YELLOW MEALWORM LARVAE (*TENEBRIO MOLITOR*) GROWN ON DEOXYNIVALENOL-CONTAMINATED WHEAT AS A FEED INGREDIENT FOR BROILER CHICKENS

A Thesis Submitted to the
College of Graduate and Postdoctoral Studies
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in the Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon

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ABSTRACT

The purpose of this project was to determine if yellow mealworm larvae (YML) grown on wheat contaminated with a high concentration of deoxynivalenol (DON) would affect broiler chicken performance. The YML were grown in containers of wheat that contained either low (LDW; <1,000 µg/kg) or high DON (HDW; 30,730 µg/kg). The DON concentration in the dried insect meals were 0 or 17.5 µg/kg for YML grown on LDW and HDW, respectively. Seventy-five male Ross 708 broilers were randomly placed into 15 cages and reared on one of three diets from day 1-35 (five replications/treatment). At day 14, bird numbers were reduced to four birds/replication. The diets consisted of a control containing no YML meal (CD) and two diets containing 5% that were grown on either LDW (LMD) or HDW (HMD). The diets were formulated to meet Ross 708 2019 performance standards and fed as a mash in two phases: starter/grower (0-21 days) and finisher (21-35 days). Titanium dioxide was included as a marker in the finisher diets to allow the measurement of crude protein (CP) and dry matter (DM) digestibility. Excreta was collected on days 33 and 34. Feed intake (FI) and body weight (BW) were measured over the duration of the experiment and used to calculate feed conversion ratio (FCR). On day 35, all birds were slaughtered and dissected to collect weights of the breasts, thighs, drums, wings, abdominal fat pads, liver, spleen, bursa of Fabricius, and gastrointestinal tract organs. A one-way ANOVA was used to assess the effect of diet on digestibility, performance, and carcass traits. Crude protein retention was higher in the LMD and HMD treatments compared to CD (68.17, 68.61, 66.17 respectively ($P = 0.0091$)). Dry matter retention was higher in the HMD diet compared to the CD and LMD diets (76.80, 74.93, 74.88 respectively; $P = 0.0046$)). Feed intake was lower in birds fed HMD compared to CD and LMD (2469.0, 2709.1, 2762.4 respectively; $P = 0.0031$)). The fatty acid profiles of the broilers fed diets containing YML differed from those on the CD ($P < 0.05$). Diet inclusion of YML did not affect the growth, meat yield or organ weights of the birds. The YML reared on DON-contaminated wheat (up to 30,730 ug/kg) and included in broiler diets at 5% could be an effective means of converting salvage wheat into a safe and sustainable source of protein.

ACKNOWLEDGEMENTS

I wholeheartedly thank my supervisor Dr. Fiona Buchanan for offering me the opportunity to work on this project. Her support, guidance, friendship, and encouragement were critical to complete this research. I also wish to thank my co-supervisor Karen Schwean-Lardner, who contributed extensively to the setup of the experiment, and guided me throughout the process of preparation. I would like to extend my thanks to Rex Newkirk and John Smillie who aided me in in the insect, nutritional, and feed related components of this research along with their enthusiasm for the insect sector. I would like to thank Natacha Hogan for her input with toxicology, histopathological, and hematological aspects of this project. I give thanks to my external Elijah Kiarie. Lab technicians Natalia Rudnitskaya and Yuguang Ying for their aid throughout the experiment. I would like to thank PDS Saskatoon for mycotoxin analysis and Debbie Puttick for the fatty acid profile laboratory analysis of our samples. Aditya Manek for all of his guidance throughout the histology work undertaken during this project. I would like to thank Duncan Thomas, Carlos Ochoa-Sanabria, Darien Deschner, Sara Thue, Abby Tillotson, Amy Gerein, Kelsey Hamilton, Jessica Jackson, and Kayla Madder for helping with yellow mealworm production. I would also like to thank Tory Shynkaruk, Kailyn Beaulac, Eugenia Herwig, Rachel Savary, Jo Ann Chew, Yousef Khanfas, Kiana Reiger, and Meghan Taylor for their help during the poultry experiment.

This research was only possible with the financial contributions of the Saskatchewan Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bi-lateral agreement.

Finally, I would like to thank my parents, sister, relatives, and friends that supported me throughout this research. It would not have been possible for me to realize my goals without the support and motivation from everyone during this project.

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LIST OF ABBREVIATIONS

15ADON	15 acetyldeoxynivalenol
3ADON	3 actetyldeoxynivalenol
ADF	Acid detergent fibre
ADG	Average daily gain
ADIN	Acid detergent insoluble nitrogen
AOAC	Association of Official Agricultural Chemists
BSFL	Black soldier fly larvae
BW	Body weight
CD	Control diet
CF	Crude fat
CFIA	Canadian Food Inspection Agency
CP	Crude protein
DM	Dry matter
DON	Deoxynivalenol
EDTA	Ethylenediamine Dipotassium Tetraacetic Acid
EFSA	European Food Safety Authority
F:G	Feed to gain ratio
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
FDK	*Fusarium* damaged kernel
FHB	*Fusarium* head blight
FI	Feed intake
FUM	Fumonisins
GC	Gas chromatography
H/L	Heterophil lymphocyte ratio
HDW	High DON wheat (30,730 µg/kg DON)
HMD	Diet containing 5% yellow mealworms reared on high DON wheat
LDW	Low DON wheat (<1,000 µg/kg DON)
LMD	Diet containing 5% yellow mealworms reared on low DON wheat
NIT	Near infrared transmittance
NIV	Nivalenol
YML	Yellow mealworm
ZEL	Zearalenol
ZEN	Zearalenone

PREFACE

This thesis is organized and formatted as per the guidelines for a manuscript-style thesis by the University of Saskatchewan College of Graduate and Postdoctoral Studies. Chapter 1 of this thesis is a general introduction. Chapter 2 is review of the current literature as it pertains to mycotoxins, animal health, insects, and animal production. Chapter 3 is a manuscript that will be published in a peer-reviewed scientific journal. Chapter 4 is a general discussion discussing topic not discussed in the other chapters. Chapter 3 is being prepared for submission to Poultry Science. References cited in each chapter are combined and listed in the References section of this thesis.

CHAPTER 1

INTRODUCTION

Fusarium fungi are a recurring issue for cereal grains for both crops and livestock production with numerous negative impacts economically. Several *Fusarium* species cause a disease called *Fusarium* head blight (FHB) causing reductions in yield and grade of grains by creating *Fusarium* damaged kernels (FDK; Dill-Macky and Jones, 2000). A large outbreak of FHB which occurred in 2016 in Western Canada resulted in an estimated one billion dollars worth of damage associated with lower grades being assigned due to the presence of FDK and/or mycotoxins (Canadian Grain Commission, 2020). On top of losses associated with FDK, some species of *Fusarium* produce secondary metabolites, mycotoxins, which can cause acute or chronic challenges in animals resulting in reductions in performance or increased mortality. In cases where the presence of FDK or mycotoxins in crops are high, the crop could be downgraded to salvage which has little or no economic value. Blending is a common practice, where wheat with high occurrence of FDK are mixed with grain that has no or a low occurrence of infection to be able to sell the crop. In years where *Fusarium* occurrence is high, salvage crops may be burnt or buried which can have negative effects on the environment. With the expected increases in temperature and unstable weather associated with climate change, outbreaks of FHB are expected to become more frequent, particularly when conditions are humid (Dweba et al. 2017).

The mycotoxin most commonly associated with *Fusarium spp.* is deoxynivalenol (DON; Tittlemier et al., 2019). Deoxynivalenol can cause reductions in feed intake, performance, or damage the gastrointestinal tracts of animals fed contaminated diets (Awad et al., 2013; Gallo et al., 2015). Recently, it has been shown that yellow mealworm larvae (YML; *Tenebrio molitor*) grown on DON-contaminated feedstuffs contained low concentrations in their bodies. Research by Ochoa-Sanabria et al. (2019) found that YML fed between 210 and 12,000 μg/kg DON retained approximately 130 μg/kg DON. Van Broekhoven et al. (2017) did not detect any DON in larvae reared on DON-contaminated feed. Yellow mealworm larvae are approximately 50% crude protein (CP) and 35% crude fat (CF) on the dry matter (DM) basis (Van Broekhoven et al., 2017; Ochoa-Sanabria et al., 2019). Yellow mealworms also have an amino acid profile that is similar to dietary needs of livestock such as poultry with the exception of methionine (Bovera et al., 2015). When considering the potential of YML they have to be produced at a low cost. Feeding salvage crops would help with this mandate and the mealworm meal could be competitive with fish meal or soybean meal as a cost-effective feed ingredient for poultry

provided it can be produced on a large scale. These results suggest that YML reared on DON-contaminated feed may be utilized as a safe, sustainable feed ingredient for use in poultry feed.

CHAPTER 2

LITERATURE REVIEW

2.1 Impact of mycotoxins

Cereals, such as wheat, are a major global crop for human and animal consumption with 2.96 billion tonnes grown worldwide in 2018 and production has been steadily increasing since 1961 (FAO, 2020). Losses due to fungal diseases represent a major economic loss due to cereals receiving lower grades or even salvage status (which has little to no monetary value), reduced yield due to damage to the kernels, or the presence of detectable mycotoxins. Mycotoxins are a secondary metabolite produced by certain fungal genera such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* which can have numerous negative effects on humans and animals (Anfossi et al., 2016). Several species of fungi can produce the same mycotoxin, and a single species may produce multiple mycotoxins. Commonly present mycotoxins include aflatoxins (AF), ochratoxin A, trichothecenes (e.g., DON; T2 toxin; HT2 toxin), fumonisins (FUM), and zearalenone (ZEN) (Freire and Sant'Ana, 2018). A ten-year study conducted by Gruber-Dorninger et al. (2019) using 74,821 feed samples found 88% were positive for a minimum of one mycotoxin and 64% contained two or more. Globally DON, FUM, and ZEN were the most detected mycotoxins at 64%, 60%, and 45% respectively (Gruber-Dorninger et al., 2019). It should be noted that not all fungi produce toxic secondary metabolites. There are over 300 identified mycotoxins however, most attention is directed towards those that negatively impact health such as AF, DON, and ZEN (Ji et al., 2016).

Mycotoxins cause large economic losses related to reductions in animal production, increased mortality, and loss of crops and feed (Tittlemier et al., 2019). Toxic consequences of mycotoxin inclusion in animal diets can include: damage to the intestine, liver and other organs, reduction in feed intake, growth and fertility, emesis, immunosuppression, teratogenic and carcinogenic effects, and neurotoxicity (Friere and Sant'Ana, 2018). The effect of mycotoxins vary based on the dose, toxicity of the compound, body weight, age, animal health, and species (Anfossi et al, 2016). There is also a risk of carryover into products such as eggs, milk, and meat (Gruber-Dorninger et al., 2019) that can have negative effects on consumers. Many mycotoxins are stable compounds and as such often remain in the final product even after processing methods such as extrusion, that generate considerable heat although concentrations may be lower than initially detected. The reduction in concentration depends on the time, heat, and moisture of the processing method (Karlovsky et al., 2016).

Due to the negative effects of mycotoxins on crop and animal production, stability and co-occurrence, methods to mitigate their impact are essential. Rejected shipments, downgrading of crops, animal and human illness have all created the impetus to find uses for mycotoxin contaminated product to reduce the economic damages associated with it.

2.2 *Fusarium*

Fusarium species are one of the most important fungal pathogens of plants, causing diseases in a multitude of crops leading to devastating losses in yields globally. In cereals, *Fusarium* species cause a devastating disease called FHB (Dweba et al., 2017). The effects of FHB in cereals are primarily the development of FDK which are chalky, shrivelled kernels that can contain high concentrations of mycotoxins (Dill-Macky and Jones, 2000). The increased presence of FDK is associated with higher concentrations of mycotoxins, although not always as contamination may vary based on environmental conditions during storage. There are several species of *Fusarium* that cause FHB, the most prevalent globally are subspecies of the *Fusarium graminearum* (Ferrigo et al., 2016).

