Fusarium* mycotoxins, such as DON, T-2 and ZEA *in vitro* (Zhu et al., 2016; Vila-Donat et al., 2018), and therefore, may also have absorptive capacity when incorporated into poultry diets to reduce effects of DON contamination.

Specific microorganisms may also be useful as feed additives through their ability to transform trichothecenes to less toxic compounds (McCormick, 2013; Ahad et al., 2017). It is suggested that the foregut microbes in poultry and ruminants can transform dietary DON to the less toxic metabolic products (Grenier and Applegate, 2013; Maresca, 2013). Poultry have higher tolerance to DON-contaminated diets compared to swine, and this higher tolerance is thought to be due to poultry having a higher foregut microbial population compared to swine (Maresca, 2013). However, direct supplementation of microorganisms may raise health safety concerns regarding potential infection for animals and farmers, as well as worry among consumers about ‘contaminating’ animal products with live microbials, such as bacteria (Kim et al., 2017).

There is currently no study demonstrating that a single type of feed additive can completely prevent the negative effects of dietary trichothecenes (primarily DON) in poultry. However, yeast cell wall products have demonstrated ability to absorb the DON and other trichothecenes *in vitro* (Boudergue et al., 2009; Zhu et al., 2016; Vila-Donat et al., 2018). Using a mixture of feed additives, such as yeast cell wall compounds, plant cell wall-based absorbents and mycotoxin degrading enzymes may provide the most effective protection against DON toxicity as compared to using a single type of additive. With the economic losses and animal health risk caused by increased occurrence of DON contamination, research to explore inactivation and detoxification methods as mitigation strategies in poultry is warranted.

2.6 Conclusions

Fusarium mycotoxins are secondary metabolites of fungi that can contaminate a wide variety of cereal crops. They are of specific concern for agriculture because of their adverse effects on humans and animals. Although more than 400 mycotoxins have been identified, only a few of them are of importance in poultry production. Feeding broiler chickens diets that are naturally contaminated with *Fusarium* mycotoxins have well-documented adverse effects on intestinal mucosal structures, digestive functions, immune function and growth performance. Although considerable research exists regarding the effect of feed-borne mycotoxins in broilers, there are several factors that have been overlooked in past research including the sensitivity of particular production stages, behavioural effects and the specific role of feed intake in reduced performance. Identifying how these particular factors affect broiler responses to the contaminated diets can provide more in-depth understanding about the *Fusarium* mycotoxins toxicosis in broilers as well as assist developing strategies to prevent negative impacts of mycotoxin-contaminated diets on broilers under an antibiotic-free production system.

2.7 Objectives and Hypotheses

The overall objective of this thesis research is to characterize the effects of *Fusarium* mycotoxins (primarily DON) on broiler chickens using naturally-contaminated diets. In order to achieve this overall objective, a series of specific objectives are identified:

1. To determine how timing and duration of exposure to feed-borne *Fusarium* mycotoxins influence growth performance and severity of changes in intestinal morphology broiler chickens.
2. To determine the effects of feed-borne *Fusarium* mycotoxins on broilers' feed preference, feeding behaviours and growth performance.
3. To determine whether the performance and gastrointestinal effects of *Fusarium* mycotoxins in broiler chickens are due to DON-induced reduction in feed intake
4. To determine the effectiveness of a new feed additive against the feed-borne *Fusarium* mycotoxins induced broiler growth suppression.

The overall hypothesis of this thesis research is that *Fusarium* mycotoxin-contaminated diets (with relatively low concentrations DON at or below legislated dietary allowance of 5 mg/kg in feed) can negatively affect growth performance and health of broiler chickens. More specifically:

1. Younger broilers are more sensitive to performance and gasterointestinal effects of *Fusarium* mycotoxins compared to older broilers.
2. Broiler chickens exhibit immediate and prolonged feed aversion to *Fusarium* mycotoxin-contaminated diets that contributes to decreased growth performance.
3. Broiler chickens provided *Fusarium* mycotoxin-contaminated diets exhibit changes in feeding and drinking behaviour that contributes to decreased growth performance.
4. Reduction of feed intake is the main cause of growth suppression observed in broiler chickens fed naturally *Fusarium* mycotoxin-contaminated diets.
5. A new yeast cell wall-based feed additive can mitigate the reduction in feed intake and behavioural effect of contaminated diets in broilers and hereby improve animal performance.

PREFACE TO CHAPTER 3

Broiler chickens may be exposed to *Fusarium* mycotoxins through consuming contaminated diets during a specific part of the production cycle or throughout the entire cycle. In this chapter, we conducted a study to determine the effects of timing and duration of dietary exposure to naturally DON-contaminated diets on broilers. In addition to measuring growth performance, mucosa structures in different segments of the small intestine were evaluated by histopathology. The results demonstrate that providing older broiler chicks (22 to 34 d) mycotoxin-contaminated feed results in a more severe reduction of growth performance and that this growth suppression may be a result of adverse effects on intestinal morphology during later growth phases of broiler.

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Author contributions:

Anhao Wang (University of Saskatchewan) designed and managed the experiment, generated and analyzed the data, prepared all figures, and drafted the manuscript.

Dr. Natacha Hogan (University of Saskatchewan) provided inspiration, scientific input and guidance, commented on and edited the manuscript, and obtained funding for the research.

CHAPTER 3

EFFECTS OF FEED-BORNE *FUSARIUM* MYCOTOXINS ON BROILER CHICKENS: INFLUENCES OF TIMING AND DURATION OF EXPOSURE

3.1 Abstract

In commercial practice, broiler chickens may be exposed to *Fusarium* mycotoxins either during a specific part of the production cycle or throughout the entire cycle. A 34-d feeding trial was conducted to identify the sensitive period for mycotoxin effects during the growth cycle of broiler chickens. A total of 420 newly-hatched Ross 308 male broilers were randomly assigned to 60 cages with seven birds/cage. Sources of clean wheat (< 0.5 mg/kg deoxynivalenol [DON]) and *Fusarium*-contaminated wheat (11.4 mg/kg DON) were used to formulate the starter diets (0.41and 6.62 mg/kg DON) provided from 1 to 21 day of age (d) and the grower diets (0.54 and 7.90 mg/kg DON) provided for 22 to 34 d. Control and DON diets were provided to broilers according to treatments (control, DON 1 to 14 d, DON 15 to 21 d, DON 22 to 34 d and DON 1 to 34 d). Birds were monitored daily for morbidity/mortality. Broiler growth performance (body weight, average daily gain, average daily feed intake and feed to gain ratio) was measured weekly. Segments of duodenum, jejunum and ileum were collected at 21 d and 34 d and morphometric parameters, such as villus height, crypt depth, villus width, muscularis thickness, villi: crypt ratio were determined. Birds fed the DON starter diet over the first 14 days did not exhibit any changes in growth performance; however, growth performance was suppressed in birds fed DON-contaminated diets during the grower period (22 to 34 d). At 34 d, birds that received the DON grower diet (DON 22 to 34 d and DON 1 to 34 d) were lighter (1433 vs. 1695 g) than birds fed the control diet. Feed to gain ratio was higher in birds fed the DON grower diet during d 22 to 28 (1.77 vs. 1.56) and d 28 to 34 (2.24 vs. 1.85) compared to corresponding controls. These results suggest that providing older broiler chicks (22 to 34 d) feed contaminated with *Fusarium* mycotoxins (specifically DON) may result in production loss to the producer. Histopathological analysis of the ileal region revealed that birds provided the DON diets throughout the entire trial (1 to 34 d) had shorter villi (506 vs. 680 µm) and shallower crypt (85 vs. 115 µm) than control birds. Taken together, these results indicate that DON-induced growth suppression may be a result of adverse effects on intestinal morphology during later growth phases of broiler.

3.2 Introduction

Fusarium fungi infect a wide range of cereal grains and corn worldwide (Yli-Mattila, 2010; Tittlemier et al., 2013). *Fusarium*-damaged grains that are down-graded cannot be used for human consumption and are often used in animal feed production. *Fusarium* fungi produce mixtures of mycotoxins, such as trichothecenes, zearalenone and fumonisin (Döll and Dänicke, 2011; Bryden, 2012). Results of a recent global survey (1384 feed samples) indicated that the trichothecene deoxynivalenol (DON), was the most prevalent with 79% of the samples testing positive, with an average contamination of 0.6 mg/kg and maximum concentration of 8.4 mg/kg (Kosicki et al., 2016). Deoxynivalenol is consistently the most prevalent mycotoxin in *Fusarium*-infected grains in Canada (Tittlemier et al., 2013) and often co-contaminates with other trichothecenes, such as 3- acetyl DON (3-ADON) and/ or 15-acetyl DON (15-ADON) (Miller and Richardson, 2013). *Fusarium* mycotoxins induce a wide range of adverse effects in food-production animals with symptoms from vomiting and feed refusal, to estrogenic effects and reduced performance, depending on the toxin and sensitivity of the animal species (Osweiler, 2014). In general, feed contamination with mycotoxins such as DON leads to economic losses in animal production (Wu, 2007).

For broiler chickens, dietary trichothecene exposure is known to increase mortality, alter immune function, increase disease susceptibility and reduce growth performance (Andretta et al., 2011; Escrivá et al., 2015). However, reported effects of DON on growth performance are often inconsistent with some studies reporting no impacts of feeding contaminated feed with high concentrations (9 to 15 mg/kg) of DON (Eriksen and Pettersson, 2004; Awad et al., 2012; Grenier and Applegate, 2013) while others found significant adverse effects of DON-contaminated feed on feed intake and weight gain, even at concentrations as low as 2 mg/kg (Awad et al., 2011; Yunus et al., 2012b). The adverse effects of DON in research trials with poultry depend on several factors, including length of exposure, timing of exposure as well as whether the poultry feed is spiked with DON or incorporated using naturally-contaminated grain (Yli-Mattila, 2010; Miller et al., 2014). This means that broilers can be subjected to dietary mycotoxins during a specific part or throughout the entire of production cycle. In addition, duration of exposure and concentration in the diet have important implications on severity of

effects observed with dietary mycotoxin exposure. For example, chronic exposure is most associated with decreased performance and nutritional efficiency and in swine, intake of 1 to 2 mg/kg vs. 12 mg/kg DON can mean the difference between decreased feed intake and total feed refusal (Pierron et al., 2016).To better understand the mycotoxin challenges faced in commercial production, studies using naturally contaminated feed and that can identify the most sensitive period for performance effects of mycotoxins are warranted.

The gastrointestinal tract is main site of nutrient digestion and absorption and is also an important immune organ to combat feed-borne pathogens. A healthy, fully functioning intestine is important for developing broilers to reach their genetic potential. It is well known that DON can alter the intestinal morphology of poultry, leading to impairment of nutrient uptake which can adversely affect energy and nutrient availability and consequently reduce growth performance (Awad et al., 2012; Ghareeb et al., 2015). This influence of DON on gut morphology and function in broilers can be observed at relatively low concentrations, even before an impairment of growth performance was observed (Awad et al., 2011). Therefore, morphological changes in intestinal structures can be used as a sensitive biological endpoint to evaluate dietary DON toxicosis.

The aim of the current study was to identify the sensitive period for adverse effects of mycotoxins during the growth cycle of broiler chickens. Diets were formulated with grain naturally-contaminated with *Fusarium* mycotoxins (primarily DON) and provided to broilers during different stages of the growth cycle. We then evaluated the effects of timing of exposure on growth performance and intestinal morphology throughout the growth cycle.

3.3 Materials and Methods

3.3.1 Diets

Diets were formulated to meet or exceed the nutrient requirements for the growing birds (Aviagen, 2014b). Starter diets were provided to birds from 1 – 21 days of age (d) and grower diets were provided from 22 – 34 d. A source of Canada Western Red Spring wheat not contaminated with mycotoxins (DON < 0.5 mg/kg) was used for the uncontaminated control diets. A source of *Fusarium*-damaged Canada Western Red Spring wheat was used to prepare mycotoxin-contaminated diets. Samples from each wheat source were analyzed for 16 common

mycotoxins (Prairie Diagnostics Services Inc., Saskatoon, SK) via ultra-high performance liquid chromatography (Agilent 1100, Agilent Technologies, Santa Clara, CA) and mass spectrometry detection (Micromass Quattro Ultima Platinum Mass Spectrometer, Waters, Milford, MA). The mycotoxin suite included: DON and metabolites (3- and 15- acetyl DON); α-zearalenol, diacetoxyscirpenol, T-2, HT-2, nivalenol, ochratoxin A, β-zearalenol, zearalenone, and aflatoxin B1. Deoxynivalenol was the major mycotoxin identified in the *Fusarium*-damaged wheat (11.4 mg/kg). Diets were formulated to contain DON at 7.5 mg/kg, the control diets were formulated to contain DON < 1.0 mg/kg. After diets were manufactured, 2 kg samples from each diet were collected. Mycotoxin concentrations were measured by Prairie Diagnostics Services Inc. (Saskatoon, SK) and nutrient compositions (dry matter, crude fiber, fat, calcium, phosphate and apparent metabolic energy (AME)) were analyzed at Central Testing Laboratory Ltd. (Winnipeg, MB). The diet ingredients, analyzed and calculated nutrients, and analyzed mycotoxin concentrations are shown in Table 3.1. The control starter and grower diet contained 0.41 mg/kg and 0.54 mg/kg DON, respectively. The starter and grower diets formulated with *Fusarium*-damaged grain contained 6.62 mg/kg and 7.90 mg/kg DON, respectively and are henceforth referred to as "DON contaminated diets". All diets were prepared at the Canadian Feed Research Centre (North Battleford, SK) in the mash form and were visually similar upon inspection.

3.3.2 Experimental procedures

Permission was granted for all experimental work by the University of Saskatchewan Animal Care Committee (protocol # 20130043), with all procedures following the recommendations of the Canadian Council on Animal Care (1993). A total 420 newly hatched, non-vaccinated, male broiler chicks (Ross 308) were obtained from a local commercial hatchery. Birds were randomly assigned to 96 battery cages (29 cm high × 48 cm wide × 83 cm long; providing 800 cm^2/bird floor space) with 7 birds/cage. Birds were kept in a temperature-controlled room at the University of Saskatchewan Poultry Research Center, Saskatoon, SK.

There were 5 treatments in this experiment and each treatment was replicated 12 times. For each treatment, the control and the DON-contaminated diets were given during different periods of growth cycle of broilers from 1 to 34 d (Fig. 3.1). Treatment 1 was given the control diets throughout the feeding trial (1 to 34 d). Treatment 2, 3, and 4 were given the DON-contaminated diet during 1 to 14 d, 15 to 21 d and 22 to 34 d, respectively. Treatment 5 was

given the DON-contaminated diets throughout the entire trial. Birds had free access to drinking water and feed during the whole trial. All mortality and culled birds were necropsied for cause of death or morbidity by Prairie Diagnostics Services Inc. (Saskatoon, SK).

3.3.3 Growth performance

Broiler body weight (BW, g) of each bird was recorded at 14, 21 and 34 d. Feed consumption was measured at 14, 21 and 34 d or as mortality occurred. Average daily gain (ADG, g/d), average daily feed intake (DFI, g/d) and feed to gain ratio (F:G, g feed/g weight gain) of each cage were then calculated. Average daily gain, DFI, F: G were calculated as followed: $ADG_{(period\ a\text{-}b)} = [BW_{day\ b} - BW_{day\ a}]$/period a-b, $DFI = [feed\ weight_{day\ a} - feed\ weight_{day\ b}]$/period a-b and F:G = DFI/ ADG.

3.3.4 Organ weight and length

At d 21 and 34 one bird from each cage was randomly selected for tissue sampling. Birds were euthanized by cervical dislocation. Liver and spleen weight were measured. Weights of the individual segments of the gastrointestinal tract were recorded after removing the surrounding fat. The intestine was divided into the duodenum, jejunum, and ileum for measuring the length of each segment. The duodenum was defined as the segment encompassing the duodenal loop (Denbow, 2015). The ileum was defined as the distal segment of the small intestine tract, starts at Meckel's diverticulum and ends at ileal cecal junction and the jejunum was defined as the segment is in between of duodenum and ileum (Denbow, 2015). Organ weights were expressed as g/kg body weight (g/kg BW, relative weight). The weight to length ratio of each intestinal segment was also calculated as an indicator of intestine density (g/cm).

2.3.5 Intestine histology

On 21 d, gastrointestinal segments (duodenum, jejunum and ileum) were collected from six randomly selected birds in the control group and birds fed the DON diets from 1 – 21 d. On 34 d, six birds from each treatment were randomly selected for intestinal morphology assessment. A segment (2 cm) of duodenum, jejunum and ileum were collected. Tissues were

fixed in 10 % neutral buffered formalin and then transferred in ethanol until processing. Tissues were paraffin embedded, sectioned at 5 μm thickness and stained with hematoxylin and eosin by Prairie Diagnostic Services Inc. (Saskatoon, SK). Two cross sections of each tissue were prepared for evaluation. Slides were examined under an Axiostar plus light microscope (Carl Zeiss Microscopy, LLC, One Zeiss Drive Thornwood, NY) and pictures of intestinal epithelial structures were acquired with 10x objective. Measurements were done on 10 – 16 villi and associated crypt per bird. All three intestinal regions were evaluated for changes in villus height, crypt depth, villus width, muscularis thickness and villi/crypt ratio.

3.3.5 Statistical analysis

Statistical analyses were performed with the PROC MIXED procedure (Littell, 2006) of SAS version 9.4 (SAS Institute Inc., Cary, NC) using different models. The experimental unit for performance was the cage, and for other analyses, the bird. Growth performance data were analyzed as repeated measures with time as a factor. Where interactions with time were significant ($\alpha = 0.05$), data were sliced by time and analyzed separately. Intestinal histological data were analyzed by using ANOVA with 6 replications (Littell, 2006). Any significant treatment effects ($\alpha = 0.05$) were analyzed using the Tukey-Kramer test to differentiate the means.

Table 3.1. Feed formulation, nutrient compositions and analyzed mycotoxins for control and DON- contaminated starter (1 – 21 d) and grower (22 – 34 d) diets.

	Starter Diet		Grower Diet	
	Control	**DON-contaminated**	**Control**	**DON-contaminated**
Ingredient (%)				
Control wheat [1]	70.3	0	75.7	0
Contaminated wheat [2]	0	70.3	0	75.7
Soybean meal	20.0	20.0	14.0	14.0
Fish meal	1.0	1.0	1.0	1.0
Corn-gluten meal	2.0	2.0	3.0	3.0
Canola oil	2.0	2.0	2.0	2.0
DiCalcium Phosphate	0.9	0.9	0.5	0.5
Limestone	1.2	1.2	1.2	1.2
Salt (as NaCl)	0.25	0.25	0.25	0.25
Vitamin/mineral premix [3]	0.5	0.5	0.5	0.5
Choline chloride	0.1	0.1	0.1	0.1
DL-Methionine	0.5	0.5	0.5	0.5
L-Lysine	0.4	0.4	0.4	0.4
Celite	0.8	0.8	0.8	0.8
Enzyme [4]	0.05	0.05	0.05	0.05
Total	100	100	100	100
Analysed composition (%) [5]				
Crude Protein	23.8	21.5	21.1	19.9
AME (kcal/kg)	3096	3251	3091	3085
Calcium	0.68	0.70	0.80	0.84
Phosphorus	0.63	0.59	0.63	0.59
Crude fiber	3.63	3.48	2.60	2.60
Fat	3.88	6.50	3.57	3.57
Ash	5.66	5.14	5.54	5.43
Calculated amino acid (%)				
Lysine	1.38	1.31	1.22	1.18
Methionine	0.56	0.51	0.54	0.52
Methionine + Cysteine	1.33	1.23	1.30	1.25
Analysed mycotoxin (mg/kg) [6]				
Deoxynivalenol (DON)	0.41	6.62	0.54	7.90
3-Acetyl DON	<0.025	0.41	<0.025	0.58
15-Acetyl DON	<0.025	0.20	<0.025	0.030
HT -2 Toxin	0.055	0.40	0.059	0.12

[1]Mycotoxins concentration in control wheat: aflatoxin B1: Not Detected (ND), 3-Acetyl Deoxynivalenol (DON): ND, 15-Acetyl DON: ND, α-Zearalenol: ND, DON: 352 µg/kg, Diacetoxyscirpenil: ND, HT-2 toxin: ND, Nivalenol: ND, Ochartoxin A: ND, T-2 toxin: ND, β-Zearalenol: ND, Zearalenone: ND.
[2]Mycotoxins concentration in control wheat: aflatoxin B1: ND, 3-Acetyl Deoxynivalenol (DON): 764 µg/kg, 15-Acetyl DON: ND, α-Zearalenol: ND, DON: 11470 µg/kg, Diacetoxyscirpenil: ND, HT-2 toxin: 107 µg/kg, Nivalenol: 59.2 µg/kg, Ochartoxin A: ND, T-2 toxin: ND, β-Zearalenol: ND, Zearalenone: ND.
[3]Vitamin/mineral premix: vitamin A: 2200000 IU, vitamin D: 440000 IU, vitamin E: 6000 IU, menadione: 400 mg, thiamine: 300 mg, riboflavin: 1200 mg, pyridoxine:800 mg, vitamin B12: 4 mg, niacin: 12000 mg, pantothenic acid: 2000 mg, folic acid: 120 mg, biotin: 30 mg. copper: 2000 mg, manganese: 16000 mg, iodine: 160 mg, zinc: 16000 mg, selenium: 60 mg, calcium carbonate: 100000 mg, antioxidant: 125 mg, wheat midds: 807879 mg. (DSM Nutritional Products Canada Inc. Ayr. ON).
[4]Enzyme: Beta-glucanase: 1000 units/g, xylanase: 1500 units/g (GNC Bioferm Inc., Saskatoon, SK).
[5]Nutrient composition were analyzed at Central Testing Laboratory Ltd., (Winnipeg, MB). AME was calculated as: AME=53+38*((Crude Protein)+(2.25*Fat)+(100-((Crude Protein)+(Crude fiber)+Fat+(Ash) (moisture)))*0.9).
[6]Mycotoxins were analyzed at Prairie Diagnostics Services Inc., (Saskatoon, SK) via ultra-high performance liquid chromatography (Agilent 1100, Agilent Technologies, Santa Clara, CA) and mass spectrometry (Micromass Quattro Ultima Platinum Mass Spectrometer, Waters, Milford, MA).

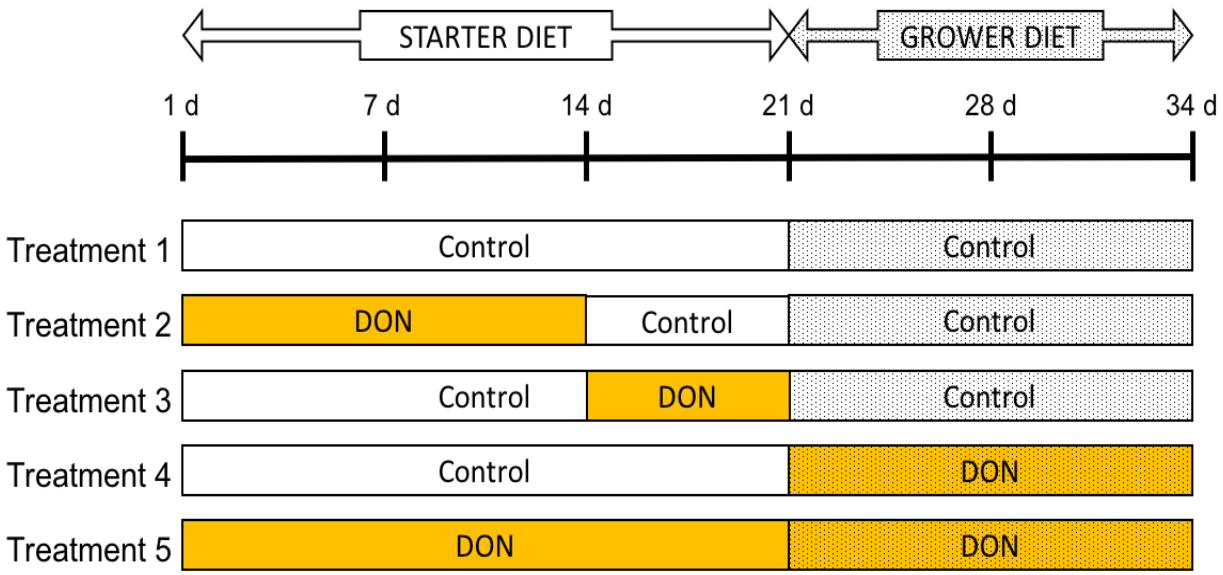

Figure 3.1 Schematic representation of the timing of different diets (control and deoxynivalenol (DON-contaminated) fed to broiler chickens during a 34-day feeding trial. The starter diets were fed from 1 to 21 d (0.41 mg/kg and 6.62 mg/kg DON) and grower diets were fed from 22 to 34 d (0.54 mg/kg and 7.90 mg/kg DON). The DON diets were prepared with wheat that was naturally-contaminated with *Fusarium* mycotoxins.

