THE IMPACT OF PROCESSING ERGOT INFECTED GRAINS ON PRODUCTION PARAMETERS AND NUTRIENT DIGESTIBILITY IN SWINE

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in the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon, Saskatchewan

By

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of the requirements for the

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by

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ABSTRACT

Ergot alkaloids (EAs), secondary metabolites produced by the fungus, *Claviceps purpurea*, infect cereal grains and grasses. The exposure of livestock to ergot contaminated feed results in symptoms ranging from reduced feed intake to death. Six EAs are commonly detected in Saskatchewan grains, and each exists as either an 'R' or 'S' epimer. Toxicity of ergot is affected by EA content, alkaloid and epimer profile, with most research indicating the R form to be more potent than the S form. There is some indication that hydrothermal processing may reduce ergot toxicity, perhaps by changing the epimer profile. The objective of this study was to investigate the effects of hydrothermal processing on the alkaloid content and epimer profile of ergot infected wheat and rye screenings and determine if this is related to toxicity. In trial 1, heavily contaminated screenings were processed with either steam explosion, pelleting, or extrusion. Samples used for trial 1 included screenings and diet samples. Screenings were heavily contaminated rye screenings that were either steam exploded for 2 or 5 mins, or, harsh or mild pelleted, and wheat screenings that were soaked or unsoaked before steam explosion. Diet samples were obtained from previous swine experiments which contained processed (steam exploded or extruded). All samples were analyzed for EA and epimer content by liquid chromatography mass spectrophotometry (LCMS). Total EA content of the screenings were reduced by 96 % in the diet containing steam exploded premix relative to the diet containing the non steam exploded premix. There was, however, no effect of extrusion on total EA content in diets containing the extruded premix. Steam explosion for 2 or 5 mins reduced the content of ergot of screenings from between 39-42 %. Mild pelleting had minimal effect (<15 %) while harsh pelleting reduced the ergot content by 25 % compared to the control. Steam explosion reduced the ergot content in wheat screenings, regardless of pre-soaking. Steam explosion resulted in greater reductions of individual EAs when than extrusion or pelleting. The relative changes were more consistent for all individual EAs with the exception of ergocristine. The change in epimer profile was not consistent comparing steam explosion and extrusion for the diets containing 4000 ppb processed contaminated screenings (premix). Steam explosion reduced both R and S epimers whereas the change observed with extrusion was due to consistent increases in S epimers but a reduction of the R. With the change in epimer profile observed with the extrusion of ergot-contaminated screenings in trial 1, a feeding trial (trial 2) was conducted by incoporating premixes(formulated for the purpose of this study) that were processed by extrusion to investigate whether a change in epimer profile will result in reduced toxicity

(negative effects) in growing pigs. In trial 2, diets containing 0 or 4000 ppb EA, extruded or not (2×2 factorial) were fed to 160 pigs (5 pigs per pen) from 65 ± 4 kg to 125 kg BW in a 56d performance trial. Blood samples collected on d7 and d56 were analyzed for prolactin, a sensitive indicator of ergot toxicity. A third trial was conducted to determine the effects on nutrient digestibility of pigs fed 1 of these 4 treatment diets with the inclusion of celite. Growth (ADG) and feed intake (ADFI) decreased when pigs consumed EA contaminated diets (1.07, 1.03 kg/d; 0 and 4000 ppb, $P<0.05$) and (2.97, 2.78 kg/d; 0 and 4000 ppb, $P<0.05$) respectively. Serum prolactin (ng/mL) was reduced on d7 (1.67, 0.57; $P<0.05$, 0 and 4000 ppb) and on d56 (0.71, 0.43; $P<0.05$, 0 and 4000 ppb) when pigs consumed EA contaminated diets. Extrusion had no effect on ADG, ADFI, FE or serum prolactin ($P>0.05$). There was a tendency for reduction of N ATTD ($P=0.06$) due to ergot. There were no ergot by extrusion interactions on any of the performance parameters, ATTD of N or serum prolactin ($P>0.05$). The results indicated that the consumption of diets by growing pigs contaminated with 4000 ppb EA had modest effects on performance and nutrient digestibility of growing pigs. Extrusion changed EA epimer profiles but did not reduce the toxicity (negative effects) of ergot-contaminated screenings. Further research is required to investigate appropriate and practical hydrothermal technologies to reduce EA toxicity.