Fusarium primarily produce trichothecenes, but some can also produce ZEN and FUM. Trichothecenes consist of two groups: A and B, differentiated by the different functional groups in the C-8 position on the trichothecene backbone (Ferrigo et al., 2016). Trichothecenes toxicity is caused by inducing apoptosis of eukaryotic cells by disrupting DNA, RNA, and protein synthesis (Gupta, 2012). *Fusarium* species that infect wheat primarily produce type B trichothecenes DON and nivalenol (NIV), but recently a type of type A trichothecene, NX toxins, have been discovered (Varga et al., 2015). Trichothecenes often co-occur with ZEN, a phenolic resorcylic acid lactone, and FUM, polyketide-derived mycotoxins, which can further compound and result in more severe repercussions to livestock health and productivity (Gupta, 2012; Ferrigo et al., 2016). ZEN is known to have carcinogenic, hepatotoxic, immunotoxic and hyper-estrogenic effects in animals. FUM induces apoptosis of cells by peroxidising membrane lipids resulting in damage to a variety of tissues as well as having carcinogenic properties (Ferrigo et al., 2016).

2.2.1 Occurrence and species in Western Canada

Years where the occurrence of *Fusarium* is high in cereals can result in substantial loss of

revenue for producers and the economy. The Western Canadian provinces, British Columbia (BC), Alberta (AB), Saskatchewan (SK), and Manitoba (MB), produce approximately 90% of the wheat produced in Canada (Stats Canada, 2020). As such, in years like 2016 where the incidence of *Fusarium* was high at 32.8, 85.2, and 90.1% for AB+BC, SK, and MB respectively, the economic implication of the losses due to FDK and DON are substantial (Canadian Grain Commission, 2020). The spread of *Fusarium* is difficult to control and almost inevitable as environmental conditions that favour fungal growth will occur for quick infection and disease progression (Dweba et al., 2017).

The predominant *Fusarium* species in Western Canada is *F. graminearum* which produces the mycotoxins DON, 3-acetyl DON (3ADON) and 15-acetyl DON (15ADON; Tittlemier et al., 2019). Other species detected in Canada include: *F. culmorum*, *F. avenaceum*, *F. crookwellense*, *F. pseudograminearum, F. asthroporoides, F. acuminatum, F. equiseti* and *F. poae*, with the additional mycotoxins NIV and ZEN being detected (Tittlemier et al., 2019; Cowger et al., 2020).

2.2.2 Deoxynivalenol

Deoxynivalenol, also called vomitoxin, is the primary mycotoxin produced by *F. graminearum*, and is one of the most prevalent in cereal crops worldwide (Gruber-Dorninger et al., 2019). The occurrence of DON is heavily associated with the presence of *Fusarium* spp. and FDK (Dweba et al., 2017; Tittlemier et al., 2019). Deoxynivalenol is stable at high temperatures and will often be present in the final product. The final concentration of DON may be lower due to the combined effects of time, moisture, and temperature of treatment (Liu et al. 2019). An increase in any of these factors can reduce DON concentrations (Liu et al., 2019). The toxicokinetics of DON is dependent on absorption, distribution, metabolism, and elimination when consumed by animals (Payros et al., 2016). Deoxynivalenol, like other trichothecenes, are amphipathic molecules which can move passively across cell membranes, allowing for rapid absorption in the gastrointestinal tract (Pinton and Oswald, 2014). In mammals, DON is metabolised by a glucuronidation pathway involving conjugation with glucuronic acid. Chickens utilize sulfonation and sulfation to reduce the toxicity of DON (Payros et al., 2016). Deoxynivalenol and its metabolites will typically be present in excreta and most species will rapidly clear them. Ruminal and intestinal bacteria can remove the epoxide group of DON,

primary determinant of toxicity, generating de-epoxy-DON (DOM-1) which is less harmful (Payros et al., 2016).

Due to the negative health impacts fusariotoxins can have on humans and livestock, the Canadian Food Inspection Agency (CFIA) has imposed regulatory limits that determine what grains can be used for depending on the concentration of mycotoxin present. If the concentration of mycotoxins exceeds the regulations set out by the CFIA for feed and single feed ingredients, the grains will be deemed salvage. Unfortunately, *Fusarium* spp. are difficult to control, requiring a mixture of biological, chemical and host resistances to adequately control incidences of FHB and as a result mycotoxin contamination (Dweba et al., 2017).

2.3 Impacts of deoxynivalenol in animals

Mycotoxins like DON can have negative effects on livestock production. Effects can result in significant losses incurred due to loss of productivity related to reduced feed intake, reduced fertility, or death of animals caused by high concentrations of DON present in feed ingredients and feed.

2.3.1 Effects on animals

Exposure to DON can have a variety of deleterious effects on animal health and productivity. The severity of mycotoxicosis can range from reduced feed intake, lesions, gastrointestinal disorders, reproductive issues, and immunosuppression (Awad et al., 2013 and Gallo et al., 2015). The severity of effects will vary dependent on the livestock species, age, dose, and duration of exposure (Freire and Sant'Ana, 2018). Subacute effects of chronic mycotoxicosis are more common as concentrations of mycotoxins in feed are typically relatively low. Mycotoxin concentrations in feed are normally not high enough to cause the acute form of mycotoxicosis (Payros et al., 2016).

2.3.1.1 Swine

Swine are particularly susceptible to the effects of DON (Awad et al., 2013). The reason for the high sensitivity is thought to be due to the rapid absorption of DON in swine, it is detectable in blood in less than 30 minutes after being ingested (Eriksen and Pettersson, 2004). Swine also have a low rate of metabolizing DON and other mycotoxins, relative to poultry and

ruminants (Pinton and Oswald, 2014). Due to their high susceptibility to DON, exposure as low as 1,000 µg/kg can result in reduced feed intake, immunosuppression, emesis, lesions in the gastrointestinal tract, and kidney problems (Cortinovis et al., 2013). The effects of DON can also continue even after contaminated feed is removed due to changes in feeding behaviour as observed by Serviento et al. (2018). Pigs grown on DON-contaminated feed were shown to feed less frequently and eat slowly. This changed feeding behaviour resulting in reduced overall feed intake (FI) causes the animals to take longer to reach market weight. Exposure to the DON-contaminated diet also caused changes in feeding behaviour which resulted in a two-to-three-day lag before FI increased once the challenge was removed (Serviento et al., 2018). Gilts can also have reduced oocyte maturation and embryo development resulting in reduced reproductive success (Cortinovis et al., 2013) which greatly affects the productivity of a swine operation.

2.3.1.2 Ruminants

Deoxynivalenol primarily causes feed refusal or reduced FI in ruminants although it can also cause gastrointestinal ulceration in the rumen (European Food Safety Authority; EFSA, 2014). The reduced FI translates into a direct decrease in productivity. Common symptoms of high DON exposure are reduced FI, reduced productivity, immunosuppression, reproductive failure, and gastrointestinal disease. Dairy cattle can have reduced milk yield, reduced fertility, and increases in somatic cell counts (Gallo et al., 2015). Deoxynivalenol impairs rumen fermentation and reduces microbial protein flowing to the duodenum (Marczuk et al., 2012).

2.3.1.3 Poultry

Relative to swine, poultry have reduced susceptibility to DON. This is partly thought to be due to lower absorption and faster metabolization rates of DON in poultry making acute effects unlikely unless mycotoxin concentrations are high (Eriksen and Pettersson, 2004; Pinton and Oswald, 2014). Dietary concentrations of DON in excess of 5,000 µg/kg can cause reductions in growth rate, productivity, and immune function, increasing susceptibility to disease. Layers and broilers will typically have reduced FI resulting in lower egg production or feed efficiency (FE) and weight gain respectively (Awad et al., 2013). Lesions may form in the gastrointestinal tract and mouth due to the cytotoxic effects of DON (Stoev, 2015). A study and Wang and Hogan (2019) observed reductions in the bodyweights ($P<0.01$) of male Ross 308

broilers fed diets containing > 6,000 µg/kg DON coinciding with reductions in FI (P<0.01). Broilers fed DON-contaminated diets for the duration of the trial were also found to have shorter ileal villi and shallower crypts (Wang and Hogan, 2019). Antonissen et al. (2014) found that exposure to DON at 3,000-4,000 µg/kg in broiler diets increased the incidence of sub clinical necrotic enteritis from 20% to 47% while increasing protein availability in the lumen, reducing intestinal barrier function which could stimulate growth and toxin production of *Clostridium perfringens*. Grenier et al. (2016) observed increased severity of coccidiosis in broilers exposed to DON and FUM below regulatory limits relative to unexposed birds. Awad et al. (2019) found that 5,000 and 10,000 µg/kg DON increased intestinal paracellular permeability in broilers which led to increased *Escherichia coli* counts in the spleens and livers indicating an increased risk of infections.

2.3.2 Regulation of DON in feed in Canada

Currently the CFIA has regulations regarding limits for contaminants such as mycotoxins in feed, under the RG-8 Regulatory Guidelines, in accordance with the Food and Agriculture Organization (FAO; Canadian Food Inspection Agency, 2017a). The RG-8 contains legislated and recommended maximum tolerated concentrations of mycotoxins including: DON, HT-2, AF, T-2, ZEN, FUM, and ochratoxin A. Regarding DON, current maximum tolerated concentrations are 2,000 µg/kg for soft wheat for human consumption, 5,000 µg/kg for cattle and poultry diets, and 1,000 µg/kg for swine and lactating dairy animal diets (Canadian Food Inspection Agency, 2017a). In 2017 the CFIA released a proposal: Contaminant Standards for Aflatoxins, Deoxynivalenol, Fumonisin, Ergot Alkaloids and Salmonella in Livestock Feeds (Canadian Food Inspection Agency, 2017b). This document proposed limits for both single feed ingredients and overall diets including additional species such as sheep, equine, and rabbits. Another document was released in 2018 with stakeholder input where salmonids were added onto the list (Canadian Food Inspection Agency, 2018). The current and proposed maximum concentrations of DON are displayed in Table 2.1.

Table 2.1 Canadian Food Inspection Agency current and proposed maximum concentrations of deoxynivalenol (DON) for feed and single feed ingredients for domestic animals

Species/class of animal	**Current dietary limits of DON (µg/kg)**	**Proposed limits of DON for single feed ingredients (µg/kg)**	**Proposed limits for diets of DON (µg/kg)**
Cattle – calves (<4 month)	1,000	5,000	1,000
Cattle – beef	5,000	10,000	5,000
Cattle – dairy	5,000	10,000	5,000
Lactating dairy animals	1,000	NA	NA
Swine	1,000	5,000	1,000
Poultry (Chickens, turkeys, ducks)	5,000	10,000	5,000
Other (Sheep, equine, rabbits)	NA	10,000	5,000
Salmonids	NA	2,000	600

Modified from Canadian Food Inspection Agency (2017a and 2017b)

2.4 Dealing with deoxynivalenol contaminated wheat

There are strategies that have been developed to mitigate the effect of *Fusarium spp.* in the food and feed industries. There are methods to prevent the occurrence of *Fusarium spp.* and mycotoxins revolving around agricultural practices such as monitoring for FHB, fungicides, and good storage practices. The creation of resistant cultivars to FHB is still in the early stages of development and still requires further research in identifying traits which work synergistically (Su et al., 2019). Wheat strains resistant to FHB have been reported to have reduced baking quality in previous studies which is undesirable (Gaikpa et al., 2019). During years with high occurrence of *Fusarium spp.* due to environmental factors involving high humidity and mild temperatures (Wegulo, 2012) crops must be managed utilizing postharvest detoxifying methods.

2.4.1 Physical methods

Current effective physical methods of reducing DON-contamination in grain revolve around separating out FDK, thereby reducing the concentration of mycotoxins. Kautzman et al. (2015) utilized near- infrared transmittance (NIT) to separate FDK utilizing kernel CP. Separating out FDK does not necessarily mean that DON concentration would be reduced however, there is a correlation between the two (Tittlemier et al., 2019). Another method of sorting grain utilizes air aspiration, which separates kernels based on density with lighter kernels (including FDK) blown further than heavier. Maygar et al. (2019) found that air aspiration reduced the detected concentrations of DON by 24%.

2.4.2 Chemical methods

Current chemical methods of reducing DON in harvest grain revolve around the use of ozone. A study by Wang et al. (2016) tested if ozonation could reduce concentrations of DON in contaminated wheat. Wheat was exposed to 75 mg/L ozone for times of 0, 30, 60, and 90 minutes. Ozone treatment reduced DON by 26.4 to 53.5% over the time intervals in the treated wheat. No changes were observed in the nutritional properties of the wheat when analyzed, although the toxicity of ozone treated wheat is not yet published.