3.4 Results

3.4.1 Growth performance

The overall mortality in current study was 7%. The mortality in each treatment at the end of the feeding trial (35 d) was 8%, 10%, 7%, 2% and 6% for control, DON 1 – 14, DON 15 – 21, DON 22 – 34 and DON 1 – 34, respectively. Mortality occurred early (within first week) and was equal across treatments (the control diet fed birds vs. the DON diet fed birds). Mortality was most often attributed to poor chick quality (i.e. yolk sec infectious and polyserositis) and in all likelihood not attributed to the presence of mycotoxins in the diet.

The initial BW of chicks did not differ among treatments at the experiment initiation ($P > 0.05$; 45.1 ± 0.4, 45.4 ± 0.4, 45.3 ± 0.5, 45.2 ± 0.5 and 44.4 ± 0.5 g for treatments 1 to 5, respectively). Body weight was not affected by treatment during first two weeks ($P > 0.05$, Table 2). At 21 d, birds in treatment 5 (DON 1 to 34 d) were lighter than control birds (724.6 vs. 650.8 g, $P < 0.01$, Table 3). At 34 d, the birds from treatment 4 (DON 22 to 34 d) and treatment 5 (DON 1 to 34 d) were lighter (1517.5 and 1433.7 g, respectively) when compared to birds fed control diets over the same time period (1695.0 g, $P < 0.01$ Table 3.2). During the first two weeks of the trial, ADG was not affected by presence of dietary mycotoxins ($P > 0.05$, Table 3.3); however, when assessed at 21 d, ADG was decreased ($P < 0.01$) for birds consuming DON-contaminated diets (treatment 5) compared to same-age birds fed control diets (Table 3.3). In addition, ADG (from 22 to 34 d) was decreased ($P < 0.01$), in birds consuming DON-contaminated diets both throughout the entire growth period (66.4 g) and during only the grower phase (66.3 g) when compared to birds fed control diets (82.8 g; Table 3.3). From 22 to 34 d, the birds fed DON diets since hatch (DON 1 to 34 d) or during the grower stage (DON 22 to 34 d) consumed less feed than the birds fed DON diet only during the starter (1 to 21 d) stages ($P < 0.01$, Table 3.4). Feed to gain ratios (F:G) were not affected by the presence of dietary DON during the starter (1 to 21 d) stages ($P > 0.05$, Table 3.5). During 22 to 34 d, DON-fed birds had higher F:G (1.98) than the birds fed control diets (1.70, $P < 0.01$, Table 3.5).

3.4.2 Organ weights and intestinal density

The means of the relative weights of organs (g/kg BW) and density (g/cm) of intestinal sections for dietary treatments are presented in Table 3.6. Among birds sampled at 21 d, relative organ weights (liver, spleen, duodenum, jejunum and ileum) were not affected by treatment ($P > 0.05$). Birds fed DON-contaminated diets from d 1 – 21 had lower jejunal density (0.20 ± 0.01 g/cm) compared to control birds (0.23 ± 0.01 g/cm, $P < 0.04$). At 34 d, relative weight of duodenum, jejunum, ileum and spleen as well as density of intestinal segments did not show any significant differences between dietary treatments ($P > 0.05$). Birds fed DON-contaminated diets during the grower period (DON 1 – 34 d or DON 22 – 34 d) had greater relative liver weight (25.28 ± 0.77 and 25.24 ± 0.77 g/kg BW, respectively) when compared to the control group (21.91 ± 1.01 g/kg BW) or groups fed DON-contaminated diets during the early starter period (1 – 14 d, 21.67 ± 0.47 g/kg BW).

3.4.3 Intestine morphological structures

On 21 d, control birds had longer jejunal (P = 0.03, Fig. 3.2A) and ileal (P = 0.01, Fig. 3.2A) villi and wider villi in duodenal region (P = 0.04, Fig. 3.2B) compare with birds subjected to the DON-contaminated diet during first 21 d. On 34 d, control birds had higher villi (P < 0.01, Fig. 3.3A) and deeper crypt (P = 0.01, Fig. 3.3A) than birds fed the DON diets in the ileum region. Ileal villi/crypt ratio were not affected by treatment (P > 0.05, Fig. 3.3B). Birds receiving the DON diet during the first 14 d had higher duodenal villi to crypt ratio than those fed DON-diets later in the growing period on 34 d (15 – 21 d and 22 – 34 d; P = 0.03, Fig. 3.3B). Birds receiving DON grower diet from 21 – 34 d had shorter jejunal villi (P < 0.01, Fig. 3.3A) and higher jejunal villi to crypt ratio (P = 0.01, Fig. 3.3B) than birds receiving the DON diet from 1 – 14 d and 15 – 21 d. Jejunal crypt depth was not affected by treatments (P > 0.05, Fig. 3.3B). Effects of feeding DON-contaminated diets to broilers for 1-34 d on ileum mucosal structures is depicted in the histological pictures in Fig. 3.4.

Table 3.2. Effects of feeding DON-contaminated diets[1] during different growth stages on body weight (g) of male broilers during a 34-day growth trial.

	Growth Stage		
Treatment	**14 d**	**21 d**	**34 d**
Control	359.5±10.0	724.6±14.2[a]	1695.0±22.5[a]
DON 1 – 14 d	330.9±14.1	673.4±17.8[ab]	1720.0±26.4[a]
DON 15 – 21 d	368.9±9.8	707.0±17.1[ab]	1717.0±28.5[a]
DON 22 – 34 d	356.7±8.6	706.6±10.4[ab]	1517.5±21.0[b]
DON 1 – 34 d	332.4±7.3	650.8±13.9[b]	1433.7±24.9[b]
ANOVA	P = 0.43	P < 0.01	P < 0.01

[a-b] Within same age group (column), mean ± SE with different superscripts (a-c) are significantly different ($P \leq 0.05$, one-way ANOVA followed by Tukey-Kramer test; n = 12/treatment)

[1]The starter diets were fed from 1 to 21 d (0.41 mg/kg and 6.62 mg/kg DON) and grower diets were fed from 22 to 34 d (0.54 mg/kg and 7.90 mg/kg DON). The DON-diets were prepared with wheat naturally contaminated with *Fusarium* mycotoxins.

Table 3.3. Effects of feeding DON-contaminated diets[1] during different growth stages on average daily gain (ADG, g/d) of male broilers during a 34-day growth trial.

	Growth Stage		
Treatment	**1 – 14 d**	**15 – 21 d**	**22 – 34 d**
Control	22.5±0.7	52.2±1.2[a]	82.8±2.2[a]
DON 1 – 14 d	20.4±1.0	48.9±1.4[ab]	86.6±1.9[a]
DON 15 – 21 d	23.1±1.1	48.3±2.0[ab]	82.6±2.0[a]
DON 22 – 34 d	22.2±0.8	50.0±1.3[ab]	66.3±2.0[b]
DON 1 – 34 d	20.6±0.6	45.5±1.1[b]	66.4±1.5[b]
ANOVA	P = 0.50	P = 0.04	P < 0.01

[a-b] Within same growth stage group (column), mean ± SE with different superscripts (a-c) are significantly different (P < 0.05, one-way ANOVA followed by Tukey-Kramer test; n = 12/treatment)
[1]The starter diets were fed from 1 to 21 d (0.41 mg/kg and 6.62 mg/kg DON) and grower diets were fed from 22 to 34 d (0.54 mg/kg and 7.90 mg/kg DON). The DON-diets were prepared with wheat naturally contaminated with *Fusarium* mycotoxins.

Table 3.4. Effects of feeding DON-contaminated diets[1] during different growth stages on daily feed intake (DFI, g/d) of male broilers during a 34-day growth trial

	Growth Stage		
Treatment	**1 – 14 d**	**15 – 21 d**	**22 – 34 d**
Control	30.7±0.7	74.5±1.8[a]	147.2±1.7[a]
DON 1 – 14 d	30.6±1.0	66.5±1.8[b]	144.1±2.1[a]
DON 15 – 21 d	30.5±1.1	73.5±2.3[a]	144.5±2.1[a]
DON 22 – 34 d	31.1±0.8	74.2±1.4[a]	137.6±2.7[b]
DON 1 – 34 d	28.9±0.6	67.3±2.2[b]	135.5±2.3[b]
ANOVA	P = 0.91	P < 0.01	P < 0.01

[a-b] Within same growth stage group (column), mean ± SE with different superscripts (a-c) are significantly different (P < 0.05, one-way ANOVA followed by Tukey-Kramer test P< 0.05; n = 12/treatment).
[1]The starter diets were fed from 1 to 21 d (0.41 mg/kg and 6.62 mg/kg DON) and grower diets were fed from 22 to 34 d (0.54 mg/kg and 7.90 mg/kg DON). The DON-diets were prepared with wheat naturally contaminated with *Fusarium* mycotoxins.

Table 3.5. Effects of feeding DON-contaminated diets[1] during different growth stages on feed to gain ratio (F:G, g feed /g gain) of male broilers during a 34-day growth trial

	Growth Stage		
Treatment	**1 – 14 d**	**15 – 21 d**	**22 – 34 d**
Control	1.37±0.02	1.46±0.01	1.70±0.03[a]
DON 1 – 14 d	1.41±0.02	1.39±0.03	1.64±0.03[a]
DON 15 – 21 d	1.33±0.02	1.52±0.02	1.70±0.03[a]
DON 22 – 34 d	1.41±0.03	1.43±0.01	1.98±0.02[b]
DON 1 – 34 d	1.41±0.03	1.48±0.03	1.98±0.05[b]
ANOVA	P = 0.22	P = 0.08	P < 0.01

[a-b] Within same growth stage group (column), mean ± SE with different superscripts (a-c) are significantly different (P < 0.05, one-way ANOVA followed by Tukey-Kramer test P< 0.05; n = 12/treatment).
[1]The starter diets were fed from 1 to 21 d (0.41 mg/kg and 6.62 mg/kg DON) and grower diets were fed from 22 to 34 d (0.54 mg/kg and 7.90 mg/kg DON). The DON-diets were prepared with wheat naturally contaminated with *Fusarium* mycotoxins.

Table 3.6. Effects of feeding DON-contaminated diets[1] during different growth stages on relative organ weights (g/kg body weight (BW)) and density of small intestine sections (g/cm) in male broilers at 21 d and 34 d.

	Relative organ weight (g/kg BW)					Intestine density (g/cm)		
Treatment	**Duodenum**	**Jejunum**	**Ileum**	**Liver**	**Spleen**	**Duodenum**	**Jejunum**	**Ileum**
21 d								
Control	7.41±0.54	15.55±0.88	11.10±0.44	33.18±1.27	0.94±0.08	0.28±0.02	0.23±0.01[a]	0.17±0.01
DON 1 – 14 d	7.32±0.63	14.88±0.43	10.59±0.43	31.37±1.78	0.92±0.06	0.26±0.01	0.21±0.01[ab]	0.15±0.01
DON 15 – 21 d	7.74±0.49	15.42±0.66	10.89±0.67	33.43±1.13	0.84±0.06	0.28±0.02	0.23±0.01[a]	0.17±0.01
DON 22 – 34 d	7.44±0.42	16.0±0.36	10.90±0.25	31.60±1.27	1.02±0.09	0.27±0.02	0.23±0.01[ab]	0.16±0.01
DON 1 – 34 d	6.84±0.66	14.70±0.67	11.0±0.50	34.38±1.37	0.89±0.07	0.23±0.02	0.20±0.01[b]	0.16±0.01
ANOVA	P = 0.85	P = 0.58	P = 0.16	P = 0.50	P = 0.54	P = 0.27	P = 0.04	P = 0.13
34 d								
Control	5.56±0.27	10.83±0.59	7.71±0.26	21.91±1.01[b]	1.04±0.05	0.35±0.01	0.28±0.01	0.21±0.01
DON 1 – 14 d	5.77±0.30	11.56±0.35	7.89±0.35	21.67±0.47[b]	1.07±0.06	0.36±0.01	0.29±0.01	0.21±0.01
DON 15 – 21 d	5.58±0.24	11.50±0.36	8.56±0.29	22.95±0.57[ab]	1.06±0.10	0.36±0.01	0.30±0.01	0.23±0.01
DON 22 – 34 d	5.78±0.15	11.81±0.43	8.84±0.39	25.24±0.59[a]	1.03±0.05	0.34±0.01	0.28±0.01	0.23±0.01
DON 1 – 34 d	5.87±0.26	11.98±0.56	8.26±0.28	25.28±0.77[a]	0.97±0.07	0.35±0.01	0.28±0.01	0.21±0.01
ANOVA	P = 0.88	P = 0.41	P = 0.14	P < 0.01	P = 0.32	P = 0.46	P = 0.54	P = 0.14

[a-c] Within same age group (column), mean ± SE with different superscripts (a-c) are significantly different (P < 0.05, one-way ANOVA followed by Tukey-Kramer test P< 0.05; n = 12/treatment).

[1] The starter diets were fed from 1 to 21 d (0.41 mg/kg and 6.62 mg/kg DON) and grower diets were fed from 22 to 34 d (0.54 mg/kg and 7.90 mg/kg DON). The DON-diets were prepared with wheat naturally contaminated with *Fusarium* mycotoxins.

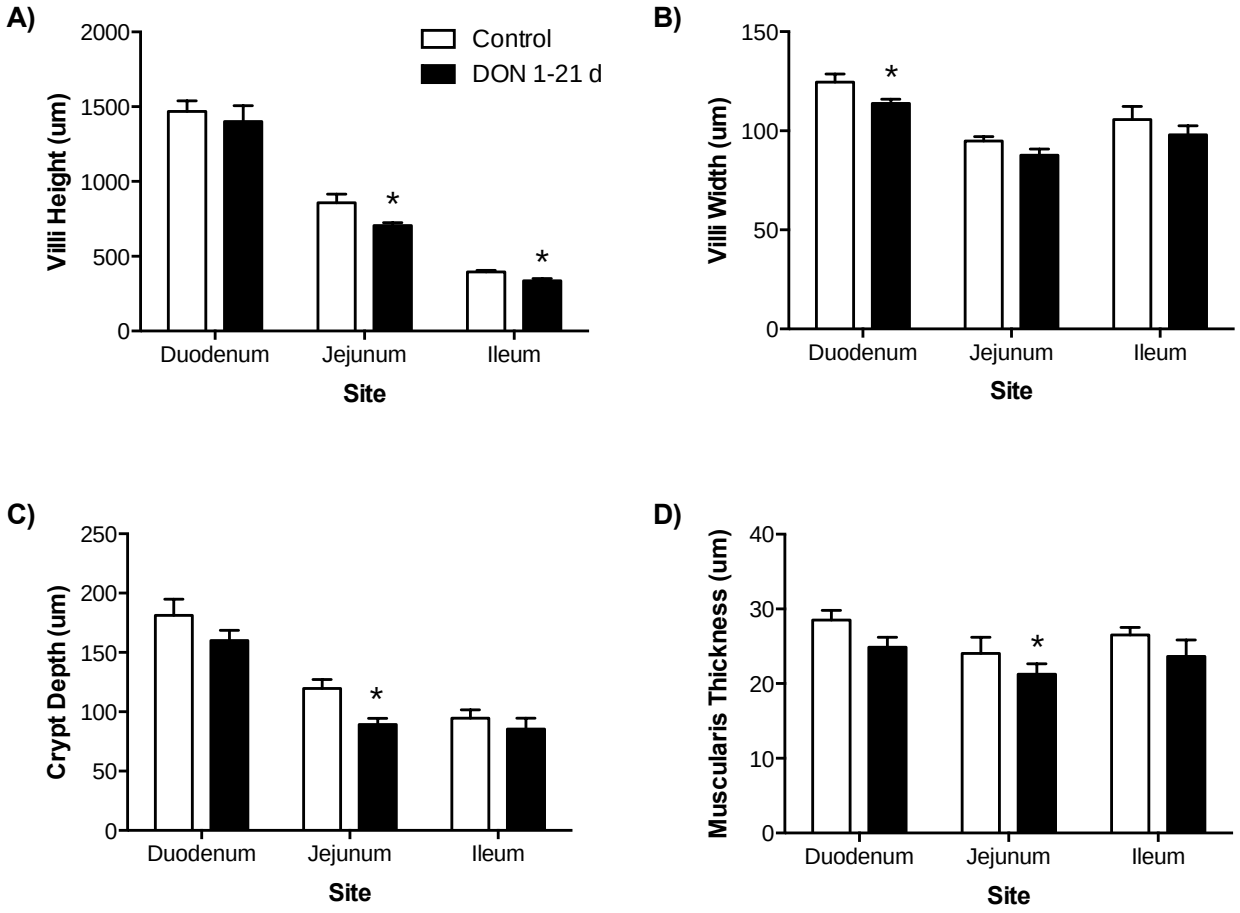

Figure 3.2 Effects of feeding a DON-contaminated diet (6.62 mg/kg) from 1-21 d on (A) villus height (µm), (B) villus width (µm), (C) crypt depth (µm), and (D) muscularis thickness (µm) in different intestinal regions of broiler chickens. Bars represent mean ± standard error of the mean (SEM) with n = 6 animals. White bars represent birds fed the control diet and black bars represent birds fed the DON-contaminated diet. Asterisk (*) represents significant difference within intestinal region (ANOVA, $P \leq 0.05$).

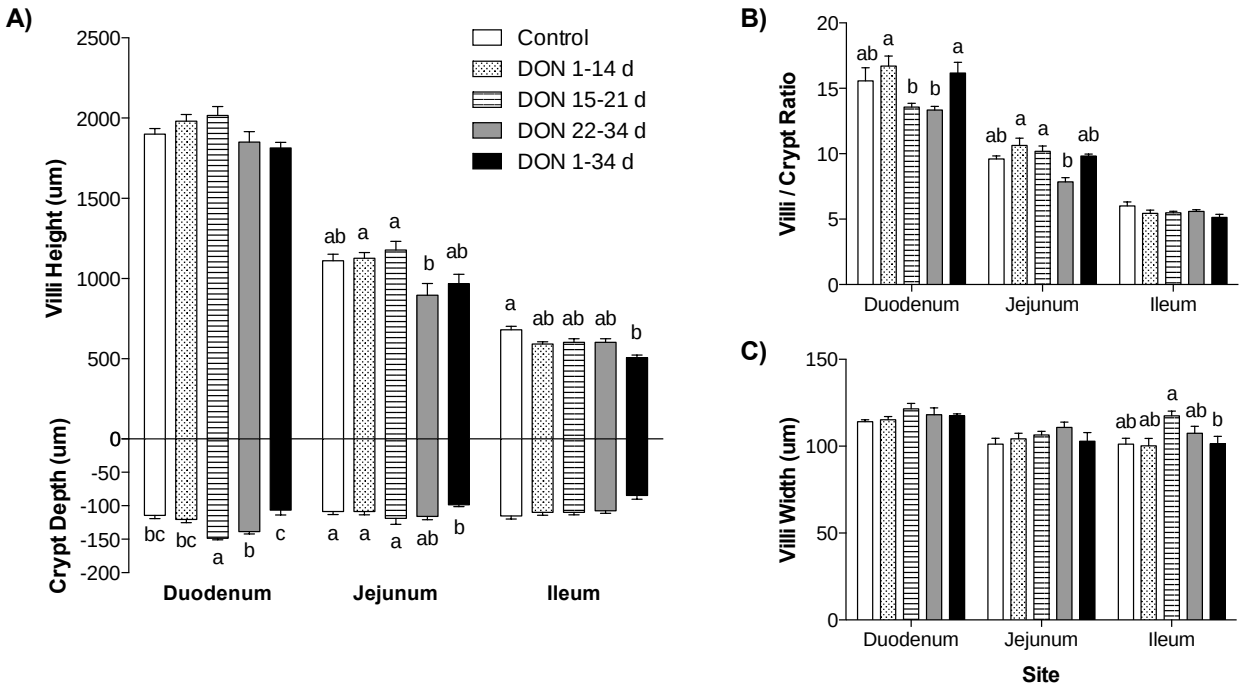

Figure 3.3. Effect of feeding DON-contaminated diets during different growth stages on (A) villi height (µm) and crypt depth (µm), (B) villi to crypt ratio and (C) villi width (µm) in different intestinal regions of broiler chickens at 34 d. Bars represent mean ± standard error of the mean (SEM) with n = 6 animals. The starter diets were fed from 1 to 21 d (0.41 mg/kg and 6.62 mg/kg DON) and grower diets were fed from 22 to 34 d (0.54 mg/kg and 7.90 mg/kg DON). (A) villi height (µm) and crypt depth (µm), (B) villi height to crypt depth ratio and (C) villi width (µm) in different intestinal regions of broiler chickens. The bars with different letters a – b are significantly different ($P \leq 0.05$, one-way ANOVA followed by Tukey-Kramer test; n = 6/treatment. are significantly different.

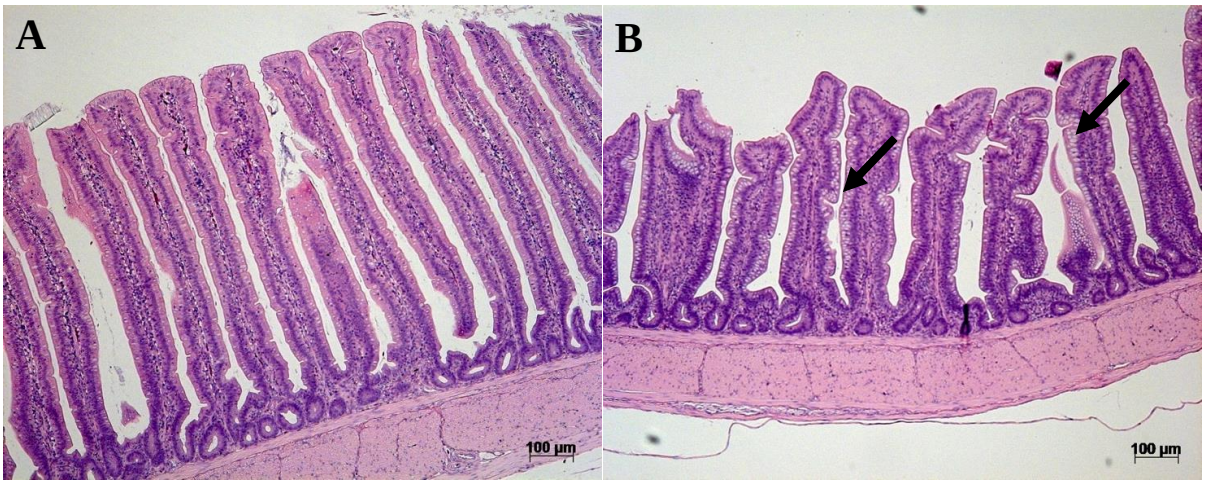

Figure 3.4. Effects of feeding DON-contaminated diets to broilers for 1-34 d on morphology of ileal mucosal structures (×10, hematoxylin and eosin staining). (A) Ileum of a control bird at 34 d with normal villi. (B) Ileum from a bird fed DON-contaminated diet (~ 7 mg/kg DON) from 1-34 d showing villi flattening and increased goblet cells (arrows).

3.5 Discussion

We investigated impacts of timing of exposure to *Fusarium* mycotoxin-contaminated feed in broiler chickens in order to determine specific periods of sensitivity to adverse effects. Broilers may be frequently exposed to *Fusarium* mycotoxin-contaminated diets during specific periods of the growth cycle under production settings. Previous studies examining growth performance effects of mycotoxins usually provided contaminated diets throughout the entire trial period (e.g. 1 – 35 days (Yunus et al., 2012b) and 1 – 56 days (Swamy et al., 2002; 2004a) and therefore, may not reflect the mycotoxins challenges faced by industry. Thus it was of interest to use targeted, stage-specific dietary exposures to determine whether broiler chickens were more sensitive to *Fusarium* mycotoxin-contaminated diets (specifically DON) during specific periods of growth.

In the current study, broilers that were provided contaminated diets in the later growth stage (22 – 34 d) had lower body weight, average daily gain, feed intake and feed efficiency than birds fed contaminated diets at early growth stages or those fed clean diets. In fact, this short-term feeding of DON-contaminated diets to older birds with no previous exposure to DON resulted in the same degree of growth suppression when compared to birds chronically fed DON-contaminated diets. These findings suggest that broilers are more sensitive to DON-contaminated diets during later stage of growth and that adverse effects on growth performance are not necessarily accumulative with continuous exposure to DON-contaminated diets. A recent meta-analysis by Andretta et al., (2011) also concluded that the effect of mycotoxins on broiler weight gain was not constant in all growth phases; however, they found that the effect of mycotoxins on growth was greater in young broilers. Our data does support findings by Swamy et al. (2002, 2004a), where DON-contaminated diets were provided during a longer feeding trial (1 – 56 d) and reduction in body weight and feed intake were observed during mid (21 – 42 d, Swamy et al., 2004a) and later (43 – 56 d, Swamy et al., 2002) growth stages. Overall, older broilers (22 – 34 d) consume significant more feed than younger birds (1 – 21 d) and feed conversion ratio increases with age in broilers (Aviagen, 2014c; Zuidhof et al., 2014). Older broilers (22 – 34 d) have higher basal metabolic rates compare to the younger (1 – 21 d) birds (Kuenzel and Kuenzel, 1977) and so one could hypothesize that mature broilers would metabolize DON faster than younger birds. Previous research in rodents found that older mice (8 – 10 weeks old) could metabolize the orally administrated DON quicker than younger mice (3 – 4 weeks old) (Pestka

and Amuzie, 2008); however, no studies to date have specifically compared toxicokinetics following DON exposure across growth stages in poultry.