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DEDICATION

I dedicate this thesis to my parents, Mr. and Mrs. Graham Halm, my sisters, Lizbeth and Elsie, for the prayers, words of encouragement and support in all forms.

"What shall I render to my God
For all His mercy's store?
I'll take the gifts he has bestowed
And humbly ask for more

My vows I will to His great name
Before His people pay,
And all I have, and all I am
Upon His altar lay

The God of all-redeeming grace,
My God, I will proclaim,
Offer the sacrifice of praise,
And call upon His name"

By

Charles Wesley

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LIST OF ABBREVIATIONS

ADF	Acid Detergent Fibre
ADFI	Average Daily Feed Intake
NH_3	Ammonia
ADG	Average Daily Gain
AIA	Acid Insoluble Ash
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATTD	Apparent Total Tract Digestibility
BW	Body Weight
CCAC	Canadian Council on Animal Care
CFIA	Canadian Feed Inspection Agency
CFRC	Canadian Feed Research Centre
°C	Degree Celsius
d	day
DM	Dry Matter
DNA	Deoxyribonucleic Acid
EA	Ergot Alkaloids
EP	Ergopeptides
FAO	Food and Agricultural Organization
FE	Feed Efficiency
GE	Gross Energy
G: F	Gain to Feed

LSD	Least Significant Difference
ME	Metabolizable Energy
N	Nitrogen
ND	Not detected
NDF	Neutral Detergent Fibre
NE	Net Energy
NRC	National Research Centre
PDS	Prairie Diagnostics Services
ppb	Parts per billion
ppm	Parts per million
PSCI	Prairie Swine Centre Inc
psi	Pounds per square inch
RCBD	Randomized Complete Block Design
RIA	Radioimmunoassay
RNA	Ribonucleic acid
rpm	Revolutions per minute
SAS	Statistical Analysis System
SEM	Standard error of means
wk	week

CHAPTER 1: INTRODUCTION

Cereals and cereal by-products provide an important source of energy and nutrients for livestock (Khaneghah *et al*., 2019). However, the contamination of grains by mycotoxins pose great risks to the health and productivity of livestock (Klotz, 2015a; Khaneghah *et al*., 2019). Mycotoxins are secondary metabolites produced mainly by fungi which affect important agricultural crops under a wide range of environmental conditions. One of the major classes of mycotoxins are ergot alkaloids (EAs) (Coufal-Majewski *et al*., 2016). Ergot alkaloids are secondary metabolites of the fungal family, Claviceps (Mulac *et al*., 2012). The alkaloids are the main toxic constituents present in ergot, the hardened sclerotia of the fungus, which infect different grains but predominantly rye, wheat, triticale, and barley (Dänicke, 2016). The Canadian Feed Inspection Agency (CFIA) has suggested feeding limits for many mycotoxins for different livestock species. For example, in pigs, a maximum of 4000-6000 ppb total EA is recommended in diets, irrespective of the stage of production (CFIA, 2015).

The proportions of the main toxic constituent in ergot, the alkaloids, vary depending on geographic region, harvesting year, cereal species, variety, and genotype (Dänicke, 2016). A substantial challenge in the observation of EA-induced effects is the highly variable individual animal response to exposure which arises in large part due to the complex plant- fungus- animal and environment interaction. The EA exist as different epimers; the 'R' or 'in' and the 'S' or 'inin', and earlier studies have indicated that under normal physiological conditions, the R epimers exert biological effects but the S-epimers are biologically inactive (Wolff *et al*., 1988). However, a recent *in vitro* study conducted by Cherewyk *et al*. (2020) demonstrated that the S epimers are also biologically active. Research, however, examining the role of the different epimers is scarce.