A study by Kong et al. (2014) found that adsorbent agents such as activated charcoal, bentonite, cellulose and microorganisms adsorbed approximately 3.24 – 22.9% *in vitro* compared to rates of over 80% in the case aflatoxin. Solís-Cruz et al. (2017) found that chitosan,

hydroxypropyl methylcellulose, sodium carboxymethylcellulose, and microcrystalline cellulose adsorbed 3.55, 31.43, 36.27, and 16.69% of DON in an *in vitro* analysis of a poultry gastrointestinal model. Adsorption rates of other mycotoxins was higher ranging from 35.42-44.58% in the case of aflatoxin B1 (Solís-Cruz et al., 2017). Synthetic magnetic nano-zeolite bound DON at a rate of 1.8% while binding >99% of aflatoxins (Karami-Osboo et al., 2020). Hahn et al. (2014) tested twenty feed additives *in vitro* to measure DON reduction over 24 hours. One product reduced DON concentration by 97% while the other products detoxified 12% or less (Hahn et al., 2014).

2.4.3 Insects

Recently some insect species have been grown on DON-contaminated feed with little to no effect on production. The DON retention within their bodies was reduced significantly or completely eliminated. A study by Van Broekhoven et al. (2017) found that YML had undetectable concentrations of DON (<100 µg/kg) with no effects on mortality and growth when grown on naturally (4,900 µg/kg) and artificially (8,000 µg/kg) contaminated wheat flour. Excreted DON concentrations were also lower than that ingested (Van Broekhoven et al., 2017). Ochoa-Sanabria et al. (2019) in contrast to Van Broekhoven et al. (2017) detected 122-136 µg/kg DON in the YML when reared on DON-contaminated wheat (200-12,000 µg/kg). There were no differences between treatments nor was the growth and survival of the YML affected. A study by Camenzuli et al. (2018) reared black soldier fly larvae (BSFL; *Hermetia illucens*) on DON-contaminated wheat ranging from 5,000 to 125,000 µg/kg that was undetectable within their bodies. Overall, rearing insects on DON-contaminated wheat could provide an excellent method of utilizing salvage wheat which has no economic viability due to exceeding regulatory limits.

2.5 Insects

Insects could fill a unique niche of converting salvage crops into a nutritious potential food and feed ingredient. Insects contain large quantities of CP, amino acids, CF and fatty acids (Makkar et al., 2004) making them an excellent source of protein and energy. With a projected world population of 9 billion people by 2050, 70% of which is expected to be urban, current farming practices are not expected to be able to meet future food demand (Alexandratos and Bruinsma, 2012). Insect production could help sustainably bridge the gap required to meet an

increased demand for animal protein and as a feed ingredient while not having as severe environmental effects compared to an increase in intensive livestock production.

Insect production has numerous benefits. They can be grown on a small land area, even in or near cities, and many insect species have reduced greenhouse gas emissions relative to traditional livestock operations (Halloran et al., 2016). Insects also have similar feed conversion ratios (FCR) to those of poultry and can accumulate protein quite efficiently. Oonincx et al. (2015) reported that YML and BSFL convert 22-45% and 43-55% of crude protein into edible body mass respectively compared to poultry at 33%. Further work is still required to optimize insect productivity and diets so this could be improved even further, although the associated costs for insect diets may not be sustainable in the long term in comparison to rearing insects on salvage crops or organic side-streams (Oonincx et al., 2015; Halloran et al., 2016). Studies in insects are also becoming more common with 147 papers published pertaining to "edible insects" in 2019, and 18 studies published between January 1-29, 2020 compared to 25 publications in 2013 (Baiano, 2020). Due to the potential to produce insects on unutilized crops and foodstuffs, insect farming has grown in interest in Canada. Enterra Feed Corporation produces BSFL on pre-consumer food waste. Insect farming is a rapidly growing industry that in time has the potential to be quite sustainable, environmentally friendly, and profitable.

2.5.1 Yellow mealworms

Yellow mealworms (*Tenebrio molitor*) are a member of Tenebriondidae family, of the order Coleoptera, also called the darkling beetle family which also include *Zophobas morio* (superworm) and *Alphitobius diaperinus* (lesser mealworm). Currently 468 species within the Coleoptera order are reported as edible, mostly it is the larvae that are eaten (Anankware et al., 2014). Yellow mealworm larvae primarily feed on and are considered a pest of starchy materials, such as wheat (Ribeiro et al., 2018). Female beetles produce a hormone, 4-methyl-1-nonanol, when ready to copulate that attracts males (Park et al., 2014). Females will typically lay 250-500 eggs singly or in clusters which they attach to substrate or walls of containers (Ghaly and Alkhoaik, 2009). The eggs will normally hatch into larvae between 4-10 days (Selaledi et al., 2019) but eggs taking up to 34 days to hatch have been reported (Kim et al., 2015). The larvae grow and go through 14-20 instars before entering the pupal stage, (Park et al., 2014) the number of instars influences the duration of the life cycle. The pupal stage lasts between 6 to 20 days

(Hill, 2002; Ghaly and Alkhoaik, 2009). Adults emerge as whitish beetles with soft exoskeletons which will then harden and darken. Oviposition begins approximately 3 days after emergence and the adult stage typically lasts for approximately 60 days but has been reported to up to 173 (Ribeiro et al., 2018). The entire life cycle takes place in the same habitat and in optimal condition can be as short as 75 days (Selaledi et al., 2019) but will typically be approximately 80-84 days (Park et al., 2014).

Yellow mealworms, like most insects are poikilothermic, thus reliant on environmental conditions for heat. Optimal temperatures are in the range of 25-28ºC (Ribeiro et al., 2018; Kim et al., 2015), below 17ºC will inhibit embryonic development and above 30ºC increases death rates (Ribeiro et al., 2018). Optimal humidity is approximately 75% (Punzo and Mutchmor, 1980). Higher growth rates in larvae have been observed at 90-100% humidity although this high humidity will favour the growth of contaminants such as fungi. In cases of extremely dry conditions, less than 10% relative humidity, larvae may stop feeding and become inactive until humidity rises (Ribeiro et al., 2018).

The YML can be grown on a wide variety of feedstuffs but grow best on diets high in protein (Van Broekhoven et al., 2015). Diets low in protein have been shown to result in a longer development time (Oonincx et al., 2015; Van Broekhoven et al., 2015), increasing the number of days to reach a harvestable size.

2.5.2 Nutrient profile of yellow mealworm larvae

Due to their high nutritive value, YML are being considered for use in animal feed and as food. Reported CP and CF typically range from 40.7-68.9% and 23.0-36.0% on a DM basis respectively (Ghaly and Alkoaik, 2009; Ravzanaadii et al., 2012; Ochoa-Sanabria et al., 2019). This variance is likely dependent on the type and composition of the feed YML are grown on (Nowak et al., 2016). A study by Van Broekhoven et al. (2015) found that dietary CP and starch content did not affect YML CP (45.1-48.6%DM) and CF (18.9-27.6%DM). This study found high protein diets (32.7% CP) improved survival rates to 88% and higher compared to the control (17.1% CP) at 71%. Another study by Oonincx et al. (2015) found diets high in protein reduced total fatty acids in YML compared to the control (26.5 vs 30.9% DM). Oonincx et al. (2015) also found that diets low in protein decreased the survival rates of YML compared to those grown on high protein diets (52 vs 79%). It was also found that the addition of a carrot as a

water source improved larvae survival (Oonincx et al., 2015). Research has shown that YML are high in amino acids (Table 2.2) and fatty acids (Table 2.3) although some amino acids and fatty acids may not be present depending on the composition of the feed the larvae were grown on (Van Broekhoven et al., 2015).

Chitin (N-acetyl-D-glucosamine), is a molecule similar in structure to cellulose, and makes up the majority of the fibre content of insects (Finke, 2007). Chitin contains acetamides at the C2 position of monomers instead of hydroxyl groups on cellulose. Chitin is a structural compound used in the exoskeleton of insects and is replaced periodically during growth and development (Doucet and Retnakaran, 2012). The similarities between chitin and cellulose allows chitin to be measured using a combination of acid detergent fibre (ADF; the ash from ADF) and acid detergent insoluble nitrogen (ADIN). The chitin content of YML have been reported between 2.7 to 6.7% DM of YML (Marono et al., 2015; Ochoa-Sanabria et al., 2019). Chitin has been shown to stimulate serum immunoglobulin G (IgG) and immunoglobulin A (IgA) concentrations in birds. Broilers fed diets containing 0.4% YML meal cultured with *Lactobacillus plantarum* and yeast (*Saccharomyces cerevisiae*) had increased survival, FI and average daily gain (ADG) than those fed the control diet when challenged with *Escherichia coli* and *Salmonella. Salmonella* and *E. coli* population were also reduced in the gastrointestinal tracts of broilers, thought to be due to the probiotic and prebiotic effects of the cultured insect meal (Islam and Yang, 2017). Chitin does have the potential to reduce nutrient digestibility (Bovera et al., 2016) which could limit the use of insects in diets to avoid the negative effects on performance. Insects have small amounts of vitamins and minerals but are not a good source for them.

Table 2.2 Amino acid profile of yellow mealworm larvae from 3 studies (% dry matter)

	Studies		
Amino acid	**Ghosh et al. (2017)**	**Ravzanaadii et al. (2012)**	**Ochoa-Sanabria et al. (2019)[1]**
Aspartic acid	2.76	3.6	4.2
Threonine	1.8	1.8	2.1
Serine	2.2	2.1	2.6
Glutamic acid	5.8	5.7	6.2
Proline	1.7	3.0	3.3
Glycine	2.6	2.4	2.7
Alanine	4.0	3.7	4.0
Cysteine	3.2	0.5	0.4
Valine	2.9	2.4	3.0
Methionine	ND	0.7	0.6
Isoleucine	2.0	3.6	2.0
Leucine	3.4	3.4	3.7
Tyrosine	3.5	3.5	4.0
Phenylalanine	1.8	1.8	1.9
Histidine	2.8	1.5	4.8
Lysine	2.0	2.9	2.6
Arginine	2.2	2.4	2.6
Crude protein	53.2	46.4	50.2

[1]Oven dried larvae
ND: not detected

Table 2.3 Fatty acid composition of yellow mealworm larvae from 3 studies (percent of crude fat)

Fatty acid		**Studies**		
Name	**Lipid number**	**Ghosh et al. (2017)**	**Finke (2015)**	**Ochoa-Sanabria et al. (2019)[1]**
Crude fat (% dry matter)		34.5	34.4	34.4
Lauric acid	C12:0	0.32	-	-
Tricedecanoic acid	C13:0	0.43	-	-
Myristic acid	C14:0	4.72	1.43	7.0
Myristoleic acid	C14:1	0.20	-	-
Pentadecanoic acid	C15:0	0.06	-	-
Palmitic acid	C16:0	13.65	12.30	20.4
Palmitoleic acid	C16:1	2.58	0.84	-
Heptadecanoic acid	C17:0	0.06	-	-
Heptadecenoic acid	C17:1	0.09	-	-
Stearic acid	C18:0	0.23	2.56	-
Oleic acid	C18:1	45.10	27.30	55.1
Linoleic acid	C18:2	21.94	24.30	18.2
Linolenic acid	C18:3	0.32	1.03	-
Arachidic acid	C20:0	0.12	-	-
Eicosenoic acid	C20:1	0.06	0.19	-
Eicosadienoic acid	C20:2	0.12	-	-
Eicosapentaenoic acid	C20:5	0.00	0.22	-
Docosadienoic acid	C22:2	0.12	-	-
Tricosanoic acid	C23:0	0.43	-	-
Lignoceric acid	C24:0	0.03	-	-

[1]Oven dried larvae
-: Not measured

2.5.3 Large scale production

Currently, insect production is costly and must compete with conventional feed ingredients like soybean meal. This is largely due to the cost of labour and implementation of automation but also part of the issue is related to the cost of the feed substrate (Van Huis, 2020). Organic side streams, such as food waste, may be an option, but laws and regulations may limit their use (Van Huis, 2020). The question comes up as to what can be used. Enterra Feed Corporation (Maple Ridge, B.C.) has been approved to use pre-consumer food waste such as urban and catering waste to raise BSFL but these sources are not suitable for other species of insect. Not all side streams work; crickets grown on straw were shown to have high mortality (Lundy and Parella, 2015). Using insects as a feed results in the addition of another conversion cycle: using organic products to produce insects (Van Huis 2020). With interest in lowering costs associated with producing animals there is interest in making feed ingredients with low cost or directly usable for production animals (Van Hal et al., 2019). Insects could be grown on substrates with little to no value for animal production and be cost competitive to conventional feed ingredients. Insects grown on substrates that are too toxic for feed or food, such as DON-contaminated grain could help alleviate this issue and reduce potential costs. This combined with insects requiring low land area, 1 kg of YML requires less than half the land area required to produce 1 kg of chicken (Miglietta et al., 2015), could allow insects to be competitive to feed ingredients like fishmeal and soybean meal.