Partial feed refusal was also observed in birds that were provided DON-contaminated diets during the grower phase and this response matched that of birds fed contaminated diets through the entire feeding trial. Trichothecenes are known to suppress feed intake through multiple pathways, such as inducing taste aversion, releasing anorexia peptides, inducing inflammatory responses, and altering liver function (Maresca, 2013; Osweiler, 2014; Lebrun et al., 2015). Long-term (1-56 d) feeding of *Fusarium* mycotoxin-contaminated diets to broilers increased the concentration of a strong satiety neurochemical, serotonin (Swamy et al., 2004b), and corresponds with DON-induced partial feed refusal and growth suppression observed in related studies (Swamy et al., 2002; 2004a). Broilers exhibit increased responsiveness to peripheral administration of serotonin throughout early chick development, as indicated by greater suppression of feed intake in older birds (Baranyiová, 1990). Although we did not measure serotonin, it is possible that the stage-specific response of partial feed refusal is due to elevated serotonin concentrations in later growth stages as there is no effect on feed intake during the early growth phase.

Deoxynivalenol exposure appeared to have accumulative adverse effects on broiler intestinal mucosa structures, where birds fed the DON-contaminated diets throughout the trial had the most severe ileal mucosa structure alterations, while other stage-specific treatments were intermediate. Specifically, we observed decreased villi height and crypt depth in the jejunum of broilers fed the DON-contaminated diets. Villi flattening are probably due to the impairment of cell proliferation and our data agrees with previous studies in piglets and broiler chickens that showed a significant decreased in villi height in duodenum and jejunum after ingestion of a diet contaminated with DON (Awad et al., 2006a; 2011; Ghareeb et al., 2015). However, our results also indicate that DON intake during the starter phase is sufficient to affect intestinal mucosa structures. Broilers have high metabolic rates for DON (Osselaere et al., 2013; Broekaert et al., 2015) and individuals can rapidly (1 – 2 d) recover from DON-induced feed refusal and growth suppression if clean diets are provided (Osweiler, 2014). This was also evident in our trial where birds provided DON-contaminated diets during the starter phase, did not exhibit any long-term effects on feed intake or growth performance.

The gastrointestinal tract is the site of nutrient digestion and absorption and a fully functioning, healthy intestine is essential for fast-growing broilers to achieve maximum growth rates with superior feed efficiency (Jin et al., 1998; Denbow, 2015). In the current study, a decrease in villi height in the ileal region was observed. Feeding broilers DON-contaminated diets is well-known to reduce villi height, crypt depth, and villi surface area (Awad et al., 2006a; 2011; Ghareeb et al., 2015). Decreased villi height is associated with a reduction of nutrient digestion and absorption (Ruttanavut and Yamauchi, 2010). Although dietary nutrient digestibility was not directly measured in the present study, the negative effects of DON on ileal mucosa structures suggest a reduction in nutrient digestion and absorption. This hypothesis is supported by the observed reduction in feed efficiency.

The reported effects of dietary *Fusarium* mycotoxins on relative organ weights in poultry are very inconsistent across studies (Swamy et al., 2004a; Girgis and Smith, 2010; Awad et al., 2011). In the present study, feeding the DON-contaminated diets during different growth stages of broilers had no effect on relative organ weights, whether evaluated at the end of the starter period (21 d) or at the end of the trial (34 d). The only exception was an increase in relative liver weight at 34 d in individuals given the DON-contaminated feed throughout the entire trial. Increased liver weight has been reported in mice given DON by oral gavage (Pestka, 2007b; Sobrova et al., 2010). Liver is the major site of DON detoxification (Sobrova et al., 2010; Maresca, 2013) and so increased relative liver weight may be due to enhanced hepatic detoxification activity. Liver is also the major organ for nutrient metabolism (Humphrey and Klasing, 2004) and DON exposure negatively affects nutrient metabolism in the liver (Pestka, 2007b; Maresca, 2013; Lebrun et al., 2015). Reduced feed efficiency observed in birds fed DON-contaminated diets may be the result of such toxic effects of DON on liver.

It is important to note that broilers used in the current study were lighter than the suggested body weight at same age (Aviagen, 2014c). The unexpected slower growth rate may be attributed to the starter diets (1-21 d) having lower than recommended (Aviagen, 2014b) calcium levels (0.70% vs. 0.96%). Calcium is an important nutrient for modern broiler to reach maximum growth performance (Rama Rao et al., 2003; Driver et al., 2005). Feeding broilers low calcium diets (approximately 80% of requirement) results in suppression of feed intake, reduced weight gain and feed efficiency (Driver et al., 2005). The calcium levels in our starter diets were

comparable in both clean and DON-contaminated diets; thus, any growth suppression effects observed in birds received DON-diets are most likely the results of DON toxicity.

Another concern is that DON-contaminated diets contained approx. 10% lower crude protein compared to control diets, which may be attributed to different wheat used in the diet preparation (Kim et al., 2005; Amerah, 2015). In the current study, naturally *Fusarium*-damaged grains were used to better reflect the mycotoxin challenges faced in broiler industry and since reduction of the crude protein level is characteristic of *Fusarium*-damaged grains (Kautzman et al., 2015b), naturally-contaminated feed used in the broiler production may contain less crude protein as compared to uncontaminated feed. It is important to note that across all experimental diets, protein levels and amino acids were sufficient to meet broiler growth requirement (Aviagen, 2014b). Previous studies reported that broiler growth performance was not affected by diets containing crude protein at 10% less than recommended levels when the essential amino acids met nutrient requirements (Bregendahl et al., 2002; Aftab et al., 2006). We are confident that the growth suppression, along with altered intestinal morphology observed in current study is due to intake of DON-contaminated feed. However, in the future, the protein levels of wheat should be measured prior to feed formulation and dietary nutrient levels within manufactured complete feed should be confirmed prior to feeding experiments.

The AME values were also different between the DON and control starter diets and this difference may have affected broiler growth performance as high AME diets can promote broiler growth (Pirgozliev et al., 2003). It is, however, possible for differences in AME to be caused by analysis and/or calculation error from the testing agency. Canola oil was added at a constant amount (2.0 %) in all diet formulations and analyzed fat content in control diets and DON-grower diet had similar fat content (~ 3.7%), with the DON-starter diet as the only exception (~ 6.5%).

The current study demonstrated that broiler chickens are more sensitive to dietary *Fusarium* mycotoxins (primarily DON) during the later stage of growth as exhibited by reduced feed intake, weight gain and feed efficiency when compared to effects at early stage (starter period). The experimental design used in this study with DON exposure at specific periods of growth showed that broilers can recover from performance effects with early exposure to DON while late exposure can have performance effects equivalent to those in chronically-fed birds. The current study also indicates that growth suppression induced from intake of DON-

contaminated diets was not accumulative. Mycotoxin-induced adverse effects on growth performance may be the result of partial feed refusal and alteration of intestinal mucosa structures. Further studies are required to further determine the predominant mechanisms (possibly taste aversion, anorexia effects or inflammatory responses) underlying *Fusarium* mycotoxin-induced feed refusal in broiler chickens. Regardless, in order to achieve maximum growth performance, *Fusarium* mycotoxin-contaminated diets should be avoided, especially during in the later stage of growth.

PREFACE TO CHAPTER 4

In the previous chapter, we identified that broilers are more sensitive to *Fusarium* mycotoxin-induced feed refusal and growth suppression during the later stages of growth. The objective of the research presented in this chapter was to determine whether decreased feed intake during the later stages of growth can be attributed to feed preference and/or changes in feeding behaviours. Our results show that broilers are sensitive to the presence of *Fusarium* mycotoxins and that moderate concentrations of DON negatively affect feed preference and growth performance when fed during the grower period. We also demonstrate that feeding behaviours can be used as a sensitive endpoint to evaluate *Fusarium* mycotoxin-toxicosis in broiler chickens.

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Author contributions:

Anhao Wang (University of Saskatchewan) designed and managed the experiment, generated and analyzed the data, prepared all figures, and drafted the manuscript.

Dr. Karen Schwean-Lardner (University of Saskatchewan) provided the guidance for experimental design, input on analysis and interpretation of behavioural data, commented on and edited the manuscript.

Dr. Natacha Hogan (University of Saskatchewan) provided inspiration, scientific input and guidance on experimental design, commented on and edited the manuscript, and obtained funding for the research.

CHAPTER 4

FEED PREFERENCE AND FEEDING BEHAVIORS IN GROWER BROILERS FED DIETS CONTAINING WHEAT NATURALLY CONTAMINATED WITH *FUSARIUM* MYCOTOXINS

4.1 Abstract

Two trials were conducted to determine the effect of feeding diets contaminated with *Fusarium* mycotoxins (primarily deoxynivalenol (DON)) on broiler chicken feed preference, feeding behaviour and growth performance. A total of 120 male Ross 308 chicks were fed a common corn-based starter diet from 1 – 20 days of age (d). At 21 d, 15 cages were randomly assigned to the feed preference trial or a feeding behaviour trial. Three wheat-based experimental diets (control, low and high DON) were prepared with a clean wheat and a naturally *Fusarium* mycotoxins contaminated wheat. The control, low and high DON diets contained DON at 0.14, 2.27 and 5.84 mg/kg, respectively. Broilers were provided experimental diets *ad libitum* fed and provided 5 hours (h) continual dark period and 19 h light period during 21 – 27 d. In the preference trial, each cage's feeder was split into two equal sized compartments so birds were provided a choice of two experimental diets (control vs. low, control vs. high and low vs. high mycotoxins). Consumption of each diet chosen per cage was compared daily. In the feeding behaviour trial, three experimental diets were randomly assigned to fifteen cages with five cages/diet. Activity of birds was recorded for 1 h before and after the dark period and a 1 h period at 9 h after the light was turned on (middle of day). Through video observations, the number of feeder and drinker visiting events and time spent at the feeder and drinker were recorded. Growth performance was assessed at 21 - 27 d. In the preference trial, broilers preferred the control diet over low (93.0 vs. 66.1 g/d, $p < 0.01$) and high (104.4 vs. 50.4 g/d, $p < 0.01$) DON diets. There was no preference observed between the low vs high DON diets (83.1 vs. 80.3 g/d, $p = 0.55$). Video observations revealed that birds fed the control diet spent less time at the feeder as compared to DON treatments during the 1 hour before (9.61 vs. 19.38 and 18.80 %, $p = 0.04$), and after (12.80 vs. 23.64 and 24.18%, $p = 0.02$) the dark period and during the middle of the day (9.79 vs. 18.89 and 19.69 %, $p < 0.01$). The number of feeder or drinker visits and time spent at the drinker were not affected ($p > 0.05$). Control birds had lower feed to gain ratio (1.65) than birds fed low (1.82) and high (1.94) mycotoxin diets ($p < 0.01$). It is clear that broilers are sensitive to the presence of *Fusarium* mycotoxins and that moderate concentrations of DON negatively affects feed preference and growth performance when fed during the grower period. Feeding behaviours can be used as a sensitive endpoint to evaluate *Fusarium* mycotoxin-toxicosis in broiler chickens.

4.2 Introduction

Trichothecene mycotoxins are secondary metabolic products of *Fusarium* fungi and are commonly detected in *Fusarium*-infected cereal grains (Miller and Richardson, 2013; Tittlemier et al., 2013). The trichothecene deoxynivalenol (DON) is the most prevalent *Fusarium* mycotoxin worldwide (Miller and Richardson, 2013) and can induce a wide range of adverse effects in monogastric animals such as partial feed refusal, suppressed weight gain, reduced feed efficiency, altered small intestine morphology and function as well as suppressed immune function (Grenier and Applegate, 2013; Maresca, 2013; Osweiler, 2014).

Deoxynivalenol-induced partial or complete feed refusal is widely reported and can have a significant impact on animal performance (Pestka, 2007a; Osweiler, 2014). Poultry are less sensitive to DON contamination compared to swine (Grenier and Applegate, 2013); however, negative effects on performance and health are reported over a wide range of concentrations (Swamy et al., 2002; Awad et al., 2011; Yunus et al., 2012b). Our previous work (Wang and Hogan, 2018) further indicated that fast growing broilers are particularly sensitive to DON induced feed refusal and growth suppression during the grower phase (22 – 34 day of age (d)). However, it remains unclear whether decreased feed intake in broilers can be attributed to feed preference and/or changes in feeding behaviours. In mammals, trichothecene-induced feed refusal occurs through a variety of actions, such as inducing taste aversion (Osweiler, 2014), increasing anorexigenic responses (Gaigé et al., 2014), and inducing inflammatory mediators such as cytokines (Maresca, 2013; Lebrun et al., 2015). Altered feeding behaviour was also observed in laboratory animals following oral exposure to trichothecenes (Girardet et al., 2011; Gaigé et al., 2014). For example, acute oral exposure to DON reduced both meal size and meal frequency in rodents (Girardet et al., 2011), and these changes in feeding behaviour were also observed in the rodents that were injected with inflammatory mediators (Langhans et al., 1993; Plata-Salamán, 2001).

Average feed intake is a commonly measured parameter to evaluate effects of DON in broiler feeding experiments (Awad et al., 2011; Yunus et al., 2012b). However, simply measuring feed intake may mask useful, dynamic information such as various feeding behaviour alterations and how quickly birds exhibit changes in feeding behaviour. Observing changes in behaviour can provide rapid and sensitive measure of sub-lethal toxicity (Melvin and Wilson,

2013). However, feeding behaviours have never been fully examined in poultry given DON-contaminated diets and could provide insight into how DON impacts feedings behaviours that contribute to reduced feed intake.

The current study was designed to determine the effect of feeding diets contaminated with *Fusarium* mycotoxins (primarily DON) on feed preference, feeding behaviours (feed preference, meal length and meal number) and growth performance in broiler chickens.

4.3 Materials and Methods

4.3.1 Animals and diets

Permission was granted for all experimental work by the University of Saskatchewan Animal Care Committee (protocol # 20130043) and followed recommended guidelines of Canadian Council on Animal Care (1993). A total of 120 day of hatch, non-vaccinated, male broiler chicks (Ross 308) were obtained from a local commercial hatchery. Birds were randomly assigned to 30 battery cages (29 cm high × 48 cm wide × 83 cm long; providing 800 cm^2/bird floor space) with 4 birds/cage in a temperature-controlled room at the University of Saskatchewan Poultry Research Center, Saskatoon, SK. At 21 d, 60 birds (4 birds/cage, fifteen cages) were randomly selected for a feed preference study and the remaining 60 birds (4 birds/cage, fifteen cages) were subjected to a feeding behaviour trial. During 1 – 7 d, birds were provided a 23 hour (h) light: 1 h continuous dark period. During 8 – 27 d, birds were provided a 19 h light: 5 h continuous dark period (Fig. 4.1). During the light period, the light intensity was 30 lux. During the dark period, the birds were subjected to complete darkness. At 21 d, the average body weight of birds was 968.0 ± 32.8 g and did not differ significantly among treatments ($P > 0.05$, ANOVA).

A common corn-soybean meal (SBM) starter diet was provided to all cages during 1 – 20 d so that all birds would be newly exposed to a wheat-SBM diet for the experimental period (21-27 d). Three experimental diets (control, low and high concentrations of *Fusarium* mycotoxins contaminated) were used. Wheat contaminated with relatively low mycotoxin concentrations (< 0.5 mg/kg DON) was used for the control diets. *Fusarium*-damaged wheat with 11.4 mg/kg DON was used to prepare the contaminated diets. All the diets were formulated to meet nutrient

requirements for broilers (Aviagen, 2014b) and birds were fed *ad libitum* during the starter period and throughout the trials.

Samples from each wheat source, corn, as well as formulated diets were analyzed for 16 common mycotoxins (Prairie Diagnostics Services Inc., Saskatoon, SK) via ultra-high performance liquid chromatography (Agilent 1100, Agilent Technologies, Santa Clara, CA) and mass spectrometry (Micromass Quattro Ultima Platinum Mass Spectrometer, Waters, Milford, MA). The mycotoxins included: DON and metabolites (3- and 15- acetyl DON); α-zearalenol, diacetoxyscirpenol, T-2, HT-2, nivalenol, ochratoxin A, β-zearalenol, zearalenone, and aflatoxin B1. The diet formulation, analyzed and calculated nutrient levels and analyzed mycotoxin concentrations are shown in Table 4.1. The corn-SBM starter diet contained DON at 0.14 mg/kg and HT-2 toxin at 0.36 mg/kg. Deoxynivalenol was the major mycotoxin in experimental diets at 0.085, 2.27 and 5.84 mg/kg in control, low and high *Fusarium* mycotoxin diets, respectively and are henceforth referred to as control, low and high DON diets (Table 4.1). The corn-based diet was prepared in crumbled form. Wheat-based experimental diets were pelleted. The pellets were with a 2 mm diameter and a 3.5 mm in length and were visually similar upon inspection.

4.3.2 Feed preference trial

In the feed preference trial, three treatments were randomly assigned to cages as: (1) control vs. low DON, (2) control vs. high DON, and (3) low vs. high DON. Each treatment was replicated five times. Each cage's feeder was split into two equal sized compartments, and different diets (control vs. low DON, control vs. high DON and low vs. high DON) were randomly allocated in each compartment in the corresponding cage. The amount of feed allocated in each compartment was recorded. During the experimental period, feed remaining in each compartment was weighed at 0900 daily. After feed weigh back, the additional diets were weighed and added into each compartment based on the treatments. The feed weigh back and feeding process were conducted within an hour. The daily and overall feed consumption of each diet per cage was calculated at 27 d.

4.3.3 Feeding behaviour trial

In the feeding behaviour trial, three experimental diets (control, low and high DON) were randomly assigned to fifteen cages with five cages per treatment. Experimental diets were offered *ad libitum* during 21 – 27 d.

The experimental design and daily activities for recording feeding behaviour are shown in Fig. 4.1. As mentioned previously, birds were subjected to 19 h light: 5 h continuous dark period with the dark period occurring between 1800 and 2300. Four birds in each cage were individually marked on the head with different signs using a black marker. During the experimental period (21 – 27 d), three cages (one cage from each treatment; one replication) were selected for the three 1 h video recording sections during a 24 h period (1000 h $_{\text{day a}}$ – 1000 h $_{\text{day a+1}}$, Fig. 4.1). Three digital cameras, one Olympus OM-D E-M10 camera (Olympus Imaging America Inc., Centre Valley, PA, USA) and two Sony α5000 cameras (Sony Corportaion, Tokyo, Japan), were set on tripods 0.5 m away from the front of the selected cages. The video recording sections were conducted at: (1) 1 h before the dark period, (2) 1 h after the end of the dark period, (3) 9 h after dark period (middle of the light period). After three video recording sections were completed, the cameras were moved to a different replication (three cages, one from each dietary treatment). During 21 – 25 d, feeding behaviours in all fifteen cages (five replications) were recorded. On 26 d and 27 d, one replication was randomly selected for behaviour recording.

Observation of videos and behaviour analyses were conducted after the trial was completed. Focal sampling (watching entire video continuously) was conducted for each bird for all three daily video recording periods. Initiation of a feeder visit event (at feeder) was defined as occurring when a bird's head was located over the edge of the feeder with the beak completely submerged into the feeder. If a feeder visit event occurred within 10 s (< 10 s), it was considered a part of the same feeding bout. If a feeder visit event occurred longer than 10 s (≥ 10 s) after another feeder visit, the two were considered separate bouts. A bout interval of 10 s was chosen based on previous work (Bokkers and Koene, 2003). Initiation of a drinker visit event (at drinker) was defined as occurring when a bird was actively pecking at the nipple drinker located at the back of the cage. The same bout interval criteria that was used for at feeder event was also used for at drinker event. If two at feeder events occurred within 10s but were separated by an at drinker event then the feeding bouts were considered as two separate bouts. The same occurred

when two at drinker events occurred within 10s but were separated by an at feeder event. The bout length (s) of every feeder or drinker visit, number of feeder or drinker visit events were recorded. Average bout length (s) of at drinker or feeder event was calculated. The percentage of time (budget) during observation spent at the feeder or the drinker was calculated as followed: (average length of at feeder or at drinker bout (s) * number of bout / 3600 s/h) * 100%. The observation was verified by a different observer and the intra-observer consistency was > 90%.

4.3.4 Growth performance

In both trials, broiler body weight (g) and total feed consumption of each cage were measured on 27 d. In the feed preference trial, feed consumption was measured daily as descripted previously. Average daily gain (ADG, g/d), average daily feed intake (ADFI, g/d) and feed to gain ratio (F:G, g feed/g weight gain) of each cage were then calculated. Average daily gain, ADFI, F: G were calculated as followed: $ADG_{(period\ a\text{-}b)} = [BW_{day\ b} - BW_{day\ a}]$/period a-b, ADFI = [feed weight $_{day\ a}$ – feed weight $_{day\ b}$]/period a-b and F:G = DFI/ ADG.

4.3.5 Statistical analyses

The feed preference and behaviour trials were a completely randomized design with five replicates. Prior to statistical analyses, data were tested for normality. If the data were not normally distributed, the log transformation was conducted. In the feed preference trial, daily proportional feed intake in each treatment (control vs low DON, control vs high DON and low vs high DON) were analyzed as repeated measure, with age (21 – 27 d) as a factor. Behaviour data (bout lengths of at feeder/ drinker events, number of feeder/drinker visiting events, budget of at feeder/drinker events) and growth performance measures (body weight, ADG, ADFI and F:G) were analyzed as completely randomized design. All data were analyzed using ANOVA by the Proc Mixed procedure (Littell, 2006) in SAS 9.4 (SAS Institute Inc., Cary, NC). Significant effects ($\alpha = 0.05$) were analyzed using the Tukey-Kramer test to differentiate the means.

Table 4.1. Feed formulations, nutrient compositions and analyzed mycotoxins for corn-based starter (1 – 20 d) diet, wheat based control, low and high mycotoxin grower (21 – 27 d) diets.

		Wheat based experimental diets		
	Corn starter	**Control**	**Low DON**	**High DON**
Ingredient (%)				
Corn [1]	50.2	0	0	0
Control wheat [2]	0	76.5	38.2	0
Fusarium-damaged wheat [3]	0	0	38.3	76.5
Soybean meal	42.3	14.0	14.0	14.0
Fish meal	0	1.0	1.0	1.0
Corn-gluten meal	0	3.0	3.0	3.0
Canola oil	2.7	2.0	2.0	2.0
DiCalcium phosphate	1.6	0.5	0.5	0.5
Limestone	1.4	1.2	1.2	1.2
Salt (as NaCl)	0.5	0.25	0.25	0.25
Vitamin/mineral premix [4]	0.5	0.5	0.5	0.5
Choline chloride	0.1	0.1	0.1	0.1
DL-Methionine	0.45	0.5	0.5	0.5
L-Lysine	0.1	0.4	0.4	0.4
L-Threonine	0.1	0	0	0
Enzyme [5]	0.05	0.05	0.05	0.05
Total	100.0	100.0	100.0	100.0
Nutrient composition (%) [6]				
Crude Protein	23.4	22.4	20.0	19.8
AME (kcal/kg)	3099	3096	3091	3085
Calcium	1.31	0.95	0.86	0.88
Phosphorus	0.90	0.62	0.60	0.58
Crude fiber	2.51	2.41	2.39	2.50
Fat	4.68	4.48	4.92	5.15
Ash	7.63	5.48	5.73	5.65
Calculated amino acid (%)				
Lysine	1.57	1.31	1.22	1.18
Methionine	0.73	0.51	0.54	0.52
Meth + Cys	1.11	1.23	1.30	1.25
Analysed mycotoxin (mg/kg) [7]				
Deoxynivalenol (DON)	0.14	0.085	2.27	5.84
3-Acetyl DON	<0.025	<0.025	<0.025	0.11
15-Acetyl DON	<0.025	<0.025	<0.025	<0.025
HT -2 Toxin	0.36	<0.025	<0.025	<0.025

[1] Mycotoxins concentration in corn: aflatoxin B1: Not Detected (ND), 3-Acetyl Deoxynivalenol (DON): ND, 15-Acetyl DON: ND, α-Zearalenol: ND, DON: 527 µg/kg, Diacetoxyscirpenil: ND, HT-2 toxin: 63 µg/kg, Nivalenol: ND, Ochartoxin A: ND, T-2 toxin: ND, β-Zearalenol: ND, Zearalenone: ND.
[2] Mycotoxins concentration in control wheat: aflatoxin B1: Not Detected (ND), 3-Acetyl Deoxynivalenol (DON): ND, 15-Acetyl DON: ND, α-Zearalenol: ND, DON: 352 µg/kg, Diacetoxyscirpenil: ND, HT-2 toxin: ND, Nivalenol: ND, Ochartoxin A: ND, T-2 toxin: ND, β-Zearalenol: ND, Zearalenone: ND.
[3] Mycotoxins concentration in *Fusarium* damaged wheat: aflatoxin B1: ND, 3-Acetyl Deoxynivalenol (DON): 764 µg/kg, 15-Acetyl DON: ND, α-Zearalenol: ND, DON: 11470 µg/kg, Diacetoxyscirpenil: ND, HT-2 toxin: 107 µg/kg, Nivalenol: 59.2 µg/kg, Ochartoxin A: ND, T-2 toxin: ND, β-Zearalenol: ND, Zearalenone: ND.
[4] Vitamin/mineral premix: vitamin A: 2200000 IU, vitamin D: 440000 IU, vitamin E: 6000 IU, menadione: 400 mg, thiamine: 300 mg, riboflavin: 1200 mg, pyridoxine:800 mg, vitamin B12: 4 mg, niacin: 12000 mg, pantothenic acid: 2000 mg, folic acid: 120 mg, biotin: 30 mg. copper: 2000 mg, manganese: 16000 mg, iodine: 160 mg, zinc: 16000 mg, selenium: 60 mg, calcium carbonate: 100000 mg, antioxidant: 125 mg, wheat midds: 807879 mg. (DSM Nutritional Products Canada Inc. Ayr. ON).
[5] Enzyme: Beta-glucanase: 1000 units/g, xylanase: 1500 units/g (GNC Bioferm Inc., Saskatoon, SK).
[6] Nutrient composition were analyzed at Central Testing Laboratory Ltd., (Winnipeg, MB). AME was calculated as: AME=53+38*((Crude Protein)+(2.25*Fat)+(100-((Crude Protein)+(Crude fibre)+Fat+(Ash) (moisture)))*0.9).
[7] Mycotoxins were analyzed at Prairie Diagnostics Services Inc., (Saskatoon, SK) via ultra-high performance liquid chromatography (Agilent 1100, Agilent Technologies, Santa Clara, CA) and mass spectrometry (Micromass Quattro Ultima Platinum Mass Spectrometer, Waters, Milford, MA).