Progress towards increased efficiency and productivity with insect rearing is possible with investment and genetic improvement. An eight-year study by Morales-Ramos et al. (2019) was able to improve growth rate, fecundity, FCR, larvae size, and pupa size in YML, although at the cost of survival. And since insects have short life spans there are more frequent opportunities to select for desired traits. It also may be possible to select for insects that can survive on certain substrates (Fowles and Nansen, 2019). Overall, the insect industry is in its early stages of development and will continue to improve with time and research.

2.5.4 Rearing yellow mealworm larvae on mycotoxin-contaminated feed

Recent studies have assessed how exposure to mycotoxins such as DON, ZEN, AF, T-2 toxin, and ochratoxin A affects the growth, breeding, and behaviour in YML (Van Broekhoven et al., 2017; Niermans et al., 2019; Ochoa-Sanabria et al., 2019). Van Broekhoven et al. (2017)

and Ochoa-Sanabria et al. (2019) reported no differences in mortality, growth and weights in YML reared on wheat contaminated with up to 8,000 μg/kg and 12,000 μg/kg DON respectively. Van Broekhoven et al. (2017) did not detect DON in the larvae while Ochoa-Sanabria et al. (2019) detected approximately 130 μg/kg regardless of the DON concentration in the diet. This was likely due to experiment by Ochoa-Sanabria et al. (2019) taking place over a longer time period (33.6 vs 15 days). Van Broekhoven et al (2017) and Ochoa-Sanabria et al. (2019) did not observe changes in mortality of YML produced on DON-contaminated wheat. There may be some impact of DON on YML as Janković-Tomanić et al. (2019) found that feeding 8,000, 16,000 and 25,000 μg/kg DON resulted in reduced weight of larvae after 2 weeks compared to larvae grown on the control diet (101.4, 113.2, and 116.34 vs 126.2 mg). Yellow mealworm larvae grown on the 25,000 μg/kg DON diet also had a lower protein content than the control (1.50 vs 1.83 mg/g). It was also found that YML grown on DON-contaminated diets had reduced locomotor activity, with reduced travel distance, speed, and time in movement (Janković-Tomanić et al. 2019). Part of the reason for this may have been due to the short duration of the experiment at 14 days, which may not have allowed the YML to adapt and recover from the initial exposure to DON.

The mechanisms pertaining to how the YML metabolize DON and other mycotoxins are currently unknown. Some residual mycotoxins are present in the frass of the larvae ranging from less than 10% (Ochoa-Sanabria et al., 2019) to 14% (Van Broekhoven et al., 2017) DON in naturally contaminated diets. Yellow mealworms produce enzymes such as cellulase, chitinase, licheninase, and β-glucosidase which have known catalytic effects on DON (Genta et al., 2006). Soil microbes such as *Pseudomonas* sp. Y1 and *Lysobacter* sp. S1 have also been shown to transform DON into 3-*epi*-DON which is less toxic (Zhai et al., 2019). Cytochrome P450 monooxygenases have been suggested as an important mechanism for the oxidation-reduction reactions involved with detoxification of mycotoxins (Scott and Wen, 2001). Yellow mealworms could possibly deal with DON utilizing a mixture of enzymes they produce complemented with microbial degradation of DON to maximize efficiency.

2.5.5 Use in animal feed

Insects for use in animal feed is a new practice for the modern animal industry. In Canada, only BSFL produced by Enterra Feed Corporation (Maple Ridge, B.C.) are approved for

use in aquaculture and poultry production. Yellow mealworms have not been approved for use in feed for animal production in Canada. Research has shown that dietary inclusion of YML does have positive effects in animal production.

In aquaculture, YML in diets has had considerable success. Defatted YML were included in red seabream diets at up to 65% of the diet (Ido, et al., 2019). Fish grown on diets containing YML meal had improved performance compared to those grown on diets containing 65% fishmeal. Body weight gain in the diet containing 65% YML was 12.8 g compared to 7.2 g for those fed 65% fishmeal. The addition of the YML fat into diets did however result in a reduction in growth rates relative to the diets containing defatted meal (Ido et al., 2019). A study by Jeong et al. (2020) found in rainbow trout fry that a YML inclusion of 14% resulted in optimal weight gain at 1,115 g compared to 943 g fed the control diet. Yellow mealworm larvae inclusion also resulted in a reduction in feed conversion ratios and feed intake indicating an increase in efficiency. Another study found that replacing fishmeal with defatted YML improved growth and immunity of pacific white shrimp (Motte et al., 2019). The study found that a 50% replacement of fishmeal with defatted YML resulted in the greatest improvement of biomass at 79.0 g compared to 63.8 g per tank in the control. Feed-to-gain ratios were also improved dropping from 1.588 in the control to approximately 1.287 in the diets containing YML (Motte et al., 2019). Yellow mealworm larvae reduced protein digestibility in the omnivorous Nile tilapia, likely due to the chitin content (Sánchez-Muros et al., 2016). Overall YML could be used to replace fishmeal in many aquatic species diets.

The use of YML in pig diets also have had positive results. Current studies have focused on weaned pigs. Jin et al. (2016) found that supplementation of YML up to 6% increased linearly FI, ADG, and body weight during days 0-14 and improved body weight and FI during days 14-35. Nitrogen retention, DM, and CP digestibility increased linearly with the concentration of YML. Inclusion of YML did not change IgA and IgG indicting no effect on immune response (Jin et al., 2016). Another study by Meyer et al. (2020) found growth parameters (weight, FI, FCR) in 5-week-old pigs were unaffected when fed diets containing 0, 5, or 10% YML. Inclusion of 10% YML did increase activation of genes, related to the urea cycle which was likely in response to the 10% reduced ileal digestibility of amino acids in pigs on that treatment relative to the control. As a result, inclusion of YML can also be beneficial for inclusion in swine diets, although more research is required for pigs as they get older.

Insects have been suggested as a high-quality alternative protein source for poultry. Other benefits include chitin, lauric acid and antimicrobial peptides present in insects as these have also been shown to improve chicken health (Gasco et al., 2018). Most research has pertained to broilers. Research has been inconsistent for poultry performance when grown on insect meal. Elahi et al. (2020) found that inclusion of YML meal at 4% performed the same as those fed a conventional diet. Ballitoc and Sun (2013) found that inclusion of YML meal at 0.5, 1, 2 and 10% increased FI while lowering the feed conversion ratio. Research by Bovera et al. (2015) found lower FI in broilers from day 46-62 with the inclusion of YML meal at 29.6% but the FCR of birds were also lower during that same period. No studies have found any impacts on carcass weight and meat yields (Ballitoc and Sun, 2013; Bovera et al., 2015; Biasato et al., 2018; Elahi et al., 2020). Yellow mealworm larvae meal has not impacted meat or carcass quality when fed to broilers (Dabbou et al., 2019). Broilers, in a study by Biasato et al. (2017), showed a quadratic response when fed diets containing 0, 5, 10 and 15% YML in erythrocyte counts, peaking at 10% YML inclusion. Albumin concentrations decreased linearly with increasing YML inclusion. Gut histology and morphological results were found not to differ with inclusion of YML. Feed intake and body weight increased in birds fed diets containing YML while FCR was not affected (Biasato et al., 2017). Another study by Biasato et al. (2019) found that inclusion of YML at 10% or higher resulted in a reduction in *Firmicutes spp.* and an increase in species such as *Clostridium*, *Sutterela*, and *Alistipes* present in the ceca. *Firmicutes* may have an impact on bird health and feed digestion so this change could be negative. The increase in *Clostridium*, *Sutterela*, and *Alistipes* are positive for bird health as they have known positive effects on bird health. Overall, the study concluded that YML inclusion above 10% had an effect on the cecal microbiota and intestinal mucin dynamics, but that more work was required to determine if this would be a positive or negative change (Biasato et al., 2019). With these results from feeding experiments and the results from growing YML on DON-contaminated wheat it might be possible to produce a cost-effective high-quality feed ingredient for use in animal production competitive with fishmeal and soybean meal.

2.6 Hypothesis and Objectives

The objective of this project was to determine if yellow mealworm larvae reared on DON-contaminated wheat could be included in the diets for broiler chickens as a safe feed

ingredient for animal production. Yellow mealworm larvae were grown on either low and high DON-contaminated wheat (<1,000 µg/kg or 30,730 µg/kg respectively), then analyzed nutritionally and for the presence of mycotoxins. The YML were included in broiler diets that were then assessed for growth rates, feed intake, meat yield, organ weight and hematological parameters.

The hypotheses of the project were:

- Yellow mealworm larvae will convert DON-contaminated wheat into a high-quality feed ingredient with less than 1,000 µg/kg suitable for poultry as they can break down DON.
- Broilers grown on diets containing YML meal (fed wheat not contaminated with DON) will perform comparably to conventional diets as insects are highly nutritious and easily digestible.
- Mealworm meals produced from low and high DON wheat will not affect broiler growth, feed intake, meat yield, organ weight and hematological parameters.

CHAPTER 3:

YELLOW MEALWORM LARVAE (*TENEBRIO MOLITOR*) REARED ON DEOXYNIVALENOL-CONTAMINATED WHEAT AS A FEED INGREDIENT FOR BROILER CHICKENS

3.1 Introduction

Fusarium contamination of crops is a global problem for food and feed production. *Fusarium* is a genus of fungi of which more than 16 species cause a disease in cereal crops called FHB (Dweba et al., 2017). *Fusarium* species produce a variety of mycotoxins, of which DON and ZEN that have toxic effects in humans and animals are the most prevalent (Ferrigo et al., 2016). In 2016, a large outbreak of FHB occurred in Western Canada which resulted in an estimated one billion dollars worth of damage associated with lower grades being assigned due to the presence of FDK and/or mycotoxins such as DON (Canadian Grain Commission, 2020). With current global warming effects related to increasing temperatures and unstable weather, major outbreaks of FHB are likely going to be more frequent in the future, especially during conditions with high humidity (Dweba et al., 2017).

Fusarium graminearum is the most prevalent species in Western Canada and is harmful due to its production of mycotoxins such as DON, 3ADON, and 15ADON (Tittlemier et al., 2019). Due to the effects DON can have in animals, the CFIA have set limits on the concentrations allowed in food and feed. The CFIA have further proposed limits on mycotoxins in single feed ingredients which would limit DON concentrations to a maximum of 10,000 µg/kg (CFIA, 2018). Any crops measuring above these concentrations would be condemned as salvage which has no economic value representing a loss to producers or would be blended into crops with low degrees of contamination. If large quantities of salvage crops are produced due to outbreaks of fungal disease, blending will not be an option, crops will be burnt or buried which can have negative environmental effects.

One novel method of utilizing DON-contaminated crops could be to rear insects such as YML (*Tenebrio molitor*) on them. Studies by Van Broekhoven et al. (2017) and Ochoa-Sanabria et al. (2019) fed YML DON-contaminated wheat, at concentrations up to 12,000 µg/kg, and reported no impacts on larval survival, fecundity, or growth. Van Broekhoven et al. (2017) and Ochoa-Sanabria et al. (2019) reported excretion of DON in frass at 14 to 41% and 6 – 15% of ingested concentrations respectively. Van Broekhoven et al. (2017) did not detect the presence of any mycotoxins in the YML reared on DON-contaminated wheat, while Ochoa-Sanabria detected approximately 130 µg/kg, which was still much less than the ingested concentrations indicating that they may have some means of metabolizing DON.

Yellow mealworms in their larval stage are rich in CF and CP at 34.4% and 50.2%

respectively (Ochoa-Sanabria et al., 2019). Yellow mealworm larvae also have an amino acid profile similar to soybean meal, with the exception of methionine, which could make them excellent as both a protein and energy source when included in animal diets (Bovera et al., 2015). These observations indicate that YML may have potential when reared on DON-contaminated crops to be a cost effective, highly nutritious, and safe ingredient for use in poultry diets. The aim of this study was to determine if YML raised on low or high DON wheat could be used as a feed ingredient for broiler diets, and investigate any effects that may occur in growth, survival, and efficiency.

3.2 Materials and methods

3.2.1 Wheat samples

Two sources of Canadian Western Red Spring wheat were purchased from producers in Saskatchewan, Canada that had low or high infection with *Fusarium*. Mycotoxin panel testing was conducted at Prairie Diagnostic Services (Saskatoon, Canada) using HPLC-tandem MS. The mycotoxin panel included mycotoxins DON, Nivalenol (NIV), 3ADON, and 15ADON. The low and high DON wheat (LDW and HDW) had <1,000 µg/kg and 30,730 µg/kg DON respectively.

3.2.2 Yellow mealworm larval production

Recently eclosed *Tenebrio molitor* beetles sourced from Bug Order Inc. (Morinville, Canada) were placed into 50 x 32 x 15 cm bins containing either LDW or HDW which was fed whole kernel. Beetles were left to breed and lay eggs which were then hatched into larvae that consumed the wheat. Larvae at a minimum weight of approximately 110 mg were harvested from bins once per week. The larvae were fasted for 24 hours to empty their gastrointestinal tract, rinsed with water to remove dust, and were euthanized by freezing and stored at -20ºC. The rearing room was maintained with an 8-hour photoperiod, with a temperature between 22-26ºC and with a minimum relative humidity of 50%. All bins had paper towels which were misted with water 3 times per week to maintain humidity. The frozen mealworm larvae were spread thinly on trays and oven-dried at 110ºC for 40 minutes. The dried larvae were then ground using a Cuisinart Model CH-4DCC food processor (Stamford, Connecticut) to produce mealworm meal and stored at -20ºC.

3.2.3 Mycotoxin and nutritional analysis of yellow mealworm larvae meal

The YML meal was analysed for mycotoxins as described by Ochoa-Sanabria et al. (2019) at Prairie Diagnostic Services (Saskatoon, Canada). Two grams of sample was combined with HPLC reagent grade 85% acetonitrile plus 15% distilled-deionized water (v/v) filtered through a Barnstead Nanopure water purification system. The mixture was stirred continuously for 10 minutes, then filtered through Whatman 41 150 mm filter paper. Three millilitres of supernatant were filtered through a MycoSEP 225 Trich cleanup cartridge, dried with nitrogen and reconstituted with 50% methanol/50% 10 mM aqueous ammonium acetate. The sample was filtered through a 0.45 µm syringe. A 10 uL sample of filtered material was added to 990 uL of the 85/15 acetonitrile/distilled-deionized filtered water. This final sample was injected into the LC/MS system. The detection limits for DON, NIV, 3ADON, 15ADON respectively were 4, 64, 16, and 16 µg/kg respectively.

Crude protein of the YML meals was determined using the Dumas-Combustion method by placing duplicate 0.11 g samples in gel capsules and combusting at 800ºC, (AOAC, 1997; method 990.03). The amino acid profile was determined by the nutrition lab in the Faculty of Agricultural and Food Sciences at University of Manitoba, (Winnipeg, Canada; AOAC, 1995; method 994.12) utilising the S2100 Sykam amino acid analyser (Eresing, Germany). Amino acid digestibility values by Matin (2019) were used to estimate those of the YML meal.

Crude fat was analysed using a Goldfisch extraction apparatus model 3500 (Kansas City, Missouri) by processing and extracting 1.3 g of samples in duplicate for 5 hours using the ethyl ether extraction gravimetric method (AOAC, 2000; method 920.39). The fatty acid profiles were determined at NRC by Linnaeus Plant Science Inc (Saskatoon, Canada) in duplicate by adding 55 mg of sample to a 16 x 100 mm glass screw cap tube containing 1.5 mL 1.5% sulfuric acid in 100% methanol (v/v) and 0.4 mL hexane. Tubes were capped tightly and incubated overnight in an 80ºC heat block, mixed occasionally. Tubes were cooled and 1 mL of 0.9% NaCl and 2.5 mL hexane were added. Samples were vortexed briefly and centrifuged at 2000 rpm in an Allegra 25R centrifuge (Indianapolis, Indiana) for 5 minutes at room temperature. Two hundred microlitres of hexane phase was transferred to gas chromatography (GC) vials and run on GC Agilent 6890N equipped with a flame ionization detector and DB23 column (0.25mm x 30 m,

0.25 um thickness; J&W Scientific, Folsom, California). A fatty acid standard mix C8-24 was used to verify peak identities.

The YML meals were also analysed for calcium, magnesium, phosphorus, potassium, sodium, copper, iron, manganese and zinc at Central Testing Laboratory Ltd. (Winnipeg, Canada; AOAC, 1996; method 985.01; AOAC 1969; method 968.08; AOAC, 1951 method 935.13). Moisture was analyzed in 2.0 g samples in aluminum dishes that were in an oven at 135ºC for 2 hours. The samples were then cooled, sealed, and weighed again to determine the loss from drying (AOAC, 1990; method 930.15). Acid detergent fibre (ADF) was determined by running duplicate 0.5 g samples in an ANKOM^{200} fiber analyzer (New York, USA; AOAC, 1977; method 973.18). The remaining portions of the samples were used to estimate CP and determine acid detergent insoluble nitrogen (ADIN). Chitin was calculated from ash-free ADF and ADIN as described by Marono et al. (2015).

3.2.4 Broiler experiment

A research exemption was obtained from the Animal Feed Division of the Canadian Food Inspection Agency to include the YML meals in the diets. Approval was obtained from the Animal Research Ethics Board at the University of Saskatchewan to conduct this experiment.

3.2.4.1 Diets

Diets were formulated (Table 3.1) to meet or exceed Ross 708 2019 performance standards (Aviagen, 2019) using the results of the analyses and produced as a mash. Corn that contained undetectable concentrations of DON and soybean meal made up the majority of the diets. The treatments consisted of the control containing no insect meal (CD), containing 5% YML grown on LDW (LMD), and 5% YML grown on HDW (HMD). Diets were fed in two phases with a starter/grower fed for the first three weeks (days 1-21) and a finisher fed during the last two weeks (days 21-35). The finisher included titanium dioxide as a marker to determine CP retention and DM digestibility. Nutrient composition (DM, moisture, CP, calcium, phosphorus, and sodium) of the diets were determined by Central Testing Laboratory LTD. (Winnipeg, Canada).

Table 3.1. Ingredients (% as fed) and nutrient composition (% as fed) of experimental diets[1]

Ingredients	**Starter diets (days 1-21)**			**Finisher diets (days 21-35)**		
	CD	**LMD**	**HMD**	**CD**	**LMD**	**HMD**
Corn	60.081	56.753	57.045	65.965	67.805	68.100
Soybean meal	29.655	32.624	32.334	22.861	21.178	20.566
Meat meal	5.973	-	-	5.942	1.968	2.238
YML LDW	-	5.000	-	-	5.000	-
YML HDW	-	-	5.000	-	-	5.000
Dicalcium phosphate	1.543	1.744	1.755	0.291	1.008	0.969
Calcium carbonate	-	1.331	1.128	0.409	0.807	0.779
Canola oil	1.000	1.000	1.000	2.864	0.422	0.546
Poultry vit/min premix	0.500	0.500	0.500	0.500	0.500	0.500
Titanium dioxide	-	-	-	0.300	0.300	0.300
Dl-methionine	0.368	0.356	0.355	0.274	0.279	0.280
Lysine HCl	0.315	0.345	0.258	0.197	0.228	0.224
Salt	0.177	0.265	0.349	0.180	0.288	0.284
Choline chloride	0.165	0.165	0.165	0.150	0.150	0.150
L-threonine	0.149	0.115	0.111	0.068	0.067	0.064
L-isoleucine	0.038	-	-	-	-	-
Valine	0.035	-	-	-	-	-
Nutrient composition (% as fed)						
AME	3105	3111	3101	3200	3200	3200
DM	87.60	87.32	87.56	87.26	87.00	87.54
CP	21.66	20.92	21.25	18.53	18.74	17.52
Crude fat	4.05	5.42	5.28	5.92	5.24	5.24
Digestible lysine	1.28	1.28	1.28	1.03	1.03	1.03
Digestible methionine	0.67	0.66	0.66	0.55	0.55	0.55
Digestible cysteine + methionine	0.95	0.95	0.95	0.80	0.80	0.80
Digestible threonine	0.86	0.86	0.86	0.69	0.69	0.69
Digestible tryptophan	0.22	0.24	0.24	0.19	0.19	0.18
Calcium	1.12	1.02	1.01	1.04	0.87	1.00

Available phosphorus	0.40	0.40	0.40	0.64	0.48	0.48
Sodium	0.14	0.18	0.16	0.13	0.15	0.17

Abbreviations: YML LDW, Yellow mealworm larvae reared on low DON wheat (<1,000 µg/kg DON); YML HDW, yellow mealworm larvae reared on high DON wheat (30,730 µg/kg DON); DM, dry matter; CP, crude protein.

[1] Three dietary treatments: CD = control diet; LMD = 5% inclusion yellow mealworm reared on low DON wheat; HMD = 5% inclusion yellow mealworm reared on high DON wheat.

[2] One kg premix contains 2,200,000 IU vitamin A, 440,000 IU vitamin D, 6,000 IU vitamin E, 400 mg menadione, 300 mg thiamine, 1,200 mg riboflavin, 800 mg pyridoxine, 4 mg vitamin B12, 12,000 mg niacin, 2,000 mg pantothenic acid, 120 mg folic acid, 30 mg biotin. 2,000 mg copper, 16,000 mg manganese, 160 mg iodine, 16,000 mg zinc, 60 mg selenium, 100,000 mg calcium carbonate, 125 mg antioxidant, 807,879 mg wheat middlings (DSM Nutritional Products Canada Inc. ON, Canada).

3.4.2.2 Growth performance

Seventy-five male Ross newly hatched 708 broilers were obtained from Lilydale Hatchery (Edmonton, Canada) and were randomly split into groups of five birds and placed into one of 15 cages (five replications/treatment, 46 cm high × 51 cm wide × 51 cm long) in a temperature controlled room at the University of Saskatchewan Poultry Research Center (Saskatoon, Canada). Bird numbers were reduced to four birds/treatment on day 14, birds were selected randomly. The removed birds were used to collect blood samples to test if flow cytometry could be used to measure blood differentials and H/L ratios, and if scalding affected the quality of histology slides of the duodenum, jejunum, and ileum. The room the broilers were housed in started at a temperature of 34ºC and gradually dropped down to 22.3ºC by day 28. Light intensity was initially 40 lux for a 22-hour photoperiod which was reduced to 20 hours on day 2. Light intensity was dropped to 20 lux on day eight, then to 10 lux on day nine. Birds had free access to feed (tray feeders) and water (nipple drinkers) for the duration of the trial. All mortalities and culled birds were necropsied for cause of death or morbidity by Prairie Diagnostic Services (Saskatoon, Canada).

Broiler body weight (BW, g) and feed intake (FI, g) was measured at 1, 7, 14, 21, 28, and 35 days. Average daily gain (ADG, g/day) was calculated on a per bird basis, and feed to gain ratio (F:G, g feed/g weight gain) was calculated per cage. Formulas used to calculate ADG and F:G were: ADG (period x–y) = (BW day y – BW day x)/Days period x–y and F:G = FI(period x–y) /(BW day y – BW day x).

3.2.4.3 Excreta

Excreta was collected over a period of 48 hours on days 33 and 34 of the trial. Samples were pooled, dried at 50ºC for 72 hours and ground using a Retsch ZM 100 Ultra Centrifugal Mill (Haan, Germany) using a 1,000-micron screen. This was used for titanium dioxide, CP, and DM analyses to determine dry matter digestibility and CP retention.