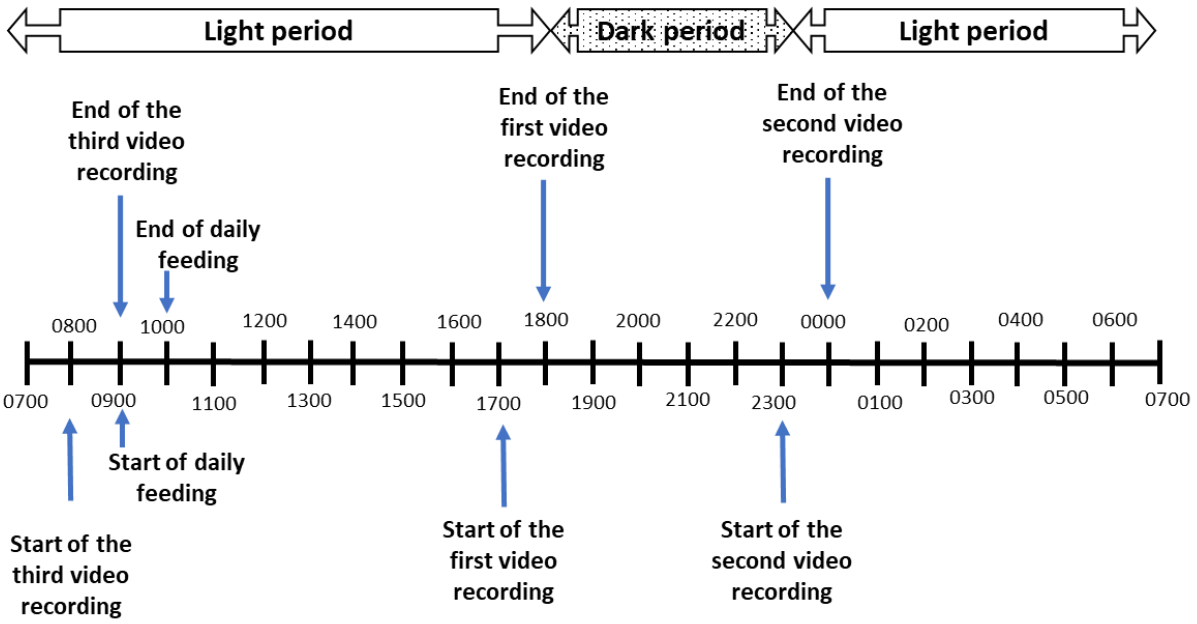

Figure 4.1. Daily activities of behavioural observation trial where broilers were provided three novel grower diets containing three concentrations of DON contamination (control, low, and high at 0.085 mg/kg, 2.27 mg/kg and 5.84 mg/kg, respectively). Diets were provided from 21 – 27 d with 5 cages assigned per diet (4 birds/cage). Three one-hour video recording sections were conducted per day. The first recoding section was conducted during the last 1 h period before the five hours uninterrupted dark period. The second recoding section was conducted during the first 1 h period after the dark period. The third video recording section was conducted for 1 h at the 9 h mark after dark period.

4.4 Results

4.4.1 Effects of DON on feed preference in broilers

Daily proportional feed intake (intake of diet/total feed consumption) during the feed preference trial is shown in Fig. 4.2. Broilers preferred control diet over low DON diet ($P < 0.01$) while age ($P = 0.99$) and diet x age ($P = 0.31$) did not affect feed preference (Fig. 4.2A). When given a choice between control and high DON diets, broilers preferred control diet over high DON diet ($P < 0.01$), and age ($P = 0.99$) and diet x age ($P = 0.35$) did not affect feed preference (Fig. 4.2B). When given a choice between the two DON diets, broilers did not exhibit a preference between low and high DON diet ($P = 0.78$), the age ($P = 0.99$) and diet x age ($P = 0.68$) did not affect feed preference (Fig. 4.2C). Across choice fed groups, control vs. low DON, control vs. high DON, and low vs. high DON, there was no difference in body weight ($P = 0.79$), daily feed intake ($P = 0.19$), average daily gain ($P = 0.38$), or feed/gain ratio ($P = 0.06$; Table 4.2). When comparing the average daily feed intake of different experimental diets within each choice-fed group, there was higher feed intake for control diet in the control vs. low DON (93.0 vs. 66.1 g, $P < 0.01$) and control vs. high DON treatments (104.4 vs. 50.4 g, $P < 0.01$), but did not differ between low and high DON diets (83.1 vs. 80.3 g, $P = 0.83$, Fig. 4.3).

4.4.2 Effects of DON on feeding and drinking behaviour in broilers

In the behaviour trial, body weight, average daily gain, and daily feed intake were not affected by dietary treatment ($P > 0.05$, Table 4.3). Birds fed the high DON diet had higher F:G ratio (1.30) compared to those fed the low DON diet (1.21) and control diet (1.10; $P < 0.01$, Table 4.3).

The effects of feeding DON-contaminated diets to broiler during 21 – 27 d on feeding behaviours are shown in Table 4.4. Broilers fed either the low DON or high DON diets spent more time at feeder during three observation periods when compared to those fed the control diet ($P \leq 0.05$, Table 4.4). During the 1 h before dark period, both low DON-fed birds and high DON-fed birds had longer at feeder bouts compared to control diet-fed birds (85.0 and 85.4 s vs. 55.2 s, $P < 0.01$). During the 1 h after dark period, the low DON-fed birds had longer at feeder bouts compared to control diet-fed birds (90.3 s vs. 51.8 s, $P = 0.05$). During the 9 h after dark

period, both low DON-fed birds and high DON-fed birds had longer at feeder bouts compared to control diet-fed birds (70.1 and 70.9 s vs. 45.9 s, $P = 0.02$). There was no effect of diet treatment on bout number for any observational period. Both low DON-fed birds and high DON-fed birds spent overall more overall time at the feeder (budget = % time spent at feeder during the observation period) when compared to control diet-fed birds at each observational period, 1 h before dark period (19.4% and 18.8% vs. 9.6%, $P = 0.04$), 1 h after dark period (23.6% and 24.2% vs. 12.8%, $P = 0.02$), and 9 h after dark period (18.9% and 19.7% vs. 9.8%, $P < 0.01$). There was no effect of diet treatment on any of the drinker behaviours assessed during the observational periods ($P > 0.05$, Table 4.5).

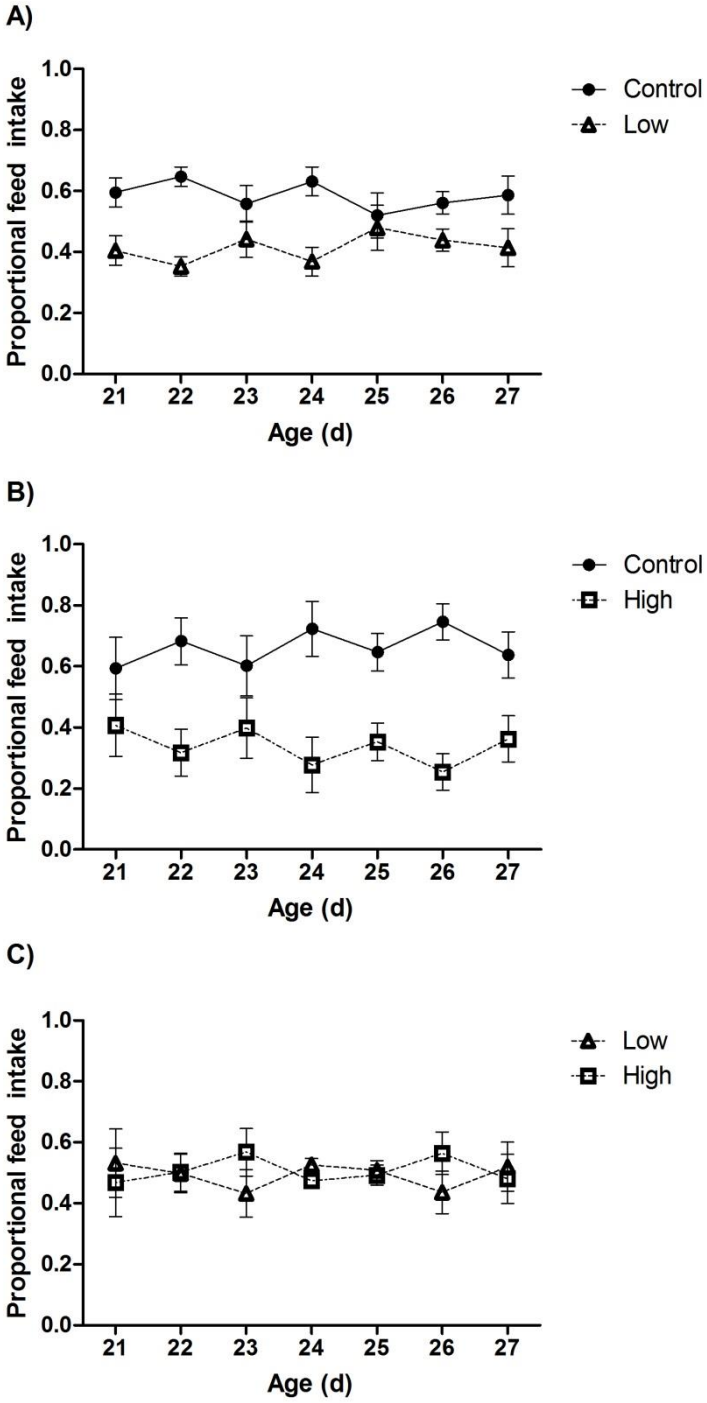

Figure 4.2. Daily proportional feed intake of broilers provided a choice of diets with varying concentrations of DON (control = 0.085 mg/kg, low = 2.27 mg/kg, high = 5.840 mg/kg) from 21-27 d. Treatments were (A) control vs. low DON, (B) control vs. high DON, and (C) low vs. high DON (n=5 cages per treatment).

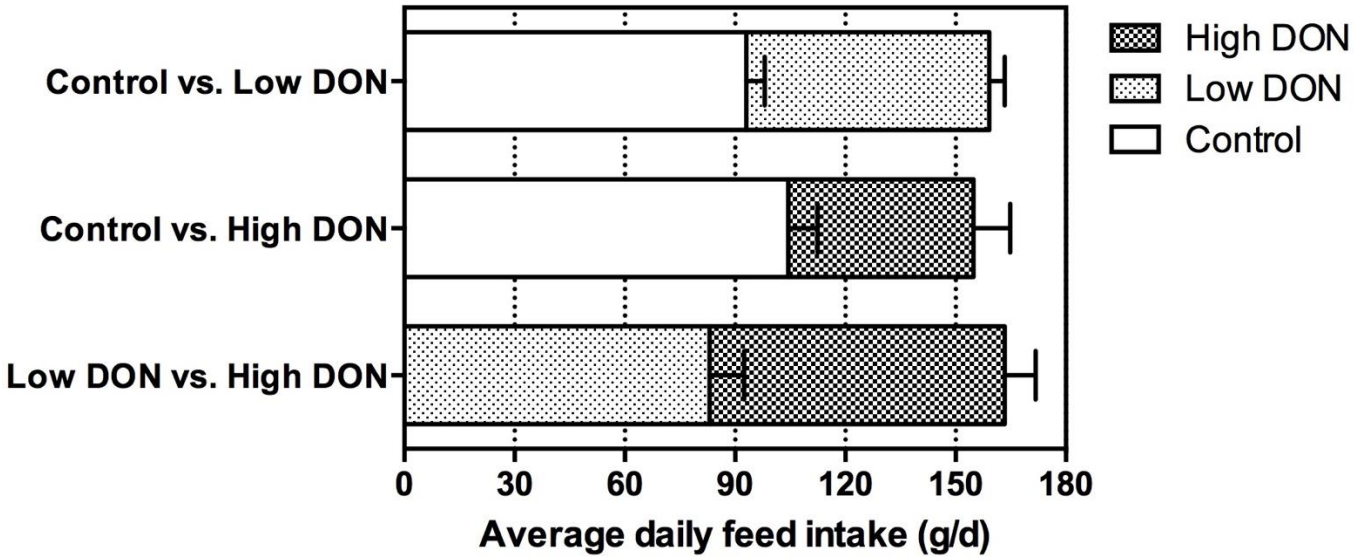

Figure 4.3. Average daily feed intake (g/d) of broilers provided a choice of diets during 21 – 27 d with varying concentrations of DON (control = 0.085 mg/kg, low = 2.27 mg/kg, high = 5.840 mg/kg. Treatments were control vs. low DON, control vs. high DON, and low vs. high DON (n=5 cages per treatment).

Table 4.2. Growth performance of broilers during 21 – 27 d in the feed preference trial.

Treatments[1]	**Body weight at 27 d (g)**	**Daily feed intake (g/d)**	**Average daily gain (g/d)**	**Feed/gain ratio (g feed/ g gain)**
Control vs Low DON	1616.5 ± 39.3	159.1 ± 5.2	95.6 ± 3.6	1.65 ± 0.03
Control vs High DON	1590.4 ± 23.6	152.2 ± 3.7	92.1 ± 2.8	1.65 ± 0.05
Low DON vs High DON	1597.6 ± 10.3	163.4 ± 2.9	91.2 ± 1.7	1.79 ± 0.05
ANOVA[2]	P = 0.79	P = 0.19	P = 0.38	P = 0.06

[1] Control, Low DON and High DON diet contained 0.085 mg/kg, 2.27 mg/kg and 5.840 mg/kg DON, respectively. Each cage's feeder was split into two equal sized compartments, and different diets were randomly offered in each compartment in the corresponding cage.
[2] Growth performance (mean ± SE) data were analyzed by one-way ANOVA; n = 5/treatment

Table 4.3. Effects of feeding broilers DON-contaminated diets during 21 – 27 d on growth performance in the feeding behaviour trial.

Treatment[1]	Body weight at 27 d (g)	Average daily gain (g/d)	Daily feed intake (g/d)	Feed/gain ratio (g feed/g gain)
Control	1621.3 ± 27.8	94.3 ± 3.4	154.7 ± 4.6	1.65 ± 0.05[c]
Low DON	1588.6 ± 66.1	86.1 ± 3.2	156.1 ± 6.3	1.82 ± 0.05[b]
High DON	1541.5 ± 27.2	83.6 ± 3.3	162.2 ± 6.8	1.94 ± 0.04[a]
ANOVA[2]	P = 0.20	P = 0.09	P = 0.40	P < 0.01

[1] Control, Low DON and High DON diet contained 0.085 mg/kg, 2.27 mg/kg and 5.840 mg/kg DON, respectively.

[2] Mean ± SE with different superscripts (a-c) are significantly different ($P \leq 0.05$, one-way ANOVA followed by Tukey-Kramer test; n = 5/treatment).

Table 4.4. Effects of feeding broiler DON-contaminated diets on feeding behaviour during 21 – 27 d in the feeding behaviour trial.

Period[1]	Treatment[2]	Bout Length (s)	Bout Number	Budget (%)
1 h before the dark period	Control	55.2 ± 6.9[a]	8.7 ± 1.7	9.6 ± 0.5[a]
	Low DON	85.0 ± 11.2[b]	10.5 ± 0.8	19.4 ± 1.4[b]
	High DON	85.4 ± 8.9[b]	10.7 ± 0.9	18.8 ± 1.0[b]
	ANOVA[3]	P < 0.01	P = 0.43	P = 0.04
1 h after the dark period	Control	51.8 ± 6.5[a]	7.8 ± 1.0	12.8 ± 1.1[a]
	Low DON	90.3 ± 13.0[b]	8.5 ± 1.1	23.6 ± 1.7[b]
	High DON	68.8 ± 8.2[ab]	9.8 ± 0.9	24.2 ± 1.2[b]
	ANOVA[3]	P = 0.05	P = 0.37	P = 0.02
9 h after the dark period	Control	45.9 ± 4.5[a]	7.8 ± 1.1	9.8 ± 0.5[a]
	Low DON	70.1 ± 5.1[b]	10.4 ± 0.7	18.9 ± 0.6[b]
	High DON	70.9 ± 4.5[b]	11.3 ± 1.3	19.7 ± 0.9[b]
	ANOVA[3]	P = 0.02	P = 0.17	P < 0.01

[1] Broilers were subjected to a five hours uninterrupted dark period during experimental period.
[2] Control, Low DON and High DON diet contained 0.085 mg/kg, 2.27 mg/kg and 5.840 mg/kg DON, respectively.
[3] Mean ± SE with different superscripts (a-b) are significantly different ($P \leq 0.05$, one-way ANOVA followed by Tukey-Kramer test; n = 5/treatment).

Table 4.5. Effects of feeding broiler DON-contaminated diets on drinking behaviour during 21 – 27 d in the feeding behaviour trial.

Period[1]	Treatment[2]	Bout Length (s)	Bout Number	Budget (%)
1 h before the dark period	Control	46.0 ± 4.5	7.8 ± 0.4	8.0 ± 1.1
	Low DON	49.3 ± 5.1	6.9 ± 1.1	6.8 ± 0.9
	High DON	38.2 ± 4.5	6.7 ± 0.6	8.9 ± 1.0
	ANOVA[3]	P = 0.66	P = 0.72	P = 0.92
1 h after the dark period	Control	35.0 ± 2.4	8.8 ± 1.5	8.4 ± 1.3
	Low DON	36.9 ± 3.6	9.2 ± 1.2	8.8 ± 1.7
	High DON	38.9 ± 2.8	9.7 ± 0.9	8.1 ± 1.2
	ANOVA[3]	P = 0.66	P = 0.72	P = 0.92
9 h after the dark period	Control	36.5 ± 5.4	6.6 ± 1.2	5.6 ± 1.1
	Low DON	35.7 ± 3.3	5.3 ± 0.6	5.0 ± 0.9
	High DON	43.5 ± 4.7	6.7 ± 0.7	7.6 ± 0.8
	ANOVA[3]	P = 0.48	P = 0.45	P = 0.19

[1] Broilers were subjected to a five hours uninterrupted dark period during experimental period.
[2] Control, Low DON and High DON diet contained 0.085 mg/kg, 2.27 mg/kg and 5.840 mg/kg DON, respectively.
[3] Data were analyzed by one-way ANOVA; n = 5/treatment.

4.5 Discussion

The current study was designed to evaluate the effect of *Fusarium* mycotoxins (primarily DON) on broiler feed preference, feeding behaviours, and growth performance and is one of the first to demonstrate DON-induced feeding behaviour changes in broilers. We demonstrated that inclusion of naturally-contaminated grain in broiler diets can reduce diet palatability, induce sensory aversion and alter feeding behaviour at concentrations lower than the recommended dietary allowance of 5 mg/kg (CFIA, 2015). Our data also indicates that broiler chickens prefer uncontaminated diets over DON-contaminated diets when given free choice. This preference was exhibited consistently over the seven day feeding trial demonstrating that broilers are sensitive to DON-induced sensory aversion. The preference exhibited by broilers for the uncontaminated diet over DON-contaminated diets observed in the current study is consistent with previous short-term feeding studies with laying hens (Hamilton et al., 1986) and broilers (Mannion and Blaney, 1988) and supports evidence that DON contamination of feed can induce taste aversion in production animals (Osweiler, 2014).

Mycotoxin-induced reduction in feed intake is often referred to as feed refusal and while feed intake is commonly used to evaluate effects of DON on broilers, feed refusal is not a consistently response observed response (Grenier and Applegate, 2013). In the current study, broiler feed intake was not affected by DON-contaminated diets. Previous studies suggest that 15 mg/kg DON in naturally-contaminated diets is required to elicit a feed refusal response in broilers under experimental setting (Awad et al., 2012; Grenier and Applegate, 2013). The relatively low concentration of DON (5.8 mg/kg) and the short-term exposure used in the current study may not have been sufficient to induce feed refusal in grower broilers although similar concentrations reduced feed intake in a previous study where broilers were fed a similar DON-contaminated diet (6.6 mg/kg) during the grower stage (22-34 d; Wang and Hogan, 2018). We also observed that when given the choice, broilers exhibited immediate and prolonged aversion to DON-contaminated diets at concentrations that are at or below recommended dietary concentrations for poultry (CFIA 2015). These same DON concentrations altered birds' feeding behaviour without affecting feed consumption, as evidenced in the behavioural trial. A previous study in rodents reported similar results where DON-spiked diets induced taste aversion and affected feeding behaviours without affecting rodents' feed intake (Clark et al., 1987). Together

our results show that feeding behaviours are a sensitive endpoint for evaluating effects of DON on broilers as changes in behaviour can occur at a lower threshold of DON exposure than feed intake.

In addition to measuring feed consumption, analysis of meal patterns by recording of food consumption and meal number allows feed intake to be examined in terms of meal size and meal frequency. We found that meal number, meal frequency (meal number/ hour) and meal size (estimated by feed intake/meal number) did not change with feeding DON-contaminated diets. However, altered broiler feeding behaviour, such as increased time that broilers spent at the feeder, was consistently observed in birds provided DON-contaminated diets, regardless of the observation period. Eating behaviours and meal patterns are typical reflections of the internal feed intake control mechanisms (Reddingius, 1980; Bokkers and Koene, 2003). Modern broilers' feed intake regulation is dominated by satiety mechanisms and broilers tend to consume feed until maximal gut physical capacity is reached (Bokkers and Koene, 2003; Buzala and Janicki, 2016). In general, activation of anorexia neurochemical pathways suppress broiler feed intake and terminate eating (Bokkers and Koene, 2003; Richards and Proszkowiec-Weglarz, 2007). In the current behaviour trial, the time (meal length) spent by the broilers to consume the similar amount of feed (meal size) was longer in DON treatments compared to the control group. This response suggests that DON induces broiler sensory aversion at concentrations which is not sufficient to induce the anorexia effects and supports previous evidence that DON contamination reduces diet palatability and induce taste aversion in broilers (Mannion and Blaney, 1988).

In the behaviour trial, a reduction of feed efficiency was observed in birds provided DON diets. This finding supports the results of a recent meta-analysis demonstrating that DON negatively affects broiler feed efficiency (Andretta et al., 2011). The gastrointestinal tract is the site of nutrient digestion and absorption as well as the primary site of dietary DON exposure (Maresca, 2013). Several studies in broilers report that consumption of DON-contaminated diets reduces villi height and surface area as well as digestion and absorption functions (Awad et al., 2004; 2006a; 2008). Although we did not measure intestinal mucosa structures or absorptive function in the present study, it is possible that the reduction of feed efficiency is due to the negative effect of DON on intestinal mucosa structures and functions. We previously reported a reduction in feed efficiency along with altered villi structure in broilers fed DON-contaminated diets during 22 – 34 d (Wang and Hogan, 2018). Together, this research suggests that modern

broilers maybe more sensitive to DON contamination than previously estimated, since seven days of exposure to relatively low concentrations of DON during the grower phase is sufficient to impair growth performance. This exposure scenario and performance effects would be relevant in a production setting where it is thought that birds may be more frequently exposed to DON at relatively low concentrations (≤ 5 mg/kg) (Grenier and Applegate, 2013; Kosicki et al., 2016).

Feed refusal is a well-documented adverse effect of trichothecenes exposure (Osweiler, 2014). Feed refusal may also negatively affect broilers by limiting the total nutrient intake. However, the relative importance of feed refusal and other trichothecenes toxic effects on broiler remain unknown. This may be in part because in previous studies that report DON-induced feed refusal and growth suppression in broilers (Swamy et al., 2004a; Awad et al., 2011; Yunus et al., 2012b), the birds were provided feed *ad libitum*. In a feeding trial with swine, the use of a paired feeding method effectively demonstrated that growth suppression was the result of DON-induced partial feed refusal (Goyarts et al., 2005). Observing broiler feeding behaviour under both *ad libitum* and restricted feeding condition may provide further insight into the relative importance of DON-induced feed refusal compared with other adverse effects in contributing to reduced growth performance in broilers.