3.2.4.4 Haematological parameters

Blood samples were collected from 2 birds per cage and were used to prepare smears for white blood cell differentials and heterophil/lymphocyte (H/L) ratios. Samples were collected from the brachial vein using an Ethylenediamine Dipotassium Tetraacetic Acid (EDTA) anti-

coagulation tube and vacutainer (BD Vacutainer). Smears were prepared using the two-slide wedge method, where a small drop of blood was transferred onto a slide from a tube utilising a stir stick and manually smeared. Differentials were measured by Prairie Diagnostic Services (Saskatoon, Canada).

3.2.4.5 Carcass traits

Meat yield, organ weights and gut lengths were collected from all birds on day 35 of the trial. Birds were euthanized by T-61 euthanasia solution injected into the brachial vein. The birds were then scalded in 66±2ºC water for 25 seconds and feathers plucked by hand. Breasts, skin on bone-in wings, skin on bone-in drums, bone in thighs, abdominal fat pads, livers, spleens, and bursa were removed from the carcasses and weighed. The fat pads were frozen and stored at -20ºC for CF and fatty acid analysis. The proventriculus and gizzard were emptied and weighed. The lengths of the ceca and colon were all measured, emptied, and weighed. Three-centimetre sections were taken from the middle of the duodenum, jejunum, and ileum after length was measured on all birds. These sections were to be used for histology however, the COVID pandemic meant that this work was postponed indefinitely.

3.2.4.6 Fat pad analyses

Abdominal fat pads were analyzed for CF using a Goldfisch extraction apparatus model 3500 (Kansas City, Missouri) by processing and extracting 1.3 g of samples in duplicate for 5 hours using the ethyl ether extraction gravimetric method (AOAC, 2000; method 920.39). The fatty acid profile of the fat pads was analyzed at the NRC by Linnaeus Plant Science Inc (Saskatoon, Canada) in duplicate by adding 80 mg of sample to a 16 x 100 mm glass cap tube containing 2.0 mL of 3% sulfuric acid in 100% methanol (v/v) and 0.4 mL toluene. Tubes were capped and incubated on an 80ºC heat block overnight. Tubes were cooled, then 2.0 mL 0.9% NaCl and 2.0 mL hexane were added. Samples were left to settle and 200 µmL of hexane phase was transferred to GC vials and run on GC Agilent 7890N equipped with a flame ionization detector and DB23 column (0.25mm x 30 m, 0.25 um thickness; J&W Scientific, Folsom, California)

3.2.5 Crude protein and dry matter retention

Excreta and feed samples were measured for CP and dry matter retention in duplicate for TiO_2 using a protocol adapted from Myers et al. (2004). Titanium dioxide was measured by placing samples weighing 0.5 and 1.0 g for excreta and feed respectively into 250 mL macro-Kjeldahl digestion tubes. A catalyst tablet containing 3.5 g of K_2SO_4 and 0.4 g of $CuSO_4$ was added to each tube. Thirteen millilitres concentrated sulfuric acid was added and samples were digested at 420ºC for 2 hours. Samples were allowed to cool for 30 minutes, 10 mL 30% H_2O_2 (v/v) was added and left to cool for 30 minutes. Samples were transferred into 125mL Erlenmeyer flasks and distilled water was added to bring the liquid weight up to 100 g. Samples were filtered using 541 Whatman paper then transferred into cuvettes and placed into a spectrophotometer set to 410 nm to measure absorbance. A standard was made using 0.2 g TiO_2 with the same procedure and was serial diluted using 1:1 standard solution to distilled water and measured in the spectrophotometer to determine the standard curve to which the samples were compared to determine TiO_2 concentrations.

Crude protein of excreta samples was determined using the Dumas-Combustion method by placing duplicate 0.11 g samples of YML meal in gel capsules and combusting at 800ºC, (AOAC, 1997; method 990.03). Moisture in excreta was analyzed by placing 2.0 g samples into aluminum dishes and placing into a 135ºC oven for 2 hours. The samples were then cooled, sealed, and weighed again to determine loss from drying from which dry matter was calculated (AOAC, 1990; method 930.15). The equation used to determine retention was: % retention = 100 – (100 x [% marker in diet/% marker in excreta] x [% nutrient in excreta/% nutrient in diet])

3.2.6 Statistics

All data were analyzed using the MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary North Carolina). Results were analyzed as a complete randomized design using a one-way ANOVA and were tested for normality using the Shapiro-Wilk test. The Grubb's test was used to test for and remove outliers. Treatment means were compared using the Tukey-Kramer HSD test at $P< 0.05$ determining significance. All tests conducted on the YML meal used a pooled sample, thus were not statistically analyzed but the values are reported.

3.3 Results

3.3.1 Yellow mealworm larvae meal nutritional and mycotoxin analyses

The YML meals produced on LDW or HDW were analyzed for multiple mycotoxins. The only detected mycotoxin was DON which was present at 17.5 µg/kg in the YML meal produced on HDW (Table 3.2). The YML meals had similar proximate nutritional compositions (Table 3.3): DM ranged from 93.60 – 94.65%, CP and CF on an as fed basis 45.28 – 47.71% and 35.66 – 38.41% respectively. The amino acid and fatty acid profiles of the two YML meals were also similar (Table 3.4, Table 3.5). The mineral profiles of the YML meals were again similar with the exception of manganese which was 9.35 mg/kg in the larvae produced on HDW compared to 1.97 mg/kg in those reared on LDW (Table 3.6).

3.3.2 Broiler performance

There were zero mortalities in the first 14 days of the experiment after which bird number was reduced to four birds per cage from five to meet animal care requirements for space. One broiler each on the LMD and HMD diets was culled for leg issues at 21 days and 30 days, respectively bringing mortality/morbidity to 0%, 5%, and 5% for CD, LMD, and HMD respectively. Growth performance of the broilers are summarized in Tables 3.7, 3.8, 3.9, and 3.10. Feed intake was reduced from days 8-14 ($P = 0.002$) at 261.8g in HMD compared to 292.0 and 293.6g in CD and LMD respectively. Feed intake 1-35 ($P = 0.003$) in birds fed HMD at 2469.0g compared to 2709.1 and 2762.4g in CD and LMD (Table .3.7) Feed intake tended to be reduced in birds fed HMD during days 15-21 ($P = 0.094$) and 29-35 ($P = 0.074$). Live weights of birds (Table 3.8) were reduced in birds fed HMD on day 14 ($P = 0.029$) with HMD averaging 344.2 while CD and LMD averaged 370.2 and 373.4g. There was a tendency to be lighter on day 21 ($P = 0.082$) although final body weights were not different ($P = 0.204$). Average daily gain was reduced in broilers fed HMD on day 8-14 at 30.6g while CD and LMD averaged 33.4 and 34.6g ($P = 0.017$; Table 3.9). The diets did not have an impact on F:G ratio in the broiler chickens ($P > 0.10$; Table 3.10).

Table 3.2. Mycotoxin concentrations of dried, ground, yellow mealworm larvae (µg/kg) reared on low or high deoxynivalenol (DON) wheat

	Treatments[1]	
Parameter	**Low (<1,000 µg/kg DON)**	**High (30,730 µg/kg DON)**
Deoxynivalenol	<4	17.5
3+15 Acetyldeoxynivalenol	<16	<16
Nivalenol	<64	<64

[1]Pooled sample of mealworm meal

Table 3.3. Proximate analyses of yellow mealworm larvae grown on low or high DON wheat (% as fed basis)

	Treatments	
Parameter	**Low (<1,000 µg/kg DON)**	**High (30,730 µg/kg DON)**
Dry Matter	94.65	93.60
Crude Protein[1]	45.28	47.71
Crude Fat	38.41	35.66
ADF	6.94	7.71
ADIN	3.69	4.18
Chitin[2]	3.25	3.53

Abbreviations: DON (Deoxynivalenol)
[1] Crude protein was analyzed using the Dumas combustion method
[2] Chitin calculated based on Marono et al. (2015)

Table 3.4. Amino acid and estimated digestible amino acid profile of yellow mealworm meal produced on low or high deoxynivalenol (DON) wheat used (% as fed basis)

	Amino acid profile			Digestible amino acid profile	
Parameter	**Low (<1,000 µg/kg DON)**	**High (30,730 µg/kg DON)**	**Digestibility (%)[1]**	**Low (<1,000 µg/kg DON)**	**High (30,730 µg/kg DON)**
Aspartate	3.638	4.009	93.10	3.387	3.732
Threonine	1.682	1.852	92.05	1.548	1.705
Serine	2.110	2.351	89.77	1.894	2.110
Glutamine	5.293	5.764	93.27	4.937	5.376
Proline	2.783	2.920	90.97	2.532	2.656
Glycine	2.315	2.412	-	2.315	2.412
Alanine	3.770	4.039	93.15	3.512	3.762
Cysteine	0.371	0.399	75.79	0.281	0.302
Valine	2.947	3.004	72.73	2.143	2.185
Methionine	0.573	0.617	92.13	0.528	0.568
Isoleucine	2.041	2.069	92.06	1.879	1.905
Leucine	3.316	3.534	93.09	3.087	3.290
Tyrosine	3.294	3.560	92.61	3.051	3.297
Phenylalanine	1.635	1.794	91.68	1.499	1.645
Histidine	2.857	3.661	90.92	2.598	3.329
Lysine	2.408	2.668	91.39	2.201	2.438
Arginine	2.469	2.651	94.64	2.337	2.509
Tryptophan	0.440	0.491	99.13	0.436	0.487

Abbreviations: DON (deoxynivalenol)
- = no value/assumed 100%
[1]Digestibility values from Matin (2019)

Table 3.5. Fatty acids detected in the yellow mealworm larvae grown on low or high deoxynivalenol (DON) wheat (% of crude fat)

		Treatments	
Fatty acid name	**Parameter**	**Low (<1,000 µg/kg DON)**	**High (30,730 µg/kg DON)**
Lauric acid	C12:0	0.36	0.54
Myristic acid	C14:0	4.37	4.96
Myristoleic acid	C14:1 n-5	0.24	0.37
Tetradecadienoic acid	C14:2 n-3	0.15	0.17
Palmitic acid	C16:0	19.10	18.76
Palmitovaccenic acid	C16:1 n-5	1.10	1.07
Palmitoleic acid	C16:1 n-7	2.47	1.99
Hexadecadienoic acid	C16:2 n-4	0.19	0.16
Stearic acid	C18:0	2.39	2.73
Oleic acid	C18:1 n-9	48.41	49.13
Vaccenic acid	C18:1 n-11	0.08	0.08
	Unknown	0.22	0.21
Linoleic acid	C18:2 n-6	20.04	19.07
Linolenic acid	C18:3 n-3	0.47	0.43
Arachidic acid	C20:0	0.23	0.23
Eicosenoic acid	C20:1 n-9	0.08	0.06
h-γ-Linolenic acid	C20:2 n-6	0.10	0.12

Table 3.6. Minerals detected in yellow mealworm larvae grown on low or high deoxynivalenol (DON) wheat on an as fed basis

	Treatments	
Parameter	**Low (<1,000 µg/kg DON)**	**High (30,730 µg/kg DON)**
Calcium (%)	0.04	0.04
Phosphorus (%)	0.74	0.68
Magnesium (%)	0.22	0.20
Potassium (%)	0.79	0.75
Sodium (%)	0.07	0.04
Copper (mg/kg)	15.46	14.42
Iron (mg/kg)	50.77	50.34
Manganese (mg/kg)	1.97	9.35
Zinc (mg/kg)	112.66	119.50

Table 3.7. Effect of the dietary inclusion of yellow mealworm larval meal produced on low or high DON wheat on feed intake of the broiler chickens (g)

	Treatment[1,2]				
Period	**CD**	**LMD**	**HMD**	**SEM**	**P Value**
Day 1-7	136.3	137.2	135.1	2.26	0.752
Day 8-14	292.0[a]	293.6[a]	261.8[b]	5.41	0.002
Day 15-21	485.2	510.6	454.1	16.62	0.094
Day 22-28	742.1	768.3	710.3	22.34	0.223
Day 29-35	1,053.6	993.8	951.8	28.48	0.074
Day 1-35	2,709.1[a]	2,762.4[a]	2,469.0[b]	45.43	0.003