PREFACE TO CHAPTER 5

In the previous chapters, we identified broilers are sensitive to *Fusarium* mycotoxin-contaminated diets through demonstration of growth suppression, feed refusal, altered intestinal morphology and changes in feeding behaviours. However, it remains unknown whether the growth suppressive effects can be attributed specifically to the reduction of feed intake or a combination of both reduced feed intake and other toxic effects of DON. In the current study, we identified that reduction in growth performance is most likely caused by reduction of feed intake rather than other toxic effects of DON. In line with Chapter 4, we also observed that DON contamination induced sensory aversion and feeding behaviour alterations in broilers during the later stage of growth. These results suggest that methods which improve feed palatibilty could prevent performance effects in broilers that results from consumption of low concentration DON-contaminated feed.

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Author contributions:

Anhao Wang (University of Saskatchewan) designed and managed the experiment, generated and analyzed the data, prepared all figures, and drafted the manuscript.

Dr. Denise Beaulieu (University of Saskatchewan) provided the guidance for experimental design and statistical analyses, commented on and edited the manuscript.

Dr. Natacha Hogan (University of Saskatchewan) provided inspiration and scientific input, commented on and edited the manuscript, and obtained funding for the research.

CHAPTER 5

EFFECTS OF *FUSARIUM* MYCOTOXINS AND RESTRICTED FEED INTAKE ON BROILER GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY AND FEEDING BEHAVIOR

5.1 Abstract

A 28-day pair-feeding trial was conducted to determine whether the performance and gastrointestinal effects of *Fusarium* mycotoxins (primarily deoxynivalenol, DON) in broiler chickens are due to DON-induced reduction in feed intake. Ross 308 eggs were randomly divided into two groups and set in incubators 24 hours apart. At hatch, 72 newly-hatched males were randomly selected from the first set of eggs and assigned to two dietary treatments (control vs. mycotoxin) with 6 birds/cage and 6 cages/diet. Birds were provided diets formulated with naturally-contaminated wheat resulting in DON concentrations of 3.0 mg/kg (starter diet, 1 – 21 day post-hatch (d)) and 2.4 mg/kg (grower diet, 22 – 28 d). The control diets had < 0.1 mg/kg DON. The first set of 72 birds were *ad libitum*-fed and feed intake was measured daily. Thirty-six newly-hatched males were randomly selected from second set of eggs and were pair-fed the control diet, where the amount of diet provided to each pair-fed cage was calculated as number of birds in a cage x average feed intake of birds fed the DON diet *ad libitum* at the same age (d). Feeding and drinking behaviors were video recorded daily for a continuous one-hour period starting three hours after feed was provided. Growth performance (body weight (g), average daily gain (ADG, g/d), feed to gain ratio (F/G, g feed/g gain)) was measured at 7, 14, 21 and 28 d. At 28 d, six birds per treatment were randomly selected and sacrificed for histopathological assessment of duodenal morphology. During 1 – 7, 15 – 21 d, DON-fed birds consumed less feed than control birds (21.6 vs. 24.4 g/d and 98.5 vs. 91.0 g/d, $P < 0.01$; respectively). However, there was no difference in ADG and BW between the DON-fed and the control birds during first 21 d ($P > 0.05$). During 22 – 28 d, DON-fed birds had lower feed intake and ADG than control birds (143.2 vs. 162.5 g/d and 89.8 vs. 101.6 g/d, $P < 0.01$; respectively). During the entire growth period, the pair-fed birds had lower ADG and were lighter than control birds ($P < 0.01$). During 1 – 21 d, DON-fed birds had higher feed intake and ADG compare to the pair-fed birds ($P < 0.01$). During 22 – 28 d, DON-fed and the pair-fed birds had similar feed intake and ADG ($P > 0.05$). Broiler at-feeder behaviours were not affected by treatment during 1 – 21 d ($P > 0.05$). During 22 – 28 d, the DON-fed birds spent longer than at feeder compare to the pair-fed birds (61.6 vs. 39.7s, $P = 0.02$). At 28 d, duodenal morphology was not affected by treatments ($P > 0.05$). The behaviour results suggest that DON intake induced anorexigenic effects and sensory aversion as DON-fed broilers consumed less feed compare to the control birds and DON-fed

birds spent longer time at feeder compares to the pair-fed treatment. Overall, since DON-fed birds and restricted-fed birds had equally reduced feed intake and suppressed performance, we conclude that the reduction in growth performance is most likely caused by reduction of feed intake rather than other toxic effects of DON.

5.2 Introduction

The trichothecene mycotoxin deoxynivalenol (DON) is the most prevalent mycotoxin of *Fusarium*-infected grains in North America (Tittlemier et al., 2013; Miller et al., 2014; Kosicki et al., 2016). DON intake induces a wide range of adverse effects in animals, including taste aversion, feed refusal, growth suppression, altered gastrointestinal (GIT) morphology and suppressed immune function (Richard, 2007; Girgis and Smith, 2010; Grenier and Applegate, 2013). Among monogastrics, poultry are considered more resistant to DON contamination, especially when compared to swine (Richard, 2007; Osweiler, 2014; CFIA, 2015). The maximum dietary DON allowance is 1 mg/kg for swine compared to 5 mg/kg for poultry (CFIA, 2015). This means that DON-contaminated grains, which are not suitable for swine, could be allocated to poultry and used at a higher inclusion level in feed production, resulting in exposure to low concentrations (< 5 mg/kg) of DON under agricultural practices. However, recent studies in our lab (Wang and Hogan, 2018; Wang et al., submitted) and by others (Awad et al., 2011; Yunus et al., 2012b; Lucke et al., 2017) show that concentrations of DON in poultry feed near or below the 5 mg/kg can significantly affect performance, feed intake, feeding behavior and GIT morphology in broiler chickens. Therefore, effects of low concentrations of *Fusarium* mycotoxins on broiler chickens cannot be underestimated.

Feed refusal is one of the most well-known responses to trichothecene exposure in animals (Vesonder et al., 1973; Osweiler, 2014). The basis of mycotoxin-induced feed refusal has not been fully elucidated but is thought to be a protective mechanism that prevents animals from developing further trichothecene toxicosis, such as disruption of metabolism and changes in intestinal morphology (Osweiler, 2014). However, feed refusal also reduces total dietary nutrient intake and prevents broilers from achieving their growth potential leading to decreased growth performance. In previous studies reporting DON-induced reduction in feed intake and growth

suppression, broilers were usually fed DON-contaminated diets *ad libitum* (e.g. (Awad et al., 2011; Yunus et al., 2012b) and this makes it difficult to ascertain whether performance effects of DON are attributed to decreased feed intake or other aspects of DON toxicity. Therefore, it remains unknown whether the growth suppression reported with feeding of DON-contaminated diets is caused by the reduction of feed intake or a combination of both reduced feed intake and the other toxic effects of DON.

Separating the DON effect on feed intake from other DON toxicities, such as effects of DON on intestinal morphology and their contribution to broiler growth performance could provide further understanding about DON-induced broiler growth suppression and direct potential methods to mitigate these adverse effects (increase birds' feed intake vs. reduce dietary mycotoxin toxicities). Consumption of the same amount of feed (i.e. a pair-feeding regimen) is necessary to differentiate the direct toxin effects from effect of feed intake. In a pair-feeding regimen, pair-fed animals were restricted in the amount of feed that was consumed by the mycotoxin group (which would be expected to be less than the control *ad libitum* group) (Goyarts et al., 2005; Hooft et al., 2011). This method was previously used in a swine study and results demonstrated that DON-induced growth suppression was the result of reduction in feed intake (Goyarts et al., 2005). However, to our knowledge, there have been no similar paired-feeding studies in broilers.

Feeding behaviours are considered a reflection of internal feed intake regulation mechanisms (Reddingius, 1980; Bokkers and Koene, 2003) and observing changes in behaviour can provide rapid and sensitive measures of sub-lethal toxin exposures (Melvin and Wilson, 2013). Trichothecenes suppress feed intake through various actions such as inducing taste aversion, anorexigenic effects and modulating immune function (Grenier and Applegate, 2013; Osweiler, 2014; Lebrun et al., 2015). Partial feed refusal and altered feeding behaviours such as reduction of meal size and meal frequency were observed in rodents following acute oral-exposure to DON (Girardet et al., 2011; Gaigé et al., 2014). We previously showed in a seven-day feeding trial (21 – 27 d) that inclusion of naturally-contaminated grain (the primary mycotoxin being DON) in broiler feed can reduce diet palatability, induce sensory aversion and alter grower broiler feeding behaviour (Wang et al., submitted) . This suggests that observing broiler feeding behaviour may provide useful information to further understand trichothecene-

induced feed refusal. However, the effects of prolonged intake of DON-contaminated diets (through the entire growth period) on broiler feeding behaviour remain to be studied.

The present study was conducted to determine the relative importance of reduction of feed intake and mycotoxins toxicosis on broilers through a 28-day continuous dietary exposure to low concentrations of DON. We compared the broiler growth performance as well as measures of GIT morphology and behaviour to determine the effects of reduction of feed intake and DON toxic effects on broiler chickens.

5.3 Materials and Methods

5.3.1 Animals, diets and feeding protocol

Permission for all experimental work was granted by the University of Saskatchewan Animal Care Committee (protocol # 20130043), with all procedures following the recommendations of the Canadian Council on Animal Care (1993). Two hundred and forty Ross 308 broiler eggs (62 ± 5 g) were purchased from a local hatchery. Eggs were randomly divided into two groups and were incubated in University of Saskatchewan hatchery 24 hours apart. The first set had one hundred and sixty eggs and the second set had eighty eggs. Eggs were candled on incubation day 16 and infertile and early embryonic dead eggs were removed. The incubation process was terminated on 21.5 day after eggs were set. The birds' age was defined as days post-hatch (d) in reference to the end of incubation process. Newly-hatched chicks were feather sexed. Seventy-two healthy males were selected from the first set of eggs and thirty-six healthy males were selected from the second set of eggs. The selected birds were randomly placed into eighteen cages (29 cm high × 48 cm wide × 83 cm long; providing 800 cm^2/bird floor space) with 6 birds/ cage. Birds were kept in a 23h light/1h dark period during 1 – 7 d (Fig. 5.1A) and an 18h light/6h dark period during 8 – 28 d (Fig. 5.1B). During the entire experimental period, birds had *ad libitum* access to water.

Diets were formulated to meet the nutrient requirements for the growing birds (Aviagen, 2014b). A source of Canada Western Red Spring wheat with DON < 0.1 mg/kg was used to prepare the control diets and *Fusarium*-damaged Canada Western Red Spring wheat (around 11.5 mg/kg DON) was used to prepare the contaminated diets. The diet formulations, calculated nutrient levels and analyzed mycotoxin concentrations, are shown in Table 4.1. Two starter (1 –

21 d) and two grower (22 – 28 d) diets were produced at the Canadian Feed Research Centre (North Battleford, SK). Samples from each wheat and subsequent diets were analyzed for 16 common mycotoxins (Prairie Diagnostics Services Inc., Saskatoon, SK) via ultra-high performance liquid chromatography (Agilent 1100, Agilent Technologies, Santa Clara, CA) and mass spectrometry (Micromass Quattro Ultima Platinum Mass Spectrometer, Waters, Milford, MA). The mycotoxins suite included: DON and metabolites (3- and 15- acetyl DON); α-zearalenol, diacetoxyscirpenol, T-2, HT-2, nivalenol, ochratoxin A, β-zearalenol, zearalenone, and aflatoxin B1. Mycotoxin concentrations of all diets were confirmed prior to feeding of animals. Mycotoxins in control diets were below the limit of detection (Table 5.1). Deoxynivalenol was the major mycotoxin measured in starter and grower diet at 3.00 and 2.40 mg/kg, respectively (Table 5.1) and these diets are henceforth referred to as DON diets. Other mycotoxins, such as 3 – ADON, HT-2 toxin and ochratoxin A were also detected but at < 0.2 mg/kg (Table 5.1). All diets were produced at the Canadian Feed Research Centre (North Battleford, SK). The starter diets were prepared in the crumbled form and the grower diets were pelleted. The pellets were 2 mm in diameter and 3.5 mm in length. The diets and were visually similar upon inspection.

The first set of birds was provided *ad libitum* access to either control or DON diet immediately after hatch (start of feeding trial) and are henceforth referred to as either control or DON-fed birds. The feed intake of *ad libitum*-fed birds was measured daily one hour after the light was turned on. The average feed intake (g/bird) of DON-fed birds was calculated daily. During the feed weigh-back, all feeders from *ad libitum*-fed groups were removed and weigh-back was conducted within ten minutes. Feeders were then immediately placed back on corresponding cages. The second set of birds was subjected to pair-feeding and are henceforth referred to as pair-fed birds where the control diet was provided based on average feed consumed by DON *ad libitum*-fed birds at the same age. Prior to providing feed to pair-fed birds, the remaining feed (residual feed) in the feeders were weighed and discarded. The total amount of feed provided to each pair-fed cage was calculated as: number of birds in a cage x average feed intake of birds provided DON diets *ad libitum* at the same age. After the control diet was added, the feeder was immediately placed back on the corresponding cage. The feed weigh-back and feeding process were conducted within a one-hour period.

5.3.2 Growth performance

On 7, 14, 21 and 28 d post-hatch, body weight (BW, g) of each bird was recorded. Average daily gain (ADG, g/d), average daily feed intake (DFI, g/d) and feed to gain ratio (F:G, g feed/g weight gain) of each cage were then calculated. Average daily gain, DFI, F: G were calculated as followed: $ADG_{(period\ a\text{-}b)} = [BW_{day\ b} - BW_{day\ a}]$/period a-b, $DFI = [feed\ weight_{day\ a} - feed\ weight_{day\ b}]$/period a-b and F:G = DFI/ ADG.

4.3.3 Feeding and drinking behaviour

The experimental design and daily activities for recording feeding behaviour are shown in Fig. 5.1. As mentioned previously, birds were subjected to 23 h light: 1 h continuous dark period during 1 – 7 d and 18 h light: 6 h continuous dark period during the remaining of the experimental period. Three birds in each cage were individually marked on the head with a black marker. On each day throughout the trial (1 – 28 d), one replicate cage per treatment (three treatments) was selected for video recording. Three digital cameras (one Olympus OM-D E-M10 camera (Olympus Imaging America Inc., Centre Valley, PA, USA) and two Sony α5000 cameras (Sony Corporation, Tokyo, Japan)) were set on tripods 0.5 m away from the front of the selected cages. The one-hour video recording section was conducted daily and started three hours after diets were provided to pair-fed birds (1200 h – 1300 h, Fig. 4.1). After daily recording was completed, a different replicate cage was selected for next day video recording. The video recording sections were divided into four 7-d periods (1 – 7 d, 8 – 14 d, 15 – 21 d and 22 – 28 d) for analyses. Within each 7-d period, behaviour was recorded at least once in the six replicate cages with one replicate recorded twice.

Observation of videos and behaviour analyses were conducted after the growth trial was completed. Focal sampling (watching entire video continuously) was conducted for each bird for all the recorded videos. Initiation of a feeder visit event (at-feeder) was defined as occurring when a bird's head was located over the edge of the feeder with the beak completely submerged into the feeder. If a feeder visit event occurred within 10 s (< 10 s), it was considered a part of the same feeding bout. If a feeder visit event occurred longer than 10 s (≥ 10 s) after another feeder visit, the two were considered separate bouts. A bout interval of 10 s was chosen based on previous work (Bokkers and Koene, 2003). Initiation of a drinker visit event (at-drinker) was

defined as occurring when a bird was actively pecking at the drinker located at the back of the cage. The same bout interval that was used for at-feeder event was also used for at-drinker event. If two at-feeder events occurred within 10s, but were separated by an at-drinker event then the feeding bouts were considered as two separate bouts. The same occurred when two at drinker events occurred within 10s, but were separated by an at-feeder event. The bout length (s) of every feeder or drinker visit, number of feeder or drinker visit events were recorded. Average bout length (s) of at drinker or feeder event was calculated. The percentage of time (budget) during observation spent at the feeder or the drinker was calculated as followed: (average length of at-feeder or at-drinker bout (s) x number of bout / 3600 s/h) x 100%. The observations were verified by a different observer and the intra-observer consistency was > 90%.

5.3.4 Organ weight and histology samples

On 21 and 28 d post-hatch, one bird from each cage was randomly selected, weighed, and euthanized by cervical dislocation. Empty weight of the individual segments of the gastrointestinal tract was recorded after removing the surrounding fat. The intestine was divided into the duodenum, jejunum, and ileum for measuring the length of each segment. The spleen and liver were weighed. The relative organ weight (g/kg BW) and intestinal density (g/cm) was calculated.

On 28 d post-hatch, six birds from each treatment were randomly selected for intestinal morphology assessment using histopathology. A 2 cm segment from the middle of the duodenum was collected. Tissues were fixed in 10 % neutral buffered formalin and then transferred in ethanol until processing. Tissues were then paraffin embedded, sectioned at 5 μm thickness and stained with hematoxylin and eosin by Prairie Diagnostic Services Inc. (Saskatoon, Saskatchewan). Three longitudinal sections were prepared for evaluation. Slides were examined under an Axiostar plus light microscope (Carl Zeiss Microscopy, LLC, One Zeiss Drive Thornwood, NY) and pictures of intestinal villi were acquired at 10x magnification. Measurements were done on 10 – 15 complete, fully developed crypt-villi axis per bird. Duodenal morphometric indices measured were villus height, crypt depth, villus width, thicknesses of muscularis, and villus-to-crypt ratio.

5.3.5 Statistical analysis

The experiment was a completely randomized design with six replicates. All data analysis was conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). Data were initially checked for normality using the UNIVARIATE procedure and if not normally distributed, log transformation was conducted. Growth performance data were analyzed as repeated measures using one-way ANOVA by the PROC MIXED procedure of SAS (Littell, 2006) and the appropriate covariance structure was selected by comparing the Akaike's Information Criteria. Organ weight, behaviour and intestinal morphometric data were analyzed using one-way ANOVA by the PROC MIXED procedure of SAS. Significant effects ($\alpha = 0.05$) were analyzed using the Tukey-Kramer test to differentiate the means.

Table 5.1. Calculated nutrient compositions and analyzed mycotoxins concentrations (mg/kg) in control and contaminated starter (1 – 21 day post-hatch, d) and grower (22 – 28 d) diets.

	Starter diets		Grower diets	
	Control	**DON[7]**	**Control**	**DON[7]**
Ingredient (%)				
Clean wheat[1]	68.20	0.00	72.90	0.00
Fusarium damaged wheat[2]	0.00	66.30	3.00	66.30
Soybean meal	16.90	15.70	12.50	16.10
Meat meal	5.00	9.20	5.00	5.00
Fish meal	3.00	3.00	3.00	3.00
Canola oil	3.00	2.00	3.00	3.00
Limestone	1.20	1.20	1.20	1.20
Di calcium phosphate	0.90	0.90	0.50	0.50
L-Lysine	0.50	0.50	0.50	0.50
Vitamin/ mineral premix[3]	0.50	0.50	0.50	0.50
DL-methionine	0.20	0.20	0.20	0.20
L-theronine	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.25	0.25
Choline chloride	0.10	0.10	0.10	0.10
Enzyme[4]	0.05	0.05	0.05	0.05
Total	100.0	100.0	100.0	100.0
Nutrient composition (%)[6]				
Crude Protein	23.0	23.0	21.5	21.5
AME (kcal/kg)	3014	3080	3102	3095
Calcium	1.20	1.20	1.10	1.10
Phosphorus	0.90	0.62	0.60	0.58
Crude fiber	2.40	2.41	2.39	2.40
Fat	4.68	4.48	4.92	5.15
Ash	7.63	5.48	5.73	5.65
Lysine	1.57	1.31	1.22	1.18
Methionine	0.73	0.51	0.54	0.52
Meth + Cys	1.11	1.23	1.30	1.25
Arginine	1.52	1.53	1.38	1.39
Analysed mycotoxin (mg/kg)[7]				
Deoxynivalenol (DON)	<0.025	3.004	<0.025	2.399
3-Acetyl DON	<0.025	0.038	<0.025	0.132
15-Acetyl DON	<0.025	<0.025	<0.025	<0.025
HT-2 Toxin	<0.025	0.055	<0.025	<0.025
Ochratoxin A	<0.025	<0.025	<0.025	0.157

[1] Mycotoxins concentration in control wheat: aflatoxin B1: Not Detected (ND), 3-Acetyl Deoxynivalenol (DON): ND, 15-Acetyl DON: ND, α-Zearalenol: ND, DON: 35 µg/kg, Diacetoxyscirpenil: ND, HT-2 toxin: 63 µg/kg, Nivalenol: ND, Ochratoxin A: ND, T-2 toxin: ND, β-Zearalenol: ND, Zearalenone: ND.
[2] Mycotoxins concentration in *Fusarium* damaged wheat: aflatoxin B1: ND, 3-Acetyl Deoxynivalenol (DON): 764 µg/kg, 15-Acetyl DON: ND, α-Zearalenol: ND, DON: 11470 µg/kg, Diacetoxyscirpenil: ND, HT-2 toxin: 107 µg/kg, Nivalenol: 59.2 µg/kg, Ochratoxin A: ND, T-2 toxin: ND, β-Zearalenol: ND, Zearalenone: ND.
[3] Vitamin/mineral premix (Per kilogram product): 2200000 IU vitamin A, 440000 IU vitamin D, 6000 IU vitamin E, 400 mg menadione, 300 mg thiamine, 1200 mg riboflavin, 800 mg pyridoxine, 4 mg vitamin B12, 12000 mg niacin, 2000 mg pantothenic acid, 120 mg folic acid, 30 mg biotin. 2000 mg copper, 16000 mg manganese, 160 mg iodine, 16000 mg zinc, 60 mg selenium, 125 mg antioxidant, 807879 mg wheat midds. (DSM Nutritional Products Canada Inc. Ayr. ON).
[4] Enzyme: Beta-glucanase: 1000 units/g, xylanase: 1500 units/g (GNC Bioferm Inc., Saskatoon, SK).
[5] Mycotoxins diets were formulated to achieve DON concentrations at around 7 mg/kg concentrations. The analyzed results indicated that dietary DON was lower than calculated concentrations.
[6] Nutrient composition were analyzed at Central Testing Laboratory Ltd., (Winnipeg, MB). AME was calculated as: AME=53+38*((Crude Protein)+(2.25*Fat)+(100-((Crude Protein)+(Crude fibre)+Fat+(Ash) (moisture)))*0.9).
[7] Mycotoxins were analyzed at Prairie Diagnostics Services Inc., (Saskatoon, SK) via ultra-high performance liquid chromatography (Agilent 1100, Agilent Technologies, Santa Clara, CA) and mass spectrometry (Micromass Quattro Ultima Platinum Mass Spectrometer, Waters, Milford, MA).

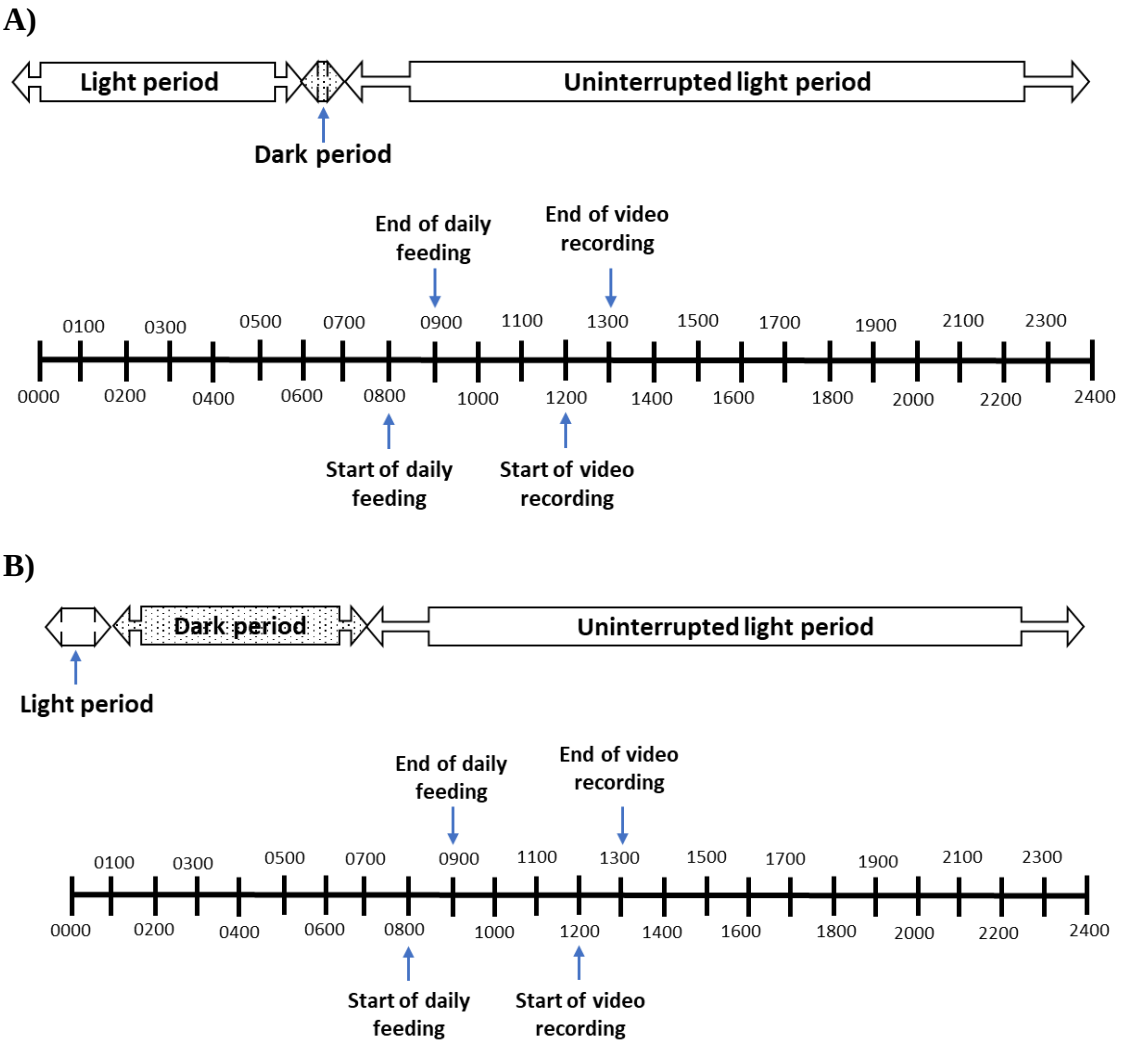

Figure 5.1. Daily activities (video recording, dark periods and feed weigh-back and feeding sections) of pair-feeding trial. Broilers were subjected to either *ad libitum* or pair-feeding and given control or DON diet during 1 – 28 day post-hatch (d). The starter diets (< 0.025 vs. 3.004 mg/kg DON) were fed from 1 to 21 d and grower diets (< 0.025 vs. 2.399 mg/kg DON) were fed from 22 to 28 d. (A). During 1 – 7 d, birds were subjected to one-hour dark period. (B). During 8 – 28 d, birds were subjected to six hours dark period. The feed intakes of *ad libitum*-fed birds were measured daily at 1 hour after light was turned on. The average feed intakes of mycotoxin diet fed birds were calculated. Both control and mycotoxin diets were provided to pair-fed birds based on average DON *ad libitum*-fed birds consumed at same age. The total amount of feed provided to each pair-fed cage was calculated as: number of birds in a cage x average bird's feed intake of DON ad libitum fed at same age. The one-hour video recording sections were conducted at three hours after feed were provided to pair-fed birds.

5.4 Results

5.4.1 Growth performance

The BW of chicks did not differ among treatments at initiation of the experiment ($P > 0.05$; 41.7 ± 0.4, 41.0 ± 0.4, 40.6 ± 0.5 for the control, DON-fed and pair-fed treatment, respectively). The mortality rate of the entire experiment was 0.9% (1 bird from pair-fed treatment).

The birds fed the DON diets had lower DFI during 1 – 7 d and 15 – 21 d compared to the control birds (21.6 vs. 24.4 g/d, and 91.0 vs. 98.5 g/d, respectively; $P < 0.01$, Table 5.2). During the first 21 days of the trial, the pair-fed birds had lower DFI compared to both the control and the DON-fed birds ($P < 0.01$, Table 5.2). During 22 – 28 d, the DFI of the DON-fed and pair-fed birds did not differ and were lower than the DFI of the control birds (143.2 and 137.8 vs. 162.5 g/d; $P < 0.01$, Table 5.2). Throughout the trial, the pair-fed birds did not consume all of the provided diet. The residual feed remained in the bottom of the feeder and was in the form of small particles (fines). The amount of fines did not differ among cages.

During 1 – 21 d, pair-fed birds had lower ADG compared to both control and DON-fed birds ($P < 0.01$, Table 5.3). During 22 – 28 d, DON-fed and pair-fed birds had lower ADG compared to control birds (89.8 and 90.2 g/d vs. 101.6 g/d; $P < 0.01$, Table 5.3). At 7, 14 and 21 d, the DON-fed birds and the control birds had similar body weight and were heavier than pair-fed birds ($P < 0.01$, Table 5.4). At 28 d, pair-fed birds were lighter than control (1477.9 vs. 1698.8 g; $P < 0.01$, Table 5.4). The body weight of the DON-fed and the control birds did not differ at 28 d ($P > 0.05$, Table 5.5). During 1 – 7 d, DON-fed and pair-fed birds had lower F/G compared to the control birds (1.10 and 1.10 vs. 1.19; $P = 0.02$, Table 5.5). During 8 – 14 d, pair-fed birds had lower F/G compared to DON-fed birds (1.22 vs. 1.31; $P < 0.01$, Table 5.5). During 15 – 21 d, 22 – 28 d and 1 – 28 d, the F/G did not differ among treatments ($P > 0.05$, Table 5.5).

5.4.2 At feeder and at drinker behavior

Broilers' at feeder behaviours were not affected by treatment during 1 – 7 d, 8 – 14 d and 15 – 21 d ($P > 0.05$, Table 5.6). During 22 – 28 d, DON-fed birds had longer at feeder bout compared to pair fed birds (61.6 vs. 39.7 s; $P = 0.02$, Table 5.6). The number of at feeder bout

and percentage time spent at feeder (budget) did not differ among treatments during 22 – 28 d ($P > 0.05$, Table 5.6).

During 1 – 7 d, drinker behaviours were not affected by treatments ($P > 0.05$, Table 5.7). During 8 – 14 d, pair-fed birds (18.6) had lower drinker visiting events compared to control birds (24.1) and DON-fed birds (22.9) were intermedia ($P = 0.05$, Table 5.6). During 15 – 21 d, pair-fed birds had more drinker visiting events and longer at drinker budget compare to control and DON-fed birds ($P < 0.01$, Table 5.7). During 22 – 28 d, at drinker behaviours were not affected by treatment ($P > 0.05$, Table 5.7).

5.4.3 Organ weights and duodenal histology

At 21 d, relative liver, spleen and duodenal weight and jejunal density did not differ among treatments ($P > 0.05$, Table 5.8). Pair-fed birds had lower relative jejunal, ileal weight and duodenal density compared to the DON-fed birds ($P < 0.01$, Table 5.8). Control birds had higher ileal density compared to pair-fed birds (0.25 vs. 0.19 g/cm; $P = 0.02$, Table 5.8).

At 28 d, relative liver, spleen and ileal weight and ileal density not differ among treatments ($P > 0.05$, Table 5.8). Pair-fed birds had lower relative jejunal and ileal weight compared to the DON-fed birds ($P < 0.05$, Table 5.8). Pair-fed birds had lower duodenal density compared to the control and the DON-fed birds (0.28 vs. 0.37 and 0.37 g/cm; $P < 0.01$, Table 5.8). Pair-fed birds had lower jejunal density compared to control birds (0.33 vs. 0.40 g/cm; $P = 0.03$, Table 5.8).

In addition to measuring small intestinal densities, the duodenal mucosa structures (villi height, width, crypt depth and villi to crypt ratio) were measured at 28 d. There was no effect of treatment on any duodenal morphological structures in 28 d broilers ($P > 0.05$, Table 5.9).

Table 5.2. Effects of providing DON-contaminated diets[1] *ad libitum*[2] or pair-fed[2] on broiler feed consumption (g/day)[3] during a 28-day growth trial.

	Age				
Treatment	**1 – 7 d**	**8 – 14 d**	**15 – 21 d**	**22 – 28 d**	**1 – 28 d**
Control	24.4 ± 0.7^{a}	55.9 ± 1.1^{a}	98.5 ± 2.0^{a}	162.5 ± 4.3^{a}	85.3 ± 4.1^{a}
DON[1]	21.6 ± 0.4^{b}	54.2 ± 0.5^{a}	91.0 ± 0.7^{b}	143.2 ± 3.4^{b}	76.4 ± 4.5^{b}
Pair-fed[2]	18.6 ± 0.6^{c}	48.8 ± 2.7^{b}	83.0 ± 2.5^{c}	137.8 ± 3.2^{b}	72.0 ± 1.4^{b}
ANOVA[3]	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

[1] The starter diets (< 0.025 vs. 3.004 mg/kg DON) were fed from 1 to 21 d and grower diets (< 0.025 vs. 2.399 mg/kg DON) were fed from 22 to 28 d.

[2] The amount of feed provided to pair-fed treatment was calculated as: number of birds in a cage x average bird's feed intake of DON diet *ad libitum*-fed at same age.

[3] Within same column, mean ± SE with different superscripts (a-c) are significantly different ($P \leq 0.05$, ANOVA followed by Tukey-Kramer test; n = 6/treatment).

Table 5.3. Effects of providing DON-contaminated diets[1] *ad libitum*[2] or pair-fed[2] on broiler average daily weight gain (ADG, g/day)[3] during a 28-day growth trial.

	Age				
Treatment	**1 – 7 d**	**8 – 14 d**	**15 – 21 d**	**22 – 28 d**	**1 – 28 d**
Control	20.4 ± 0.4^{a}	43.7 ± 0.4^{a}	73.8 ± 1.5^{a}	101.6 ± 3.4^{a}	59.2 ± 1.3^{a}
DON	19.6 ± 0.4^{a}	43.6 ± 0.4^{a}	69.8 ± 2.0^{a}	89.8 ± 2.7^{b}	54.9 ± 1.4^{ab}
Pair-fed	16.9 ± 0.5^{b}	37.1 ± 0.5^{b}	61.1 ± 0.6^{b}	90.2 ± 3.1^{b}	51.3 ± 1.1^{b}
ANOVA	$P < 0.01$	$P = 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

[1] The starter diets (< 0.025 vs. 3.004 mg/kg DON) were fed from 1 to 21 d and grower diets (< 0.025 vs. 2.399 mg/kg DON) were fed from 22 to 28 d.

[2] The amount of feed provided to pair-fed treatment was calculated as: number of birds in a cage x average bird's feed intake of DON diet *ad libitum*-fed at same age.

[3] Within same column, mean ± SE with different superscripts (a-b) are significantly different ($P \leq 0.05$, ANOVA followed by Tukey-Kramer test; n = 6/treatment).

Table 5.4. Effects of providing DON-contaminated diets[1] *ad libitum*[2] or pair-fed[2] on body weight (g)[3] of male broilers during a 28-day growth trial.

	Age			
Treatment	**7 d**	**14 d**	**21 d**	**28 d**
Control	184.7 ± 3.1[a]	490.5 ± 10.7[a]	1007.0 ± 18.0[a]	1698.8 ± 40.6[a]
DON	177.9 ± 2.8[a]	470.6 ± 12.3[a]	959.3 ± 24.8[a]	1588.9 ± 34.0[ab]
Pair-fed	159.3 ± 2.8[b]	418.9 ± 5.8[b]	846.5 ± 8.7[b]	1477.9 ± 31.7[b]
ANOVA	P < 0.01	P < 0.01	P < 0.01	P < 0.01

[1] The starter diets (< 0.025 vs. 3.004 mg/kg DON) were fed from 1 to 21 d and grower diets (< 0.025 vs. 2.399 mg/kg DON) were fed from 22 to 28 d.
[2] The amount of feed provided to pair-fed treatment was calculated as: number of birds in a cage x average bird's feed intake of DON diet *ad libitum*-fed at same age.
[3] Within same column, mean ± SE with different superscripts (a-b) are significantly different (P ≤ 0.05, ANOVA followed by Tukey-Kramer test; n = 6/treatment).

Table 5.5. Effects of providing DON-contaminated diets[1] *ad libitum*[2] or pair-fed[2] on broiler feed to gain ratio (F/G, g feed/ g weight gain)[3] during a 28-day growth trial.

	Age				
Treatment	**1 – 7 d**	**8 – 14 d**	**15 – 21 d**	**22 – 28 d**	**1 – 28 d**
Control	1.19 ± 0.03^{a}	1.28 ± 0.03^{ab}	1.34 ± 0.01	1.61 ± 0.03	1.44 ± 0.02
DON	1.10 ± 0.01^{b}	1.31 ± 0.01^{a}	1.30 ± 0.01	1.50 ± 0.04	1.41 ± 0.02
Pair-fed	1.10 ± 0.02^{b}	1.22 ± 0.03^{b}	1.36 ± 0.02	1.58 ± 0.05	1.38 ± 0.04
ANOVA	P = 0.02	P = 0.03	P = 0.07	P = 0.11	P = 0.45

[1] The starter diets (< 0.025 vs. 3.004 mg/kg DON) were fed from 1 to 21 d and grower diets (< 0.025 vs. 2.399 mg/kg DON) were fed from 22 to 28 d.

[2] The amount of feed provided to pair-fed treatment was calculated as: number of birds in a cage x average bird's feed intake of DON diet *ad libitum*-fed at same age.

[3] Within same column, mean ± SE with different superscripts (a-b) are significantly different ($P \leq 0.05$, ANOVA followed by Tukey-Kramer test; n = 6/treatment).

Table 5.6. Effects of providing DON-contaminated diets[1] *ad libitum*[2] or pair-fed[2] on broiler at feeder behaviours[3] during 1 – 28 d.

	Treatment	Length (s)	Bout number	Budget (%)
1 – 7 d	Control	59.0 ± 16.7	24.0 ± 3.9	8.55 ± 1.81
	DON	56.3 ± 9.8	13.4 ± 1.1	7.49 ± 1.70
	Pair-fed	51.2 ± 9.2	22.6 ± 4.1	8.93 ± 1.04
	ANOVA	P = 0.84	P = 0.07	P = 0.80
8 – 14 d	Control	50.2 ± 6.2	37.7 ± 4.7	16.4 ± 2.27
	DON	70.1 ± 10.0	25.6 ± 3.3	18.9 ± 3.73
	Pair-fed	63.0 ± 8.5	34.9 ± 6.5	15.7 ± 3.27
	ANOVA	P = 0.22	P = 0.19	P = 0.75
15 – 21 d	Control	69.9 ± 12.1	28.4 ± 4.4	15.30 ± 1.94
	DON	71.2 ± 6.1	28.4 ± 5.0	17.57 ± 3.62
	Pair-fed	64.3 ± 7.0	39.9 ± 1.3	22.62 ± 1.57
	ANOVA	P = 0.84	P = 0.08	P = 0.14
22 – 28 d	Control	41.1 ± 4.2^{ab}	30.6 ± 3.6	10.73 ± 1.46
	DON	61.6 ± 7.1^{a}	31.0 ± 3.2	15.88 ± 1.45
	Pair-fed	39.7 ± 7.0^{b}	37.9 ± 7.8	12.63 ± 2.24
	ANOVA	P = 0.02	P = 0.56	P = 0.14

[1] The starter diets (< 0.025 vs. 3.004 mg/kg DON) were fed from 1 to 21 d and grower diets (< 0.025 vs. 2.399 mg/kg DON) were fed from 22 to 28 d.

[2] The amount of feed provided to pair-fed treatment was calculated as: number of birds in a cage x average bird's feed intake of DON diet *ad libitum*-fed at same age.

[3] Mean ± SE with different superscripts (a-b) are significantly different ($P \leq 0.05$, ANOVA followed by Tukey-Kramer test; n = 6/treatment).

Table 5.7. Effects of providing DON-contaminated diets[1] *ad libitum*[2] or pair-fed[2] on broiler at drinker behaviours[3] during 1 – 28 d.

	Treatment	Length (s)	Bout number	Budget (%)
1 – 7 d	Control	16.3 ± 2.4	23.7 ± 2.9	3.43 ± 0.59
	DON	19.8 ± 3.3	18.9 ± 2.5	3.55 ± 0.44
	Pair-fed	21.5 ± 1.8	20.3 ± 2.9	4.4 ± 0.54
	ANOVA	P = 0.11	P = 0.46	P = 0.57
8 – 14 d	Control	24.0 ± 1.5	24.1 ± 1.5^{a}	5.04 ± 0.24
	DON	23.0 ± 2.2	22.9 ± 1.3^{ab}	4.43 ± 0.68
	Pair-fed	27.1 ± 1.2	18.6 ± 1.8^{b}	4.81 ± 0.43
	ANOVA	P = 0.23	P = 0.05	P = 0.68
15 – 21 d	Control	32.4 ± 3.2	18.4 ± 0.8^{b}	5.24 ± 0.53^{b}
	DON	30.1 ± 2.2	20.6 ± 1.3^{b}	5.33 ± 0.30^{b}
	Pair-fed	33.6 ± 4.2	27.9 ± 3.0^{a}	7.76 ± 0.76^{a}
	ANOVA	P = 0.75	P < 0.01	P < 0.01
22 – 28 d	Control	38.6 ± 4.6	19.1 ± 2.7	6.03 ± 1.00
	DON	31.5 ± 2.0	24.4 ± 2.3	6.54 ± 0.63
	Pair-fed	34.5 ± 4.5	26.9 ± 2.2	8.63 ± 0.68
	ANOVA	P = 0.46	P = 0.31	P = 0.29

[1] The starter diets (< 0.025 vs. 3.004 mg/kg DON) were fed from 1 to 21 d and grower diets (< 0.025 vs. 2.399 mg/kg DON) were fed from 22 to 28 d.

[2] The amount of feed provided to pair-fed treatment was calculated as: number of birds in a cage x average bird's feed intake of DON diet *ad libitum*-fed at same age.

[3] Mean ± SE with different superscripts (a-b) are significantly different (P ≤ 0.05, ANOVA followed by Tukey-Kramer test; n = 6/treatment).

Table 5.8. Effects of providing DON-contaminated diets[1] *ad libitum*[2] or pair-fed[2] on broiler relative organ weights (g organ/ kg body weight) and small intestinal segment densities (g/ cm)[3] on 21 and 28 d.

	Relative organ weight (g/kg body weight)					Segment density (g/cm)		
Treatment	Liver	Spleen	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
21 d								
Control	26.93±0.66	0.88±0.05	8.51±0.23	15.47±0.41^{ab}	12.46±0.68^{a}	0.35±0.01^{ab}	0.29±0.01	0.25±0.01^{a}
DON	27.93±0.41	0.91±0.03	8.93±0.04	16.60±0.42^{a}	12.03±0.82^{a}	0.36±0.01^{a}	0.29±0.01	0.24±0.01^{ab}
Pair-fed	28.32±0.73	0.87±0.07	8.47±0.23	13.63±0.49^{b}	8.23±0.23^{b}	0.31±0.01^{b}	0.26±0.01	0.19±0.01^{b}
ANOVA	P = 0.29	P = 0.80	P = 0.53	P < 0.01	P < 0.01	P = 0.02	P = 0.09	P = 0.02
28 d								
Control	24.24±0.57	0.86±0.07	5.93±0.28^{ab}	12.54±0.29^{ab}	9.03±0.27	0.37±0.02^{a}	0.40±0.01^{a}	0.31±0.01
DON	24.40±0.16	0.91±0.04	6.14±0.33^{a}	13.12±0.58^{a}	9.15±0.12	0.37±0.02^{a}	0.38±0.02^{ab}	0.28±0.01
Pair-fed	24.33±0.71	0.88±0.08	4.95±0.34^{b}	11.06±0.42^{b}	9.27±0.48	0.28±0.01^{b}	0.33±0.01^{b}	0.28±0.01
ANOVA	P = 0.98	P = 0.86	P = 0.04	P = 0.01	P = 0.87	P < 0.01	P = 0.03	P = 0.13

[1] The starter diets (< 0.025 vs. 3.004 mg/kg DON) were fed from 1 to 21 d and grower diets (< 0.025 vs. 2.399 mg/kg DON) were fed from 22 to 28 d.
[2] The amount of feed provided to pair-fed treatment was calculated as: number of birds in a cage x average bird's feed intake of DON diet *ad libitum*-fed at same age.
[3] Mean ± SE with different superscripts (a-b) are significantly different (P ≤ 0.05, ANOVA followed by Tukey-Kramer test; n = 6/treatment).

Table 5.9. Effects of providing DON-contaminated diets[1] *ad libitum*[2] or pair-fed[2] on broiler duodenal mucosa structures[3] at 28 d.

	Duodenal mucosa structures			
Treatment	Villi height (µm)	Crypt depth (µm)	Villi width (µm)	Villi to crypt ratio
Control	1898.04 ± 92.78	157.13 ± 5.56	115.30 ± 5.16	13.99 ± 0.95
DON	1951.01 ± 77.57	141.23 ± 2.27	123.19 ± 2.95	12.38 ± 0.64
Pair-fed	1897.33 ± 75.84	137.14 ± 13.04	125.18 ± 2.68	12.11 ± 0.26
ANOVA	P = 0.89	P = 0.34	P = 0.26	P = 0.24

[1] The starter diets (< 0.025 vs. 3.004 mg/kg DON) were fed from 1 to 21 d and grower diets (< 0.025 vs. 2.399 mg/kg DON) were fed from 22 to 28 d.
[2] The amount of feed provided to pair-fed treatment was calculated as: number of birds in a cage x average bird's feed intake of DON diet *ad libitum*-fed at same age.

5.5 Discussion

Reduction in feed intake is a characteristic sign of trichothecene toxicosis and is commonly referred as feed refusal (Osweiler, 2014). However, the relative importance of feed refusal on growth suppression in broilers has not been determined in the previous studies where feed was provided *ad libitum*. The current study used a pair-feeding approach to determine whether the DON-induced reduction in feed intake and its contribution to growth suppression in broilers is independent from other effects of DON.

In the present study, DON-fed birds consumed less feed over most of the 28-day feeding trial. The reduced feed intake may be attributed to the DON contamination reducing diet palatability. In a previous study (Wang et al. submitted), we found that DON contamination reduced palatability, induced sensory aversion and altered broiler feeding behaviour. However, there was no difference in ADG and BW between the control and the DON-fed birds despite differences in feed intake during the early stage of growth. In studies with swine, DON-fed animals appeared to have improved nutrient digestibility and absorption as a compensatory mechanism for a reduction in feed intake (Dänicke et al., 2004; Goyarts et al., 2005), and this mechanism may be active in broilers. During the later growth stages, we observed a reduction in feed intake and weight gain in DON-fed birds compared to the control birds, while these measures did not differ between the DON-fed and the pair-fed birds. The amount of control feed provided to the pair-fed birds was based on the feed consumption of DON-fed birds at the same age. Since the DON-fed birds consumed less feed than the control birds, the pair-fed birds were subjected to feed restriction as the amount of uncontaminated feed provided was less than the amount they would typically consume *ad libitum*. We observed that the pair-fed birds had lower feed intake and weight gain compared to DON-fed birds during the starter period and did not differ during the grower period. Taken together, this suggests that reduction of broiler weight gain in DON-fed birds is most likely due to reduction of feed intake rather than due to other toxic effects of DON. Our findings are in accordance with a recent meta-analysis that concluded growth suppression effects observed in the DON-fed broilers are due to reduction of feed intake (Andretta et al., 2011).

The current study is the first to evaluate the effects of long-term exposure (28 day) to *Fusarium* mycotoxins (primarily DON) on broiler feeding behaviour. In addition to measuring feed consumption, recording broiler at-feeder behaviour allows feed intake to be examined in terms of meal size (estimated by feed intake/meal number) and meal frequency (meal number/ hour). In the present study, feeding broilers DON-contaminated diets increased broiler meal length (estimated by at-feeder bout length) and agrees with our previous study where short-term feeding of DON-contaminated diets increased meal length, a response previously attributed to a reduction in feed palatability (Wang et al., submitted). In addition, we observed a reduction of meal size in DON-fed birds. Broiler feed intake is controlled by complex orexigenic/anorexigenic neurochemical pathways (Richards and Proszkowiec-Weglarz, 2007). In general, the activation of anorexigenic neurochemical pathways suppress feed intake and terminate the broiler feeding behaviour (Bokkers and Koene, 2003; Richards and Proszkowiec-Weglarz, 2007). Feeding DON-contaminated diets increased anorexigenic neurochemical serotonin concentrations in broilers (Swamy et al., 2004b), which corresponded with DON-induced partial feed refusal observed in related studies (Swamy et al., 2002; 2004a). Although we did not measure the anorexigenic neurochemicals, it is possible that the reduced meal size observed is due to DON-induced anorexia effects. Based on behavioural observations, our findings suggest that DON-induced reduction in feed intake is caused by both anorexia effects and sensory aversion.

The current study is one of the first to evaluate the effects of DON intake on broiler drinking behaviour. In line with our previous study (Wang et al., submitted), broiler drinking behaviour did not differ between the control and the DON-fed birds. As mentioned previously, the pair-fed birds were subjected to feed restriction in the current study as the amount of control diet provided to the pair-fed birds was less than what the birds would consume *ad libitum*. It is suggested that altered drinking behaviour in restricted-fed birds, such as increased water consumption and drinking frequency, is because broilers redirect their feeding motivation to drinking (Hocking et al., 1996; Mench, 2002). Such responses may explain the differences in drinking behaviour observed in the pair-fed birds subjected to feed restriction.

A naturally-contaminated diet containing approximately 3 mg/kg DON was sufficient to suppress broiler feed intake and growth in the current study. This is in contrast to previous claims that 15 mg/kg DON in naturally contaminated diets is the threshold for broiler feed

refusal and growth suppression in controlled research studies (Chowdhury et al., 2005; Awad et al., 2012). However, several recent studies showed that low concentrations of naturally DON-contaminated diets (< 5 mg/kg) are sufficient to suppress feed intake and growth (Yunus et al., 2012b; Shang et al., 2016; Lucke et al., 2017). Reduction of broiler feed intake may be the result of DON-induced anorexia as it has been shown that feeding DON-contaminated diets to broilers increases the concentration of the anorexia neurochemical serotonin (Swamy et al., 2004b). Broiler chickens are selected for superior growth performance, such as increased appetite and weight gain (Havenstein et al., 2003) and the increased feed intake is achieved by suppression of anorexia neurochemical pathways (Bokkers and Koene, 2003; Honda et al., 2012). It is possible that selection towards broiler growth performance also increases broiler sensitivity to DON-induced anorexia effects.