Abbreviations: DON (deoxynivalenol); CD (Control Diet); LMD (Diet containing mealworm meal grown on <1,000 µg/kg DON wheat); HMD (Diets containing mealworms grown on 30,730 µg/kg DON wheat)
[1]Mean of replicates (n=5) per treatment
[2]Presented on a per bird basis
Different letters indicate significance: $P < 0.05$

Table 3.8. Effect of the dietary inclusion of yellow mealworm larval meal produced on low or high DON wheat on body weight of broiler chickens (g)

	Treatment[1,2]				
Period	**CD**	**LMD**	**HMD**	**SEM**	**P Value**
Day 0	40.0	39.4	38.4	0.86	0.775
Day 7	136.0	131.4	130.3	2.37	0.236
Day 14	370.2[a]	373.4[a]	344.2[b]	7.31	0.003
Day 21	723.8	788.9	698.6	26.47	0.082
Day 28	1,232.9	1,252.1	1,173.5	31.69	0.229
Day 35	1,908.7	1,877.0	1,786.1	47.19	0.204

Abbreviations: DON (deoxynivalenol); CD (Control Diet); LMD (Diet containing mealworm meal grown on <1,000 µg/kg DON wheat); HMD (Diets containing mealworms grown on 30,730 µg/kg DON wheat)
[1]Mean of replicates (n=5) per treatment
[2]Presented on a per bird basis
Different letters indicate significant difference: $P < 0.05$

Table 3.9. Effect of the dietary inclusion of yellow mealworm larval meal produced on low or high DON wheat on average daily gain of the broiler chickens (g)

	Treatment[1,2]				
Period	**CD**	**LMD**	**HMD**	**SEM**	**P Value**
Day 1-7	13.7	13.1	13.1	0.31	0.336
Day 8-14	33.4[a]	34.6[a]	30.6[b]	0.86	0.017
Day 15-21	50.5	59.4	49.2	3.43	0.154
Day 22-28	72.7	66.2	67.8	2.68	0.228
Day 29-35	96.5	89.3	87.5	2.93	0.229
Day 1-35	53.3	52.5	49.9	1.33	0.200

Abbreviations: DON (deoxynivalenol); CD (Control Diet); LMD (Diet containing mealworm meal grown on <1,000 µg/kg DON wheat); HMD (Diets containing mealworms grown on 30,730 µg/kg DON wheat)
[1]Mean of replicates (n=5)
[2]Presented on a per bird basis
Different letters indicate significance: $P < 0.05$

Table 3.10. Effect of the dietary inclusion of yellow mealworm larval meal produced on low or high DON wheat on feed to gain ratios of the broiler chickens

	Treatment[1]				
Period	**CD**	**LMD**	**HMD**	**SEM**	**P Value**
Day 1-7	1.422	1.497	1.485	0.0288	0.190
Day 8-14	1.247	1.215	1.225	0.0175	0.438
Day 15-21	1.375	1.252	1.286	0.0481	0.213
Day 22-28	1.480	1.609	1.500	0.0874	0.593
Day 29-35	1.562	1.594	1.552	0.0301	0.606
Day 1-35	1.448	1.443	1.440	0.0134	0.413

Abbreviations: DON (deoxynivalenol); CD (Control Diet); LMD (Diet containing mealworm meal grown on <1,000 µg/kg DON wheat); HMD (Diets containing mealworms grown on 30,730 µg/kg DON wheat)
[1]Mean of replicates (n=5) per treatment

3.3.3 Meat and organs

The diets did not influence meat yield, organ lengths or weights in the male broilers (Table 3.11 and 3.12; $P > 0.10$), although fats pads (g) of birds fed the CD tended to be heavier than birds fed LMD or HMD ($P = 0.055$). Gizzard weights (% BW) had a tendency to be heavier relative to body weight of broilers fed HMD (Table 3.11). Four females were removed from analysis in total: one from CD and three from HMD.

3.3.4 Abdominal fat pads

The results for the CF and fatty acid profiles of the broiler chickens are displayed in Table 3.13. The CF levels in the fat pads did not differ between treatments ($P = 0.879$). Lauric acid (C12:0) was present at 0.049 and 0.047% in the fat pad of broiler fed LMD and HMD compared to 0.004% in CD ($P = 0.0002$). Myristic acid (C14:0) was 0.954 and 0.959% in broilers fed LMD and HMD with 0.442% in in fat of CD ($P < 0.0001$). Myristoleic acid (C14:1 n-5) was present in higher levels in the fat pads of broilers fed LMD and HMD at 5.129 and 5.682% compared to 0.144 in CD ($P < 0.001$). Palmitovaccenic acid (C16:1 n-5) was increased in the fat pads of broilers fed LMD and HMD at 0.544 and 0.559% compared to 0.411% in CD ($P = 0.0005$). Vaccenic acid (C18:1 n-11) was present in high levels in CD at 2.124% compared to 1.566 and 1.657% in LMD and HMD respectively ($P < 0.0001$). Linolenic acid (C18:3 n-3) was present in the fat pad of CD at 1.643% compared to 0.808 and 0.882% in LMD and HMD ($P < 0.0001$). Arachidic acid (C20:0) was increased in CD at 0.123% relative to LMD (0.101%) and HMD (0.103%; $P = 0.0002$). Eicosenoic acid (C20:1 n-9) was also increased in CD at 0.374% compared to LMD and HMD at 0.280 and 0.269% ($P < 0.0001$). Eicosadienoic acid (C20:2 n-6) was present in higher levels in fat pads of broilers fed CD (0.249%) compared to LMD (0.221) and HMD (0.222%; $P < 0.05$). Stearic acid (C18:0) had a tendency to be higher in the fat pads of broilers fed LMD compared to HMD ($P = 0.078$).

3.3.5 Hematological parameters

White blood cell differentials and H/L ratios (Table 3.14) did not differ between treatments ($P > 0.10$).

Table 3.11. Effects of dietary inclusion of yellow mealworm larval meal produced on low or high DON wheat on meat yield and organs weights of male broiler chickens

	Treatment[1]				
Parameter	**CD (n=19)**	**LMD (n=19)**	**HMD (n=16)**	**SEM**	**P Value**
Body weight (BW) (g)	1,916.6	1,886.6	1,789.4	59.04	0.329
Breasts (g)	434.7	453.8	414.7	21.37	0.470
Breasts (% BW)	22.6	23.8	23.0	0.54	0.263
Thighs (g)	200.0	192.8	186.5	6.96	0.426
Thighs (% BW)	10.4	10.2	10.4	0.14	0.419
Drums (g)	176.1	175.0	166.4	5.15	0.392
Drums (% BW)	9.2	9.3	9.3	0.14	0.835
Wings (g)	145.6	145.7	141.3	4.37	0.749
Wings (% BW)	7.6	7.7	7.9	0.11	0.158
Fat pad (g)	21.9	17.3	17.5	1.48	0.055
Fat pad (% BW)	1.15	0.92	0.97	0.077	0.103
Bursa (g)	3.8	3.6	3.6	0.22	0.734
Liver (g)	46.7	45.4	42.2	1.52	0.131
Liver (% BW)	2.45	2.42	2.37	0.067	0.683
Spleen (g)	1.9	1.7	1.9	0.10	0.270
Proventriculus (g)	6.8	6.6	6.7	0.20	0.655
Gizzard (g)	22.5	23.6	23.5	0.56	0.355
Gizzard (% BW)	1.18	1.28	1.33	0.044	0.092
Ceca (g)	6.3	6.2	6.5	0.24	0.751
Colon (g)	2.1	2.2	2.3	0.19	0.778

Abbreviations: DON (deoxynivalenol); CD (Control Diet); LMD (Diet containing mealworm meal grown on <1,000 µg/kg DON wheat); HMD (Diets containing mealworms grown on 30,730 µg/kg DON wheat)
[1]Mean of replicates
Different letters indicate significance: $P < 0.05$
n=number of birds

Table 3.12. Effects of dietary inclusion of yellow mealworm larval meal produced on low or high DON wheat on gastrointestinal tract lengths of male broiler chickens

	Treatment[1]				
Parameter	**CD (n=19)**	**LMD (n=19)**	**HMD (n=16)**	**SEM**	**P Value**
Duodenum Length (cm)	23.7	23.6	23.6	0.47	0.963
Jejunum Length (cm)	61.2	61.7	61.4	1.27	0.964
Ileum length (cm)	63.8	63.9	63.2	1.17	0.427
Colon Length (cm)	30.1	30.9	29.9	0.70	0.535
Ceca Length (cm)	5.7	6.1	6.2	0.30	0.495

Abbreviations: DON (deoxynivalenol); CD (Control Diet); LMD (Diet containing mealworm meal grown on <1,000 µg/kg DON wheat); HMD (Diets containing mealworms grown on 30,730 µg/kg DON wheat)
[1]Mean of replicates
n=number of birds

Table 3.13. Effect of dietary inclusion of yellow mealworm larval meal produced on low or high DON wheat on crude fat (% as is) and fatty acid profile (% as is) of fat pads of broiler chickens

		Treatment[1]				
Parameter		**CD**	**LMD**	**HMD**	**SEM**	**P Value**
Crude fat (% as is)		86.2	85.4	85.7	1.17	0.879
Lauric acid	C12:0	0.004[b]	0.049[a]	0.047[a]	0.0016	0.0002
Myristic acid	C14:0	0.442[b]	0.954[a]	0.959[a]	0.0310	<0.0001
Myristoleic acid	C14:1 n-5	0.144[b]	0.190[a]	0.169[a]	0.0097	0.019
Tetradecadienoic acid	C14:2 n-3	0.108	0.107	0.113	0.0043	0.506
Palmitic acid	C16:0	19.466	20.091	20.248	0.4312	0.425
Palmitovaccenic acid	C16:1 n-5	0.411[b]	0.544[a]	0.559[a]	0.0209	0.0005
Palmitoleic acid	C16:1 n-7	5.689	5.129	5.682	0.2241	0.170
Hexadecadienoic acid	C16:2 n-4	0.088	0.101	0.104	0.0053	0.113
Stearic acid	C18:0	4.125	4.455	3.936	0.1473	0.078
Oleic acid	C18:1 n-9	37.780	37.040	36.082	0.5672	0.148
Vaccenic acid	C18:1 n-11	2.124[a]	1.566[b]	1.657[b]	0.0635	<0.0001
	Unknown	0.089[c]	0.119[a]	0.105[b]	0.0037	0.0003
Linoleic acid	C18:2 n-6	12.570	13.686	12.817	0.4443	0.217
	Unknown	0.125	0.141	0.129	0.0091	0.444
Linolenic acid	C18:3 n-3	1.643[a]	0.808[b]	0.882[b]	0.0503	<0.0001
Arachidic acid	C20:0	0.123[a]	0.101[b]	0.103[b]	0.0028	0.0002
Eicosenoic acid	C20:1 n-9	0.374[a]	0.280[b]	0.269[b]	0.0107	<0.0001
Eicosadienoic acid	C20:2 n-6	0.249[a]	0.221[b]	0.222[b]	0.0064	0.016
h-γ-Linolenic acid	C20:3 n-6	0.127	0.125	0.124	0.0087	0.974
Arachidonic acid	C20:4 n-6	0.084	0.097	0.100	0.0063	0.206
Eicosatrienoic acid	C20:3 n-3	0.110	0.135	0.135	0.0130	0.326

Abbreviations: DON (deoxynivalenol); CD (Control Diet); LMD (Diet containing mealworm meal grown on <1,000 µg/kg DON wheat); HMD (Diets containing mealworms grown on 30,730 µg/kg DON wheat)
[1]Mean of replicates (n=5)
Different letters indicate significance: $P < 0.05$

Table 3.14. Effect of dietary inclusion of yellow mealworm larval meal produced on low or high DON wheat on heterophil/lymphocyte ratios and blood differentials

	Treatment[1]				
Parameter	**CD**	**LMD**	**HMD**	**SEM**	**P Value**
HL ratio (H/L)	1.12[2]	1.11[2]	0.89[2]	0.18	0.596
Heterophils (%)	43.6	41.5	46.7	3.84	0.911
Lymphocytes (%)	40.9	42.5	42.2	4.03	0.955
Basophils (%)	7.8	8.0	6.4	0.88	0.391
Eosinophils (%)	3.6	5.3	5.7	0.89	0.215
Monocytes (%)	4.3	3.2	4.2	0.62	0.389

Abbreviations: CD (Control Diet); LMD (Diet containing mealworm meal grown on <1,000 µg/kg DON wheat); HMD (Diets containing mealworms grown on 30,730 µg/kg DON wheat)

[1]Mean of replicates (n=10) per treatment

[2]Mean of samples (n=9)

3.3.6 Crude protein and dry matter retention

Dry matter and CP retention results are displayed in Table 3.15. Crude protein retention was increased in broilers fed LMD and HMD relative those fed CD (P = 0.0091). Retention in broilers fed LMD and HMD was 68.17 and 68.61% respectively while CD was 66.17%. Dry matter retention was increased in HMD at 76.80% compared to LMD and CD at 74.93 and 74.88% respectively (P = 0.0046).