It should also be noted that pair-fed birds did not consume the entire quantity of the control diets provided. The residual feed was allocated in the bottom of feeder and was in the form of small particles (fines). The lower feed intake observed in the pair-fed birds may therefore be due to the inability of birds to consume the fines (Dozier et al., 2010; Lv et al., 2015).

Pair-fed birds that had restricted feed intake had reduced intestinal segment weights and density and this agrees with a previous study where restricted-fed broilers (30% restriction compared to *ad libitum*-fed birds) had reduced intestinal weight and density as the result of a reduction of feed intake (Gilbert et al., 2008). We found no difference in organ weights and intestinal densities between control and DON-fed birds. The effects of feeding DON-contaminated diets on organ weights in broilers are very inconsistent across studies (Swamy et al., 2004a; Girgis and Smith, 2010; Awad et al., 2011) and may be due to different experimental conditions, such as mixtures and concentrations of the naturally-occurring mycotoxins, timing and duration of exposure and genetic background of birds (Li et al., 2012).

Broilers fed relatively low concentration DON-contaminated diets can exhibit changes in intestinal mucosa structures, such as reduced duodenum villi height, crypt depth and villi surface area, even before reduction of feed intake and impairment of growth performance are apparent (Awad et al., 2006b; 2011; 2012). We did not, however, observe any changes in duodenal mucosa structures in DON-fed birds, which supports the proposal that feed refusal is a protective mechanism that limits the total mycotoxin intake and prevents the animal from developing

further toxicosis (Osweiler, 2014). Our previous study showed that feeding broiler chickens a naturally DON-contaminated diet (approx. 7 mg/kg) from 1 – 34 d was associated with reduced villi height in the ileum and jejunum (Wang and Hogan, 2018). There may be a site-specific effect of DON on intestinal mucosal structures but it is most likely that the concentrations of DON used and length of exposure in the present study were not adequate to cause morphological damage. Given the similar performance between DON-fed birds (who reduced their feed intake) and those birds restricted in feed to the same concentration, it appears that the presence of DON did not negatively affect intestinal absorptive functions or nutrient utilization.

The current study demonstrates that relatively low concentrations of *Fusarium* mycotoxins (primarily DON) can alter broiler feeding behaviours and suppress broiler feed intake and growth. Through behaviour observations, we demonstrated that reduction of broiler feed intake is caused by DON-induced sensory aversion and anorexia effects. Since DON-fed birds and restricted-fed birds with similar (reduced) feed intake had equally suppressed performance, we conclude that the contribution of dietary DON to growth suppression in broilers is independent from other toxic effects of DON.

PREFACE TO CHAPTER 6

In the previous chapters, we identified that low to moderate *Fusarium* mycotoxin contamination consistently induces feed refusal and growth suppression in broilers. In research presented in this chapter, we evaluated the efficacy of a new yeast cell wall-based feed additive in counteracting the performance effects of *Fusarium* mycotoxins in broilers, specifically the growth suppressive effects. Our results indicate that supplementation with this feed additive can mitigate the adverse effects of feeding low concentration DON-contaminated diets on broiler performance.

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Author contributions:

Anhao Wang (University of Saskatchewan) designed and managed the experiment, generated and analyzed the data, prepared all figures, and drafted the manuscript.

Rob Patterson (Canadian Bio-Systems Inc.) provided diet ingredients (enzyme mix and feed additive), scientific input, commented and edited the manuscript.

Dr. Natacha Hogan (University of Saskatchewan) provided inspiration, scientific input and guidance, commented on and edited the manuscript, and obtained funding for the research.

CHAPTER 6

EVALUATION OF A FEED ADDITIVE IN REDUCING THE ADVERSE EFFECTS OF *FUSARIUM* MYCOTOXINS ON BROILER CHICKEN GROWTH PERFORMANCE

6.1 Abstract

A study was conducted to determine the efficacy of a yeast cell wall-based feed additive in counteracting the performance effects of mycotoxins in broilers fed a naturally-contaminated diet with the primarily mycotoxin being deoxynivalenol (DON) at 1.8 – 2.0 mg/kg. Two hundred and forty newly hatched Ross 308 chicks were randomly assigned to 40 cages with 6 birds per cage. Four starter diets (1 – 21 day (d)) and four grower diets (22 – 32 d) were formulated to meet broiler nutritional requirements. The treatments were: 1) basal diet (control), 2) DON diet, 3) 0.25 % additive (2.5 kg/1000 kg) and 4) DON diet + additive. Body weight (g), average daily gain (ADG, g/d), average daily feed intake (DFI, g/d) and feed to gain ratio (g feed intake/ g weight gain) were measured on 7, 14, 21, 29 and 32 d. On 21 and 32 d, one bird from each cage was randomly selected and relative weight (g/kg BW) of liver, spleen and small intestine segments and small intestine segment densities (g/cm) were measured. At the end of the trial (32 d), birds fed the DON-diets were lighter (1806.6 g) than those that that received the additive (1929.6 g) and DON diet + additive (1917.8 g, $P < 0.01$). During the grower stage, ADG was higher in birds consuming the diet with the DONM + additive (94.7 g/d) compared with birds that consumed the DON diet (84.1 g/d, $P = 0.03$). Over the entire feeding trial, birds fed the DON diet had lower DFI (82.3 g/d) than birds fed the control diet (90.3 g/d), additive diet (91.0 g/d) and DON + additive diet (88.7 g/d, $P = 0.05$). Feeding broilers the DON-contaminated diet did not affect broiler organ weight or intestinal densities ($P > 0.05$). The results of this study indicate that feeding low concentration DON-contaminated diets for 32 d can adversely affect broiler growth, and that supplementation with a yeast cell wall-based feed additive might be beneficial in counteracting the adverse effects of mycotoxins on broiler performance.

6.2 Introduction

Small cereal grains and corn are commonly used as a dietary energy source in modern broiler feed production; however, these feedstuffs are often infected by pathogenic *Fusarium* fungi (Bryden, 2012; Miller et al., 2014). Deoxynivalenol (DON) is the most prevalent mycotoxin in *Fusarium* fungi-infected cereal grains and the most frequently occurring mycotoxin in feed (Kosicki et al., 2016). Poultry are considered to be relatively tolerant to DON

(Grenier and Applegate, 2013) and the maximum dietary DON allowance is 5 mg/kg for broiler chickens (CFIA, 2015) – higher than that of other monogastrics. While acute DON toxicosis is rare in poultry, chronic and sub-lethal effects of DON intake include reduction of feed intake and performance, intestinal damage and malabsorption of nutrients, and increased disease susceptibility, and flock mortality (Andretta et al., 2011; Osselaere et al., 2013). Studies by our group (Wang and Hogan, 2018; Wang et al., submitted) and others (Awad et al., 2011; Yunus et al., 2012b; Shang et al., 2016) have demonstrated reduced feed intake, reduced weight gains and feed efficiency in broilers fed naturally-contaminated diets with DON concentrations near or below the 5 mg/kg. Moreover, under field conditions with additional stress factors, the toxicity of DON could be exacerbated and so it would not be surprising to observe adverse effects in the health and performance of poultry even at considered "safe" concentrations (Vila-Donat et al., 2018). Therefore, practical and effective strategies are required to mitigate negative effects of DON in naturally-contaminated feed.

Economic losses and animal health risk caused by increased occurrence of mycotoxin contamination have spurred research to explore inactivation and detoxification methods as mitigation strategies. These include eliminating mycotoxins from contaminated grains, decreasing the bioavailability of such mycotoxins in the gastrointestinal tract of animals, or directly degrading mycotoxins in feeds. Feed additives, such as mineral clays, yeast cell wall products (YCW) and plant cell wall fibers are among those tested for their ability to mitigate *Fusarium* mycotoxin-induced adverse effects (Boudergue et al., 2009; Zhu et al., 2016; Vila-Donat et al., 2018). Although mineral clays have limited binding ability against *Fusarium* mycotoxins, yeast cell wall products have demonstrated ability to absorb the DON and other trichothecenes effects *in vitro* (Boudergue et al., 2009; Zhu et al., 2016; Vila-Donat et al., 2018). Dietary supplementation of YCW restored feed intake and weight gain in turkeys (Girish et al., 2008b) and broilers (Swamy et al., 2002; Shang et al., 2016) fed DON-contaminated diets as well as preventing DON-induced immune suppression in broilers (Swamy et al., 2004a). A new feed additive containing YCW polysaccharides along with vitamins and mineral clays has been developed. This feed additive has demonstrated potential to reduce the negative effects of *Fusarium* mycotoxins in swine and ruminants (unpublished data) but it has yet to be tested in broilers. Therefore, the purpose of the present study was to investigate the effects of low concentration naturally-contaminated diets containing primarily DON on feed intake,

performance and organ weight of broiler chickens and to test efficacy of this feed additive in preventing DON-induced adverse effects.

6.3 Materials and Methods

6.3.1 Animals and diets

Animal care procedures followed the guidelines of the Canadian Council on Animal Care (1993) and the protocol was approved by the University of Saskatchewan Animal Care Committee (protocol # 20130043). Birds were weighed and examined at the beginning of the experiment. Two hundred and forty newly-hatched Ross 308 were purchased from a local hatchery and randomly assigned to four dietary treatments with sixty birds per treatment. The treatments were as follows: 1) basal diet (control, CON), 2) DON diet (DON), 3) 0.25 % additive (2.5 kg/1000 kg control diet) (ADD) and 4) DON diet + additive (DON + ADD). Birds were places in battery cages (29 cm high × 48 cm wide × 83 cm long; providing 800 cm^2/bird floor space) with six birds per cage and ten cages per treatment in a temperature-controlled room at the University of Saskatchewan Poultry Research Center (Saskatoon, SK, Canada). Birds had free access to drinking water and were provided feed *ad libitum* during the entire trial. Birds were kept in a 23h light/1h dark period during 1 – 7 d and an 18h light/6h dark period during 8 – 32 d. Temperature regimen was in accordance with the recommendations of the breeder (Aviagen, 2014a). Morbidity and mortality were monitored daily. All mortalities were necropsied for cause of death or morbidity by Prairie Diagnostics Services Inc. (Saskatoon, SK, Canada).

All diets were formulated to meet nutrient requirements for broiler chickens (Aviagen, 2014b) and composed of wheat at approximately 70% inclusion level. A source of Canada Western Red Spring wheat with DON < 0.1 mg/kg was used to prepare the control diets. A source of *Fusarium*-damaged Canada Western Red Spring wheat (approximately 11.5 mg/kg DON) was used to prepare the contaminated diets. The diet formulations, calculated nutrient levels and analyzed mycotoxin concentrations are shown in Table 5.1. Four starter (1 – 21 d) and four grower (22 – 32 d) diets (CON, DON, ADD, DON + ADD) were produced at the Canadian Feed Research Centre (North Battleford, SK). After diets were prepared, representative 2 kg samples were collected and analyzed for 16 common mycotoxins at Prairie Diagnostics Services Inc., (Saskatoon, SK) via ultra-high performance liquid chromatography (Agilent 1100, Agilent

Technologies, Santa Clara, CA) and mass spectrometry (Micromass Quattro Ultima Platinum Mass Spectrometer, Waters, Milford, MA) prior to apply to corresponding cages. The mycotoxins included DON and metabolites (3- and 15- acetyl DON); α-zearalenol, diacetoxyscirpenol, T-2, HT-2, nivalenol, ochratoxin A, β-zearalenol, zearalenone, and aflatoxin B1. Mycotoxin concentrations were confirmed for all diets prior to feeding of animals. The control starter and grower diets (with and without additive) contained < 0.1 mg/kg DON while the contaminated diets contained 1.8 – 2.0 mg/kg DON and are henceforth referred to as "DON-contaminated diets". The starter diets were prepared in crumbled form and the grower diets were pelleted. The pellets were 2 mm in diameter and 3.5 mm in length. Diets were visually similar upon inspection.

6.3.2 Growth performance and organs weights

At 7, 14, 21, 29 and 32 d post-hatch, birds were weighed using cage as the unit. Body weight (g) and feed consumption (g) were measured. Average daily gain (ADG, g/d), average daily feed intake (DFI, g/d) and feed to gain ratio (F:G, g feed/g weight gain) of each cage were then calculated as follows: $ADG_{(period\ a\text{-}b)} = [BW_{day\ b} - BW_{day\ a}]$/period a-b;, $DFI_{(period\ a\text{-}b)} = [feed\ weight_{day\ a} - feed\ weight_{day\ b}]$/period a-b and F:G = DFI/ ADG.

At 21 and 32 d post-hatch, one bird from each cage was randomly selected, individually weighed, and euthanized by cervical dislocation. Empty weight of the individual segments of the gastrointestinal tract was recorded after removing the surrounding tissues. The intestine was divided into the duodenum, jejunum, and ileum for measuring the weights and length of each segment. The duodenum was defined as the segment encompassing the duodenal loop. The ileum was defined as distal segment of small intestine tract, starts at Meckel's diverticulum and ends at ileal cecal junction and the jejunum was defined as the segment is in between of duodenum and ileum (Denbow, 2015). Organ weights were expressed as g/kg body weight (g/kg BW, relative weight). The weight to length ratio of each intestinal segment was also calculated as an indicator of intestine density (g/cm).

6.3.3 Statistical analysis

The experiment was a 2 × 2 factorial arrangement in a completely randomized design with 10 replications. All data analysis was conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). Data were initially checked for normality using the UNIVARIATE procedure and if not normally distributed, log transformation was conducted. Growth performance data were analyzed as repeated measures using one-way ANOVA with 10 replications by the PROC MIXED procedure of SAS (Littell, 2006) and the appropriate covariance structure was selected by comparing the Akaike's Information Criteria. Organ weight and intestinal density data were analyzed using one-way ANOVA by the PROC MIXED procedure of SAS. Significant effects ($\alpha = 0.05$) were analyzed using the Tukey-Kramer test to differentiate the means.

Table 6.1. Diet formulation, nutrient composition and mycotoxins concentrations (mg/kg) in experimental diets.

	Starter (1 – 21 d)				Grower (22 – 32 d)			
	No Additive		Additive		No Additive		Additive	
	Control	DON	Control	DON	Control	DON	Control	DON
Ingredients (%)								
Control wheat[1]	68.2	0	67.95	0	72.9	3.0	72.65	2.75
Fusarium-damaged wheat[2]	0	66.3	0	66.2	0	66.3	0	66.2
Feed additive[3]	0	0	0.25	0.25	0	0	0.25	0.25
Soybean meal	16.9	15.7	16.9	15.45	12.5	16.1	12.5	16.1
Meat meal (55%)	5.0	9.2	5.0	9.2	5.0	5.0	5.0	5.0
Fish meal	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Canola oil	3.0	2.0	3.0	2.0	3.0	3.0	3.0	3.0
Limestone	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Dicalcium phosphate	0.9	0.9	0.9	0.9	0.5	0.5	0.5	0.5
L-Lysine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin/mineral premix[4]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methionine	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3
Threonine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Enzyme[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nutrient Composition (%)								
Crude protein	23.0	23.0	23.0	23.0	21.5	21.5	21.5	21.5
AME (Kcal/Kg)	3080	3014	3080	3014	3095	3102	3095	3102
Crude fiber	2.40	2.40	2.40	2.40	0.40	2.30	0.40	2.30
Phosphorus	0.50	0.50	0.50	0.50	0.51	0.48	0.51	0.48
Calcium	1.20	1.00	1.20	1.00	1.10	1.10	1.10	1.10
Lysine	1.49	1.49	1.49	1.49	1.37	1.40	1.37	1.40
Methionine	0.56	0.56	0.56	0.56	0.51	0.51	0.51	0.51
Arginine	1.53	1.52	1.53	1.52	1.39	1.38	1.39	1.38

Mycotoxins[6] *(mg/kg)*								
DON	0.100	1.940	0.090	1.940	0.090	1.840	0.110	2.010
3-ADON	<0.025	0.050	<0.025	0.050	<0.025	0.050	<0.025	0.050
OTA	<0.025	<0.025	0.040	<0.025	<0.025	<0.025	<0.025	<0.025

[1] Mycotoxin concentrations in *Fusarium*-damaged wheat: aflatoxin B1: Not Detected (ND), 3-Acetyl Deoxynivalenol (DON): 764 µg/kg, 15-Acetyl DON: ND, α-Zearalenol: ND, DON: 11470 µg/kg, Diacetoxyscirpenil: ND, HT-2 toxin: 107 µg/kg, Nivalenol: 59.2 µg/kg, Ochratoxin A: ND, T-2 toxin: ND, β-Zearalenol: ND, Zearalenone: ND. The mycotoxin diets were formulated to be contaminated around 7.500 mg/kg DON.

[2] Mycotoxins concentration in control wheat aflatoxin B1: ND, 3-Acetyl Deoxynivalenol (DON): ND, 15-Acetyl DON: ND, α-Zearalenol: ND, DON: 352 µg/kg, Diacetoxyscirpenil: ND, HT-2 toxin: ND, Nivalenol: ND, Ochrartoxin A: ND, T-2 toxin: ND, β-Zearalenol: ND, Zearalenone: ND.

[3] Feed additive contains yeast autolysate dehydrated, montmorillonite clay, silicon dioxide along with dextrose, sodium bisulfite complex, 1250000 IU vitamin A, 500000 IU vitamin D, 30000 IU vitamin E, 1000 mg menadione, 5000 mg thiamine, 4000 mg riboflavin, 22000 mg niacin, 12000 mg calcium d-pantothenate, 2500 mg pyridoxine, 10 mg biotin, 22 mg cobalamin (Canadian Bio-Systems Inc. Calgary, AB).

[4] Vitamin/mineral premix: 2200000 IU vitamin A, 440000 IU vitamin D, 6000 IU vitamin E, 400 mg menadione, 300 mg thiamine, 1200 mg riboflavin, 800 mg pyridoxine, 4 mg vitamin B12, 12000 mg niacin, 2000 mg pantothenic acid, 120 mg folic acid, 30 mg biotin. 2000 mg copper, 16000 mg manganese, 160 mg iodine, 16000 mg zinc, 60 mg selenium, 100000 mg calcium carbonate. (DSM Nutritional Products Canada Inc. Ayr. ON).

[5] Enzyme: Superzyme-W ™ contains: 300 units/g glucanase, 1000 units/g xylanase, 1900 units/g cellulase, 4200 units/g amylase, 150 units/g invertase (Canadian Bio-Systems Inc. Calgary, AB).

[6] Mycotoxins were analyzed at Prairie Diagnostics Services Inc., (Saskatoon, SK) via ultra-high performance liquid chromatography (Agilent 1100, Agilent Technologies, Santa Clara, CA) and mass spectrometry (Micromass Quattro Ultima Platinum Mass Spectrometer, Waters, Milford, MA).

6.4 Results

The overall mortality was 4.5% and the mortality in each treatment at the end of the feeding trial (32 d) was 3.3%, 3.3%, 8.3% and 3.3%, respectively. Mortality occurred early (within first week) and was attributed to poor chick quality (i.e. yolk sec infections and polyserositis) and not to the presence of mycotoxins in the diet.

The initial BW of chicks did not differ among treatments at the experiment initiation of experiment and were 43.0 ± 0.4, 42.8 ± 0.5, 43.1 ± 0.4 and 43.3 ± 0.4 g for CON, DON, ADD and DON + ADD respectively ($P > 0.05$, Table 6.2). Broiler BW was not affected by dietary treatments during starter stage (1 – 21 d, $P > 0.05$, Table 6.2). At 32 d, the birds fed the ADD diet and DON + ADD diet were heavier (1929.6 g and 1917.8 g, respectively) than the DON-fed birds (1806.6 g, $P < 0.01$ Table 6.2). During 1 – 21 d, broiler ADG was not affected by treatment ($P > 0.05$, Table 6.3). During 22 – 32 d, the birds fed DON + ADD had higher ADG (94.7 g/d) than DON-fed birds (84.1 vs. 94.7 g/d, $P = 0.03$, Table 6.3). During 22 – 32 d, DON-fed birds had lower DFI (135.1 g/d) when compared to both the control group (135.1 vs. 152.8 g/d, $P < 0.01$, Table 6.3). During 1 – 32 d, the DON-fed birds had better F:G ratio when compared to birds fed the control diet (1.48 vs. 1.55, $P < 0.01$, Table 6.3)

The relative weights of organs (g/kg BW) and density of intestinal sections (g/cm) for each treatment are presented in Table 6.4 and Table 6.5, respectively. At 21 and 32 d, the relative organ weights and small intestinal segment densities were not affected by treatment ($P > 0.05$).

Table 6.2. Body weight (BW, g) of broilers as influenced by feeding a low concentration deoxynivalenol (DON)-contaminated diets[1] with or without yeast cell wall additive (ADD)[2] for 32 d.

	Control (C)	DON	C + ADD	DON + ADD	p-values		
					DON	ADD	DON*ADD
day 1	43.0±0.4	42.8±0.5	43.1±0.4	43.3±0.4	P = 0.98	P = 0.54	P = 0.69
day 7	181.5±1.8	183.9±2.9	193.7±3.5	183.7±2.5	P = 0.33	P = 0.54	P = 0.50
day 14	473.6±5.0	481.7±5.7	502.3±9.1	481.7±7.8	P = 0.15	P = 0.55	P = 0.22
day 21	906.2±11.5	925.4±13.5	953.6±17.9	906.6±14.3	P = 0.13	P = 0.36	P = 0.11
day 29	1630.7±25.1^{ab}	1544.4±29.4^{b}	1655.3±21.6^{a}	1661.4±25.6^{a}	P < 0.01	P = 0.04	P < 0.01
day 32	1895.3±32.6^{ab}	1806.6±33.4^{b}	1929.6±32.5^{a}	1917.8±32.6^{a}	P < 0.01	P = 0.01	P < 0.01

[1] The control diets were contaminated with DON at around 0.1 mg/kg DON, the DON diets were contaminated with around 2.0 mg/kg DON.
[2] Feed additive contains yeast autolysate dehydrated, montmorillonite clay, silicon dioxide along with dextrose, sodium bisulfite complex, 1250000 IU vitamin A, 500000 IU vitamin D, 30000 IU vitamin E, 1000 mg menadione, 5000 mg thiamine, 4000 mg riboflavin, 22000 mg niacin, 12000 mg calcium d-pantothenate, 2500 mg pyridoxine, 10 mg biotin, 22 mg cobalamin (Canadian Bio-Systems Inc. Calgary, AB).
[3] Within same age group (row), mean ± SE with different superscripts (a-b) are significantly different (ANOVA P<0.05 followed by Tukey-Kramer test; n=10/treatment).

Table 6.3. Average daily grain (ADG, g/d), average daily feed intake (ADFI, g/d), and feed efficiency (g feed/g gain) of broilers as influenced by feeding a low concentration deoxynivalenol (DON)-contaminated diets[1] with or without yeast cell wall additive (ADD)[2] for 1 - 32 d.

		Control (C)	DON	C + ADD	DON + ADD	p-values		
						DON	ADD	DON*ADD
ADG, g/d	1-7 d	19.8±0.3	20.0±0.4	21.5±0.5	20.0±0.3	P = 0.37	P = 0.49	P = 0.54
	8-14 d	41.7±0.6	42.5±0.5	44.1±0.9	42.7±0.8	P = 0.36	P = 0.82	P = 0.66
	15-21 d	61.8±0.9	62.3±1.5	64.2±1.1	61.8±1.5	P = 0.58	P = 0.55	P = 0.69
	22-29 d	93.4±1.5^{a}	83.3±2.9^{b}	89.3±2.5^{ab}	94.4±2.5^{a}	P = 0.38	P = 0.39	P < 0.01
	30-32 d	85.3±3.2^{b}	87.5±4.1^{ab}	91.3±3.4^{a}	87.3±3.4^{ab}	P = 0.16	P = 0.63	P = 0.02
	1-21 d	41.1±0.5	41.6±0.6	43.3±0.7	41.5±0.6	P = 0.49	P = 0.65	P = 0.69
	22-32 d	91.4±1.7^{ab}	84.1±2.1^{b}	90.5±2.3^{ab}	94.7±1.5^{a}	P = 0.03	P = 0.33	P = 0.03
	1-32 d	58.5±0.9	56.2±1.1	60.0±1.1	59.9±0.8	P = 0.03	P = 0.32	P = 0.26
ADFI, g/d	1-7 d	24.9±0.4	23.5±0.2	25.6±0.4	24.8±03	P = 0.29	P = 0.28	P = 0.48
	8-14 d	54.4±1.4	54.7±0.6	58.8±0.9	57.1±0.9	P = 0.02	P = 0.69	P = 0.11
	15-21 d	93.6±1.7^{ab}	88.4±1.6^{b}	96.9±1.9^{a}	93.1±1.7^{ab}	P = 0.03	P = 0.04	P = 0.02
	22-29 d	144.5±3.0^{a}	123.4±3.4^{b}	138.9±2.4^{a}	138.3±2.5^{a}	P = 0.04	P < 0.01	P < 0.01
	30-32 d	170.4±5.0^{a}	152.8±3.4^{b}	175.2±4.4^{a}	159.5±3.9^{ab}	P = 0.03	P < 0.01	P < 0.01
	1-21 d	57.6±0.8	55.5±0.7	60.4±0.9	58.6±0.9	P = 0.29	P = 0.14	P = 0.32
	22-32 d	152.8±2.3^{a}	135.1±2.9^{b}	148.3±2.3^{a}	144.1±2.5^{ab}	P = 0.26	P < 0.01	P < 0.01
	1-32 d	90.3±1.3^{a}	82.3±1.5^{b}	91.0±1.3^{a}	88.7±1.1^{a}	P < 0.01	P = 0.02	P = 0.05
Feed efficiency (g feed/g gain)	1-7 d	1.26±0.02^{a}	1.18±0.02^{b}	1.19±0.01^{b}	1.24±0.02^{ab}	P = 0.98	P = 0.56	P < 0.05
	8-14 d	1.30±0.02	1.29±0.01	1.33±0.01	1.34±0.02	P = 0.06	P = 0.85	P = 0.28
	15-21 d	1.52±0.03^{a}	1.42±0.02^{b}	1.47±0.01^{ab}	1.48±0.02^{ab}	P = 0.71	P = 0.05	P < 0.05
	22-29 d	1.58±0.02^{a}	1.57±0.03^{ab}	1.56±0.03^{ab}	1.48±0.03^{b}	P = 0.09	P = 0.01	P < 0.01
	30-32 d	1.93±0.04^{a}	1.81±0.05^{b}	1.91±0.05^{ab}	1.85±0.05^{b}	P = 0.34	P < 0.01	P < 0.01
	1-21 d	1.40±0.02	1.34±0.01	1.37±0.01	1.40±0.01	P = 0.36	P = 0.26	P = 0.15
	22-32 d	1.68±0.02^{a}	1.61±0.02^{ab}	1.68±0.02^{a}	1.58±0.02^{b}	P = 0.40	P = 0.01	P = 0.04
	1-32 d	1.55±0.01^{a}	1.48±0.01^{b}	1.52±0.01^{ab}	1.52±0.01^{ab}	P = 0.75	P < 0.01	P < 0.01

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[1] The control diets were contaminated with DON at around 0.1 mg/kg DON, the DON diets were contaminated with around 2.0 mg/kg DON.
[2]Feed additive contains yeast autolysate dehydrated, montmorillonite clay, silicon dioxide along with dextrose, sodium bisulfite complex, 1250000 IU vitamin A, 500000 IU vitamin D, 30000 IU vitamin E, 1000 mg menadione, 5000 mg thiamine, 4000 mg riboflavin, 22000 mg niacin, 12000 mg calcium d-pantothenate, 2500 mg pyridoxine, 10 mg biotin, 22 mg cobalamin (Canadian Bio-Systems Inc. Calgary, AB).
[3] Within same age group (row), mean ± SE with different superscripts (a-b) are significantly different (ANOVA $P<0.05$ followed by Tukey-Kramer test; n=10/treatment).

Table 6.4. Relative organ weight (g/kg BW) of broilers fed a low concentration deoxynivalenol (DON)-contaminated diet[1] with or without yeast cell wall additive (ADD).

	Control (C)	DON	C + ADD	DON + ADD	p-values		
					DON	ADD	DON*ADD
21 day							
Liver	30.97±0.84	29.69±1.22	29.63±0.61	29.71±0.78	P = 0.51	P = 0.46	P = 0.45
Spleen	0.72±0.03	0.76±0.06	0.75±0.04	0.83±0.05	P = 0.19	P = 0.29	P = 0.62
Duodenum	9.21±0.36	9.63±0.30	9.06±0.030	8.67±0.030	P = 0.97	P = 0.09	P = 0.21
Jejunum	16.55±0.60	16.29±0.59	16.60±0.51	15.95±0.52	P =0.42	P = 0.80	P = 0.73
Ileum	10.31±0.15	10.38±0.15	10.04±0.18	10.27±0.14	P = 0.33	P = 0.23	P = 0.60
32 day							
Liver	25.61±0.68	25.94±0.85	25.33±0.97	25.80±0.96	P = 0.65	P = 0.82	P = 0.94
Spleen	0.89±0.08	1.03±0.07	0.87±0.05	0.90±0.09	P = 0.26	P = 0.29	P = 0.44
Duodenum	6.90±0.31	6.91±0.33	7.00±0.30	6.61±0.31	P = 0.19	P = 0.31	P = 0.19
Jejunum	12.64±0.46	12.50±0.70	13.31±0.70	11.94±0.43	P = 0.21	P = 0.92	P = 0.31
Ileum	8.80±0.13	8.73±0.09	8.82±0.12	8.72±0.11	P = 0.72	P = 0.98	P = 0.95

[1] The control diets were contaminated with DON at around 0.1 mg/kg DON, the DON diets were contaminated with around 2.0 mg/kg DON.
[2] Feed additive contains yeast autolysate dehydrated, montmorillonite clay, silicon dioxide along with dextrose, sodium bisulfite complex, 1250000 IU vitamin A, 500000 IU vitamin D, 30000 IU vitamin E, 1000 mg menadione, 5000 mg thiamine, 4000 mg riboflavin, 22000 mg niacin, 12000 mg calcium d-pantothenate, 2500 mg pyridoxine, 10 mg biotin, 22 mg cobalamin (Canadian Bio-Systems Inc. Calgary, AB).

Table 6.5. Intestinal segment density (g/cm) of broilers fed a low concentration deoxynivalenol (DON)-contaminated diet[1] with or without yeast cell wall additive (ADD) for 32 d.

	Control (C)	DON	C + ADD	DON + ADD	p-values		
					DON	ADD	DON*ADD
21 day							
Duodenum	0.34±0.02	0.35±0.02	0.33±0.01	0.32±0.01	P = 0.92	P = 0.31	P = 0.61
Jejunum	0.31±0.02	0.30±0.01	0.31±0.01	0.32±0.02	P = 0.64	P = 0.41	P = 0.93
Ileum	0.20±0.01	0.19±0.01	0.19±0.01	0.19±0.01	P = 0.50	P = 0.59	P = 0.48
32 day							
Duodenum	0.45±0.02	0.44±0.01	0.45±0.01	0.42±0.02	P = 0.21	P = 0.45	P = 0.63
Jejunum	0.44±0.02	0.40±0.01	0.42±0.02	0.40±0.01	P = 0.10	P = 0.37	P = 0.53
Ileum	0.26±0.01	0.24±0.01	0.25±0.01	0.25±0.01	P = 0.30	P = 0.88	P = 0.10

[1] The control diets were contaminated with DON at around 0.1 mg/kg DON, the DON diets were contaminated with around 2.0 mg/kg DON.
[2] Feed additive contains yeast autolysate dehydrated, montmorillonite clay, silicon dioxide along with dextrose, sodium bisulfite complex, 1250000 IU vitamin A, 500000 IU vitamin D, 30000 IU vitamin E, 1000 mg menadione, 5000 mg thiamine, 4000 mg riboflavin, 22000 mg niacin, 12000 mg calcium d-pantothenate, 2500 mg pyridoxine, 10 mg biotin, 22 mg cobalamin (Canadian Bio-Systems Inc. Calgary, AB).

6.5 Discussion

Mycotoxins are a cause of concern in the poultry industry due to the potential for major health problems and decreased performance. Poultry producers need methods to assist them in protecting their flocks from these toxic metabolites. The aim of this study was to evaluate the effects of low DON concentrations in naturally-contaminated feed on performance parameters in broiler chickens and determine whether a yeast cell wall-based feed additive could mitigate DON-induced adverse effects.

Both feed intake and weight gain were reduced in DON-fed birds during the later stages of growth (past 21 days of age). These performance effects were observed with feeding naturally contaminated diets with 2 mg/kg DON, which is much lower than the 15 mg/kg DON concentration claimed to be the threshold for feed refusal and reduced growth in poultry (Chowdhury et al., 2005; Awad et al., 2012; Grenier and Applegate, 2013). Our past research (Wang and Hogan, 2018) and work by others (Awad et al., 2011; Yunus et al., 2012b; Shang et al., 2016) have demonstrated that naturally-contaminated diets with DON concentrations near or below the 5 mg/kg effectively reduces feed intake and weight gain in broilers. Based on these findings, broilers appear to be more sensitive to DON-contaminated diets than previous estimations and even low concentrations of DON should be avoided in broiler production.

Feed intake, weight gain and body weight in birds were increased when a YCW-based feed additive was included in DON-contaminated diets during the grower stage. Several other studies also report that dietary supplementation of a YCW-based feed additive can prevent DON-induced growth suppression in poultry (Swamy et al., 2002; Girish et al., 2008b; Shang et al., 2016). Yeast cell wall products, such as yeast β-d-glucan and mannan oligosaccharides, typically have a large surface area that provide numerous potential sites for mycotoxin binding and have demonstrated high adsorptive capacity for DON and other *Fusarium* mycotoxins (Shang et al., 2016; Zhu et al., 2016; Vila-Donat et al., 2018). In addition to increased adsorption capacity, dietary inclusion of YCW can also provide additional beneficial health effects in broilers, such as enhanced immune functions (Li et al., 2012), increased small intestinal barrier functions (Gomez-Verduzco et al., 2009), reduced intestinal inflammation (Xue et al., 2017), increased recovery after a coccidiosis challenge (M'Sadeq et al., 2015) as well as overall enhancing broiler

growth (Gomez-Verduzco et al., 2009). It appears that the feed additive used in this study was advantageous for the mycotoxin-fed birds, either by improving the nutrients digestibility or perhaps improving "access" to the nutrients in the feed that they did consume. The enhanced growth performance observed in the birds fed the additive-supplemented diets in the present study may be due to the additive not only absorbing feed-borne *Fusarium* mycotoxins but also through enhancing broilers' immune capacity and improving intestinal mucosa function.

We found that broilers fed DON-only diets had greater feed efficiency during most of the starter stage and through the entire grower stage. Increased nutrient digestibility and feed efficiency was observed in broilers (Dänicke et al., 2003) and pigs (Goyarts et al., 2005) fed naturally *Fusarium* mycotoxin-contaminated diets and it is suggested that this is a compensatory mechanism for the mycotoxin-induced reduction of feed intake (Goyarts et al., 2005). *Fusarium* fungi infection also damages cereal grains starch granules structures which may improve starch digestibility and thus feed efficiency (Jackowiak et al., 2005; Grenier and Applegate, 2013; Schmidt et al., 2016). A common concern regarding mycotoxin feed additives with binding abilities is that they may also bind dietary nutrients (e.g. vitamins and minerals) reducing their availability, and thereby suppressing animal growth and decreasing feed efficiency (Zhu et al., 2016; Vila-Donat et al., 2018). However, we and others (Swamy et al., 2002; Girish et al., 2008b; Shang et al., 2016) did not find that dietary supplementation of YCW-based feed additive affected broiler growth and feed efficiency.

There was no effect of DON on organ weight and intestinal densities at the end of starter and grower stage. The effects of feeding DON-contaminated diets on broiler organ weights are very inconsistent across studies (Swamy et al., 2004a; Girgis and Smith, 2010; Awad et al., 2011). Awad et al. (2011) also found that broiler organ weights were not affected when birds were fed naturally contaminated diets with 5 mg/kg DON. In contrast, an increase in liver weight was observed in broiler chickens fed 2 mg/kg DON (Li et al., 2012) and 7 mg/kg DON in naturally-contaminated diets (Wang and Hogan, 2018). Inconsistency in effects on liver weight may be due to potential mycotoxin mixtures in naturally contaminated diets and well as the timing and duration of exposure across studies. There was no effect of the YCW-based feed additive on any of the organ weights.

The results obtained in our study indicate that feed formulated with naturally-contaminated wheat to contain a low concentration of DON (approx. 2 mg/kg) significantly

depressed growth performance in broilers. The yeast cell wall additive added to the naturally-contaminated diet increased performance. These results suggest that this additive at 0.25% might be sufficient to counteract the adverse effects of *Fusarium* mycotoxins. Further research would be required to evaluate the efficacy of this feed additive against higher concentrations of *Fusarium* mycotoxin contamination (> 5 mg/kg DON).

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

7.1 Overview and Objectives

Fusarium fungal species produce mycotoxins that contaminate cereal grains and consequently the animal feed produced using the contaminated grains. The mycotoxin deoxynivalenol (DON) is the most frequently occurring *Fusarium*-mycotoxin in cereal grains and complete feed and presents an ongoing challenge for maintaining production animal health. In the case of broiler chickens, consumption of feed formulated with DON-contaminated grain can cause various adverse effects such as reduction of feed intake, growth and, immune suppression as well as alteration of GIT mucosa structures and functions. In Canada, the maximum dietary allowance of DON is 5 mg/kg in poultry feed and is five times higher than the maximum allowance for swine (CFIA, 2015). With increasing prevalence of *Fusarium* mycotoxin-contaminated grain worldwide (Kosicki et al., 2016), we will likely see a greater proportion of this grain utilized in compounded diets for poultry as poultry are considered resistant to *Fusarium* mycotoxin toxicity, specifically DON. Considering the industrial aim to produce high-performing broiler chickens under antibiotic-free conditions, it is important to re-evaluate threshold concentrations for *Fusarium* mycotoxins in poultry feed which are reflected in the current applicable guidance values.

In this thesis research, five animal feeding trials were conducted to better characterize sub-lethal responses to *Fusarium* mycotoxins (primarily DON) in broiler chickens and understand how exposure factors affect broiler responses to the contaminated diets. In addition to measuring broiler feed intake and growth performance (frequently used experimental endpoints), feeding behaviours were evaluated as a novel endpoint to evaluate the effects of DON-contaminated diets on broilers and provide new insight into how DON contamination contributes to the reduced feed intake. It is anticipated that this thesis research will provide more in-depth understanding about the *Fusarium* mycotoxin toxicosis in broilers and aid in developing strategies to reduce or prevent their negative impacts on broiler performance and health.

7.2 Low concentrations of DON suppress broiler growth performance

Chickens have historically been considered to have a low sensitivity towards DON (as compared to swine) with claims that feed refusal and reduced weight gain are only found when

concentrations reach >15 mg DON/kg feed (Awad et al., 2012; Grenier and Applegate, 2013). In the research conducted for this thesis, we formulated poultry feed using naturally *Fusarium*-mycotoxin contaminated grain with the intent to achieve diets with DON contamination at concentrations near to or below the CFIA legislation (5 mg/kg) to more accurately reflect the mycotoxin challenges faced in poultry production. We consistently observed a reduction of feed intake, weight gain and feed efficiency in response to low (< 5 mg/kg) to moderate (7 to 8 mg/kg) concentrations of DON-contaminated diets across the individual feeding trials conducted for this thesis research. This indicates that broiler chickens are more sensitive to DON-contaminated diets than previous estimations and contradicts previous claims that 15 mg/kg DON in naturally-contaminated diets is the threshold for broiler feed refusal and growth suppression in controlled research studies (Awad et al., 2012; Grenier and Applegate, 2013). Moreover, these results demonstrate that it is not necessary to use high concentrations of naturally-contaminated diets (> 15 mg/kg DON) to achieve reduced growth performance in research studies, and in fact this reduces the practical relevance of such data for informing industry practice.

Another important aspect of the current research is that the contaminated diets used across individual feeding trials were manufactured with the same source of naturally-contaminated wheat (around 11 mg/kg DON). Since *Fusarium* fungi can produce different combinations of mycotoxins depending on many intrinsic and extrinsic factors, using the same source of contaminated wheat may have reduced the inter-experimental variability in animal response and allowed us to replicate and compare DON-induced effects across trials. Lastly, the experimental diets used in this thesis research are formulated without inclusion of a subclinical antimicrobial to more accurately reflect the challenges poultry industry may face when moving toward antimicrobial-free production systems.

7.3 Grower stage broilers are most susceptible to *Fusarium* mycotoxins

Reduced feed intake and growth suppression was observed in DON-fed grower broiler chickens when compared to feeding during other growth stages and this effect was consistent across the different feeding trials. This data appears to confirm that grower broilers are more sensitive to DON-contaminated diets than the younger birds in terms of reduced performance.

Furthermore, our results indicate that *Fusarium* mycotoxins (primarily DON) do not have accumulated growth suppression effects in broiler chickens and a short period of exposure (7 – 14 days) during the grower stage is sufficient to impair broiler growth performance and alter feeding behaviours. In the previous DON-feeding studies, broilers were subjected to contaminated diets through the entire exposure period and the most sensitive period of DON-induced growth effects was never identified.

Identifying broiler critical sensitive period to naturally DON-contaminated diets has applied significance in the context of animal production. Under production settings, broilers may be frequently exposed to contaminated diets during a specific part of production, since *Fusarium* mycotoxins are not evenly distributed within contaminated diets and multiple diets are frequently used in the production cycle. The current thesis research highlights that DON-contaminated feed should be avoided in broiler production, especially during later stage of growth. In the context of basic research in animal science and toxicology, the specific growth suppression effects observed during the grower stage (22 – 34 d) also shows that the length of time for feeding DON-contaminated diets can be shortened in the future feeding studies with broiler chickens.

7.4 *Fusarium* mycotoxins reduce diet palpability and alter broiler feeding behaviours

This thesis research is the first to examine the effects of dietary *Fusarium* mycotoxins (primarily DON) on broiler feeding behaviours. Feeding behaviours are an external reflection of internal feed intake regulation (Reddingius, 1980; Bokkers and Koene, 2003), so short-term behavioural observations were conducted to identify possible actions (i.e. sensory aversion and anorexia effects) underlying the reduction of feed intake in DON-fed birds. Broilers exhibited consistent preference towards the uncontaminated diet over DON-contaminated diets when given free choice. Changes in feeding behaviours (e.g. increased time spent at feeder) were observed in the DON-fed birds in both the feeding behaviour study (Chapter 4) and the feed restriction study (Chapter 5). It was postulated that these behavioural alterations were due to a reduction in diet palatability. In addition, short term exposure (7 days) to low concentration DON-contaminated diets (< 5 mg/kg) was sufficient to alter broiler feeding behaviours, while in these same birds feed intake and weight gain were not affected. Taken together, our research highlights that

feeding behaviours can be used as sensitive experimental endpoints to evaluate *Fusarium* mycotoxin-toxicosis in broiler chickens.

In addition to changes in feeding behaviours, this research provides evidence that consumption of DON-contaminated diets induces anorexia effects in broiler chickens as indicated by a reduction of meal size (estimated by feed intake/meal number). Swamy et al. (2004b) found that feeding broilers DON-contaminated diets resulted in an elevation of anorexia neurochemical concentrations (Swamy et al., 2004b). Although we did not measure anorexia neurochemicals, it is possible that the reduction of meal size observed in our pair-feeding study is due to DON-induced anorexia effects. From this research, we propose that increasing feed palatibility and/or enhancing appetite in broilers may be an effective strategy for overcoming DON-induced feed refusal and preventing growth suppression in broilers.

7.5 Feed refusal induces the growth suppression in low concentration DON-fed broiler chickens

Reduction of feed intake is one of the most well-known responses observed in animals that consume DON-contaminated diets and is frequently referred to as feed refusal. Although the effects of contaminated diets on broiler performance are often evaluated in feeding studies, the specific contribution of feed refusal and reduction in feed intake has never been addressed. Understanding how DON suppresses broiler growth performance could provide important information to help direct potential methods to mitigate these adverse effects (i.e. increase feed intake and/or reduce dietary mycotoxin toxicities).

The research findings presented in Chapter 5 represent the first work in poultry to examine the contribution of feed refusal and other DON-induced toxicities on decreased performance through a pair-feeding approach. The data indicate that DON-induced growth suppression is primarily caused by reduction of feed intake rather than other DON toxic effects, such as alteration of intestinal mucosa structures and nutrient metabolisms. These results also support a recent meta-analysis that found that growth suppression observed in DON-fed birds is the result of a reduction of feed intake (Andretta et al., 2011). The pair-feeding study also supports the concept that feed refusal is a protective mechanism, which prevents animals developing further DON-induced systemic toxicity (Osweiler, 2014). In Chapter 3 however,

reduction of feed intake, reduction of feed efficiency as well as changes in ileal mucosa structures were observed in DON-fed broilers. The concentrations of DON differed between the diets used for these studies; in the Chapter 3 birds were subjected to naturally-contaminated diets with moderate concentration of DON (7 to 8 mg/kg), while in the Chapter 5, birds were subjected to naturally-contaminated diets with lower concentrations of DON (2 to 3 mg/kg). From this comparison, it could be concluded that partial feed refusal can prevent DON-induced adverse effects when birds are subjected to low concentrations; however, the magnitude of feed refusal exhibited by broilers (partial feed refusal) is not sufficient to protect birds from other DON toxic effects when faced with a moderate DON challenge. Taken together, this suggests that different concentrations of DON in naturally-contaminated diets suppress broiler growth performance through different mechanisms. Low concentrations of DON (e.g. less than CFIA legislated concentration) that can induce growth suppression do so through a reduction of feed intake, while moderate concentrations of DON can suppress broiler growth through suppressing birds' feed intake, altering intestinal mucosa structures and possibly altering the nutrient uptake and metabolism. Different mitigation strategies to prevent DON-induced broiler growth suppression could also be targeted based on DON-concentrations; improving feed palatability (i.e. increase birds' feed intake and appetite) may overcome effects of low concentrations of DON, while improving feed palatability and providing a feed additive to improve gut health and protect against potential intestinal damage would better prevent effects from higher concentrations of DON. The yeast cell wall-based feed additive tested in Chapter 6 appeared to restore feed intake concentrations and weight gain in broilers fed naturally DON-contaminated diets. Whether this additive acted through an ability to bind DON or indirectly prevented DON effects on performance by maintaining barrier function and improving intestinal health remains unknown.

7.6 Future Research

This thesis research investigated some important factors involved low level DON-induced broiler growth suppression, such as timing and duration of exposure and the relative importance of reduction of feed intake, and other mycotoxin toxic effects. Considerable research efforts have addressed the mechanisms by which trichothecenes such as DON reduce feed intake

and growth performance, with significant attention being recently paid to gut-brain interactions and dysregulation in neuroendocrine signaling (Lebrun et al., 2015). Although we confirmed that broilers demonstrated partial feed refusal, reduced feed intake, and altered feeding behaviours when fed DON-contaminated diets, mechanisms underlying these responses were not assessed. Future research should focus on determining the effects of DON on neuroendocrine mechanisms involved in the changes of feeding behaviours and feed refusal. Understanding how *Fusarium* mycotoxins (primarily DON) affect feed intake regulation signaling pathways could help in developing strategies to prevent feed refusal and growth suppression, such as reducing DON effects on peripheral organs such as liver and intestine and/or mitigating DON effects on the central nervous system.

The poultry industry is moving towards antibiotic-free poultry production due to consumer demand. The challenge for commercial poultry production is to achieve the same growth performance in a cost-efficient manner under an antibiotic-free system as under regular production practices. The increasing occurrence of highly resistant strains of pathogens worldwide further emphasizes the importance of maintaining adequate immune function in broilers that are raised in an antibiotic-free system. *Fusarium* mycotoxins, such as DON, are well-characterized immune modulators and can negatively affect immune function in broilers, leaving them more vulnerable to disease outbreaks and reducing vaccine efficacy (Awad et al., 2013). Although considerable research has been conducted to evaluate the effects of mycotoxins and DON on immune function in broilers (Awad et al., 2013), questions related to timing and duration of mycotoxin exposure should be addressed with regards to immune function, likewise, the contribution of reduced nutrient intake (adequate vs. restricted). Understanding how such factors influence mycotoxin-induced immune effects will aid in reevaluating of the effects of lower doses of mycotoxins, especially subclinical doses that do not induce clinical signs but may predispose birds to metabolic and immunologic disorders.

The adverse effects of *Fusarium*-contaminated diets on performance and health is not limited to broilers but is applicable to other economically important poultry types such as laying hens, turkeys and ducks. The experimental design and approaches developed and used in this research with broilers could be extended to address similar research questions with other poultry and to compare sensitivity to mycotoxins across types of poultry. Different poultry types can be adversely affected by *Fusarium* mycotoxins, especially when contaminated diets are fed for

extended periods. It is maintained that turkeys are the most sensitive poultry to *Fusarium* mycotoxicosis while ducks are the most resistant (Girgis and Smith, 2010). Parallel studies using naturally-contaminated diets, which is more representative of field conditions, across different poultry types would provide more robust and comparable data from which to evaluate differences in sensitivity among poultry.

7.7 Conclusion

The work presented here is a comprehensive and broad effort aimed at elucidating the underlying exposure and physiological factors associated with adverse effects of *Fusarium* mycotoxins (specifically DON) in broiler chickens. These findings could be used to develop mitigation strategies to reduce the impact of *Fusarium* mycotoxins on in broiler production, such as avoiding mycotoxin exposure during periods of greater sensitivity, enhancing broiler appetite and feed intake, reducing toxic effects on the small intestine as well as targeting supplementation of yeast-based feed additives. This research not only provides a more in-depth understanding about how mycotoxins affect broiler growth, but will hopefully assist in refining future research studies that will help in the production of healthier broiler chickens and other poultry in the face of changing industry practices.

8. REFERENCES

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