Table 3.15. Effect of dietary inclusion of yellow mealworm larval meal produced on low or high DON wheat on dry matter and crude protein retention in broiler chickens

	Treatment[1]				
Parameter	**CD**	**LMD**	**HMD**	**SEM**	**P Value**
Dry matter (%)	74.88[b]	74.93[b]	76.80[a]	0.37	0.0046
Crude protein (%)	66.17[b]	68.17[a]	68.61[a]	0.49	0.0091

Abbreviations: CD (Control Diet); LMD (Diet containing mealworm meal grown on <1,000 µg/kg DON wheat); HMD (Diets containing mealworms grown on 30,730 µg/kg DON wheat)
[1]Mean of replicates (n=5)
Different letters indicate significance: $P < 0.05$

3.4 Discussion

3.4.1 Yellow mealworm larvae meals nutrient and mycotoxin profiles

Deoxynivalenol was the only mycotoxin detected in the wheat and was identified in the YML meal produced on HDW at a concentration of 17.5 µg/kg. These results are much lower than Ochoa-Sanabria et al. (2019) detected, approximately 130 µg/kg when feeding up to 12,000 µg/kg DON. This could be due to genetic differences in the breeding colonies as the YML were obtained from two different sources. These results are similar to those reported by Van Broekhoven et al. (2017) that did not detect any presence of mycotoxins in YML grown on up to 8,000 µg/kg DON. The exact mechanisms used by the YML to metabolize DON are currently unknown, it is most likely a mixture of microbial and gut enzymatic activity. Genta et al. (2006) noted an adaptation of the gut microbiota in YML and that certain digestive enzymes disappeared in larvae treated with antibiotics indicating that they might have been microbial in origin. It was suggested that some of the microbes may have unessential digestive roles which can help the larvae adapt to dietary changes (Genta et al., 2006). These microbes may also play a role in the adaptation process to exposure to mycotoxins.

Crude protein values of the YML meals were consistent with previous studies that assessed nutritional profiles (Van Broekhoven et al., 2015; Ochoa-Sanabria et al., 2019). The amino acids profiles of our YML meals were similar to those measured by Ochoa-Sanabria et al. (2019), Ghosh et al. (2017), and Ravzanaadii et al. (2012). This study however, detected a larger number of fatty acids than Ochoa-Sanabria et al. (2019) but were similar in profile to those measured by Ghosh et al. (2017). The fatty acid profiles of the mealworms reared on LDW and HDW were similar. The mineral profiles were also similar between the two treatments; however, manganese was increased in the YML meal produced on HDW, likely due to differences in the nutrient composition of the wheat (Van Broekhoven et al., 2015).

3.4.2 Broiler performance

The primary objective of this study was to determine if YML reared on high DON wheat (30,730 µg/kg) were safe for consumption when included in poultry diets and if any effects on growth performance, organ weights, organ size, and haematological measures could be observed. Final body weight and feed intake in the broilers were lower by 14.5 to 19.9% and 13.4 to 22.6% respectively when compared to the performance objective set out by Aviagen (2019). This was

likely due to the physical form of the feed which was fed as a mash instead of a crumble or pellet which have been shown to improve body weights, feed intake, and F:G ratios in broilers (Abdollahi et al., 2018). Feed intake was reduced in broilers fed HMD during days 8-14 which likely led to the reduction in body weight measured on day 14. Feed intake also had a tendency to be lower during days 15-21 and days 29-35. Average daily gain was also reduced in the HMD treatment during days 8-14 likely due to the reduction in feed intake. This may be indicative of the presence of DON-like metabolites or a modified mycotoxin present in the YML which could be accumulating through the mechanisms used in detoxification. Due to the difference in structure of the molecules, the mycotoxin would not be detected when using traditional means (Freire and Sant'Ana, 2018). The broilers fed CD and LMD had similar growth performance throughout the trial which agrees with the results found by Biasato et al. (2016). Elahi et al. (2020) also found no differences in broiler performance with inclusion of 0, 2, 4, and 8% YML meal in broiler diets. Bovera et al. (2015 and 2016) however, found improved growth performance with reductions in feed intake and F:G ratios observed when completely replacing soybean meal with YML meal. Most studies looking at YML meal as a feed ingredient have formulated diets based on total amino acids which is not ideal when formulating for broiler chickens (Elahi et al., 2020). Matin (2019) recently published results pertaining to amino acid digestibility of various insects including YML, therefore more research formulating based on digestible amino acid profiles of insects will likely come out in the future.

Crude protein retention was increased in broilers fed LMD and HMD relative to those fed CD. Improved CP retention has been associated with a reduction in abdominal fat deposition in broilers (Rao et al., 2018). Crude protein retention in CD was 66.17% which is higher than 53.5 to 57.2% with 0 to 9% inclusion of meat and bone meal in diets reported by Bolarinwa et al. (2012). Dry matter retention was increased in broilers fed HMD, although broiler performance was not improved, the exact reasons for this increase are unknown.

3.4.3 Meat yield and organ weights

Meat yield and organs were not affected by the diets analysed on a weight or as a percentage of live weight basis indicating no effects of carcass traits. This agrees with research by Bovera et al. (2016), Biasato et al. (2016 and 2018), and Elahi et al. (2020) where YML inclusion ranging from 0 to 15% had no observed effects on carcass traits. Elahi et al. (2020) and

Khan et al. (2018) also observed no effects of YML meal on meat quality. The tendency for the YML meals to reduce abdominal fat pad weights may in part be caused by the hypolipidemic and hypocholesterolemic properties of chitin that can result in reductions in body fat of broilers (Gasco et al., 2018). Marono et al., (2017) reported lower serum cholesterol and triglyceride concentrations in layer hens which were attributed to chitin being able to bind bile acids and free fatty acids in the gastrointestinal tract. Chitin can also act as a prebiotic to increase *Lactobacillus* populations in the gastrointestinal tract (Islam and Yang, 2017) which has been observed to reduce carcass fat of broilers (Kalavathy et al., 2008). The lengths of the small intestine sections, ceca, and colon were not affected by any of the treatments.

3.4.4 Fat pads

The level of CF in the fat pads were similar between all diets. The differences between the fatty acid profiles of the fat pads are likely due to the fat sources and compositions in the diets. Poultry directly absorb and deposit fatty acids (Çalik et al., 2018), thus changes in composition directly affect the fat pad. The YML meals were the primary fat source in LMD and HMD while canola oil was in the CD. The YML meals had higher concentrations of lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1) and palmitoleic acid (C16:1) at approximately 0.45, 4.67, 0.30, 2.23% compared to canola oil which had approximately 0, 0.1, 0, and 0.2% of crude fat respectively (Eskin, 2016). Canola oil in the CD had higher levels of oleic acid (C18:1), linolenic acid (C18:3), arachidic acid (C20:0), and eicosenoic acid (C20:1) at 61.6, 9.6, 0.6, and 1.4% (Eskin, 2016) while the YML meals contained approximately 48.77, 0.45, 0.23, and 0.07% of crude fat respectively.

3.4.5 Hematological parameters

White blood cell differentials and H/L ratios were not affected by any of the treatments. Heterophil/lymphocyte ratios were the same across all treatments which is similar to observations by Biasato et al. (2017 and 2018) indicating that YML inclusion did not impact the health status of the birds, stimulate immune response or induce stress in the broilers (De Marco et al., 2013). Bovera et al. (2015) reported a decrease in albumin/globulin ratios, which was attributed to the chitin content of the YML meal, in broilers which is typically indicative of improved disease resistance and immune response. The proportion of heterophils, lymphocytes,

basophils, eosinophils, and monocytes were not different between treatments which indicates that the chitin did not have an effect on immune response. Chitin has been reported to have a bacteriostatic effect in Gram-negative bacteria such as *Escherichia coli*, *Vibrio cholerae*, and *Salmonella typhimurium* (Lopez-Santamarina et al., 2020). Antifungal and antimicrobial effects have been reported as well by Khoushab and Yamabhai (2010). Chitin also has prebiotic effects and has been shown to increase populations of *Bifidobacterium* and *Lactobacillus* species in gut microbiota (Imathiu, 2020). These effects of chitin could help reduce the severity and duration of some infections by decreasing the efficacy of the causative agent which in turn could result in the use of it as an antimicrobial agent.

3.4.6 Summary

With the need to improve sustainability of agricultural processes, the use of by-products and crops that are unsuitable for human and animal consumption for insect production could help bridge the gap required for food and feed requirements. Conventional livestock production requires a large landmass and resources such as water to sustain production. Insects on the other hand require much less land mass and can potentially be grown on crops which may not be suitable for feed. Insects have a nutritional profile that can meet most requirements for animal production. Insects are naturally consumed by livestock such as poultry, and entomophagy is a common practice in some regions of the world. The safety of insects produced on potentially toxic feed is a concern for animal and human safety as they could potentially relay these metabolites on.

This project has shown that YML reared on DON-contaminated wheat up to 30,730 can be a feed ingredient with less than 1,000 µg/kg suitable for use in poultry diets. Inclusion of YML reduced overall broiler feed intake, but did not impact F:G ratios, BW, meat yield, organ weights, and haematological parameters.

In conclusion, this research suggests that YML grown on wheat contaminated with DON up to 30,7300 ug/kg can be used as an effective feed ingredient for use in poultry production. Larger scale experiments with high inclusion levels of YML should be conducted to further assess safety. Further research is required to establish how YML metabolize DON, if any metabolites are present in the larvae, and if any of those metabolites have toxic effects in animals.

CHAPTER 4

FUTURE DIRECTIONS

This project demonstrated that YML reared on DON-contaminated wheat can be utilized as a feed ingredient for poultry. Feed intake was reduced while no effects were observed in final BW and meat yield. It is possible that DON-like metabolites were present in YML meal produced on high DON wheat causing the reduction in FI. Further research is required to determine how YML metabolize mycotoxins such as DON and what metabolites accumulated in the larvae. It may be possible that the derivatives formed in the metabolism of DON could be as or more toxic than DON. This may also occur when mealworms are fed other mycotoxins such as aflatoxins and ZEN even though quantities detected in larvae are much less than what is fed (Bosch et al., 2017; Camenzuli et al., 2018). As such, the metabolism pathways utilized by insects must be further researched to determine pathways and accumulation of mycotoxins if insects produced on contaminated feed are to be used for food and feed. Currently there is a group researching the metabolism of DON in the YML at the National Research Council of Canada (Saskatoon, Canada). Further research is required to test if YML reared on mycotoxin contaminated feedstuffs are safe for animal consumption.

I believe that if insects produced on salvage crops are to be produced economically, they will likely need to be grown on unbalanced diets that are not optimized for maximum efficiency and productivity. The reason for this is that balancing diets typically involves the use of more expensive feed ingredients that could counter the low costs associated with using solely salvage crops allowing insects to better compete against ingredients such as soybean meal and fish meal while remaining profitable. Currently, the cost of producing insects is high due to the high levels of manual labour, unoptimized production systems, and lack of automation. This results in prices of insect meals to be high making it difficult to compete with feed ingredients like soybean meal (Van Huis et al. 2020).

It would be interesting to research further optimizations in insect production. Optimal average particle size might be interesting to determine if insect larvae prefer certain particle sizes or have improvements to performance. Morales-Ramos et al. (2019) improved average YML larvae size though breeding, so it would be interesting to see if further improvements can be made to factors such as feed efficiency and fecundity. Overall, the insect industry is still in the early stages of growth and has potential to further improve production as our understanding of insects improves. Our research shows that YML can be a viable feed ingredient for broiler production, but the safety of YML reared on high DON wheat needs to be further assessed.

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