The consumption of EA can cause a range of effects such as convulsions, gangrene, hyperthermia, agalactia, and reduced feed intake and growth (Carson, 1977; McMullen and Stoltenow, 2002; Burrows and Tyrl, 2012; Klotz, 2015a). Decreased feed intake is the most sensitive indicator of the presence of EA for poultry (Dänicke, 2017) while reduced feed intake and growth, reproductive problems and agalactia are observed in both cattle and swine following ergot ingestion (Coufal-Majewski *et al*., 2016). Ergot alkaloids interfere with prolactin production which is important for lactation. The decrease in prolactin is a sensitive indicator of ergot toxicity (Klotz, 2015a). Due to concerns of the negative impacts of ergot on livestock performance, it is essential to either reduce total ergot content in diets or reduce ergot toxicity. Successful mitigation

strategies to reduce the impact of ergot have been investigated and there is some evidence that hydrothermal processing of contaminated grains may reduce ergot toxicity (Kabak, 2009). Coufal-Majewski *et al*. (2017) reported that pelleting diets reduced the negative impacts of EAs, possibly by changing alkaloid profiles.

This study seeks to evaluate the impact of hydrothermal processing of ergot-infected feed on toxicity, defined as effects on performance parameters, prolactin output, and nutrient digestibility in grow-finishing pigs. The findings from this research will provide producers with information to consider testing ergot amounts with the inclusion of ergot-contaminated grains in the feed of livestock (particularly, swine).

1.1 General objectives

The overall objective of this research is to examine the impact of hydrothermal processing on toxicity of ergot contaminated screenings and whether this could be related to changes in the EA profile and/or epimers. The specific objectives were to investigate the effect of various hydrothermal processing techniques on EA contents and their epimers and to determine the effect of extruding ergot contaminated wheat screenings on performance parameters, serum prolactin and nutrient digestibility when included in diets for growing swine.

1.2 Research hypothesis

It was hypothesized that extrusion of ergot contaminated grains will reduce ergot toxicity, measured as feed intake, growth, and prolactin output. It was hypothesized that this will occur through changes in EA and epimer ratios. The negative effects of ergot on growth performance and nutrient digestibility will be reduced if the feed has been subjected to hydrothermal processing.

CHAPTER 2: LITERATURE REVIEW

2.1 Mycotoxin contamination

The infestation of grains and grasses with mycotoxins is a significant global problem (Hussein and Brasel, 2001). Estimations from the Food and Agricultural Organization (FAO) are that 25 % of the world's crop is typically contaminated with mycotoxins (Waliyar *et al.*, 2015). Mycotoxins are low molecular-weight compounds that are secondary metabolites produced by fungi (Kabak, 2009). One of the earlier reports of mycotoxins affecting livestock was in 1962 when almost 100,000 turkeys died after they were fed peanut meal contaminated with the secondary metabolites of fungi of the *Aspergillus flavus* genera (Lin, 1976). Fungi are pervasive and all feedstuffs can be affected by mycotoxins under certain conditions. Environmental factors such as humidity, temperature, insect damage, and drought can influence the diversity and level of mycotoxin contamination (Hussein and Brasel, 2001; Zain, 2011). Not all moulds are toxigenic, and not all secondary metabolites from these moulds are harmful. Although mycotoxins are difficult to identify and classify (Lazicka and Orzechowski, 2010), more than 300 secondary compounds from fungi have been identified and research is underway to determine which ones are deleterious to health (Zain, 2011).

Mycotoxins associated with cereal grains which are of agro-economic importance include aflatoxins, ergot alkaloids, ochratoxins, trichothecenes, zearalenone and fumonisins (Lazicka and Orzechowski, 2010) and the consequences of mycotoxin contamination are of concern in both developed and developing countries (Shephard, 2008). Contamination of cereals and cereal related products by mycotoxins can pose serious risks to human and animal health and have international trade implications (Wu, 2006; Wild and Gong, 2009; Bryden, 2012) which adversely affects the supply of animal and animal by-products.

2.2 Prevalence and exposure to feedstuff contamination by mycotoxins

The increasing prevalence of contamination of feedstuff with mycotoxins can be attributed to changes in climate and agricultural practices (Fink-Gremmels, 2008). Unlike other mycotoxins prevalent in Western Canada such as deoxynivalenol or zearalenone which are of concern primarily to swine and poultry, ergot is a concern for all classes of livestock, including ruminants (Coufal-Majewski *et al.*, 2016). Ergot can develop either in the field or during storage (Yiannikouris and Jouany, 2002) and can parasitize plants particularly during the flowering period,

and replace grain with bodies known as sclerotium that contains the alkaloids (Krska and Crews, 2008; Lee, 2009).

2.3 Mycotoxins and ergot alkaloids

Mycotoxins usually enter an organism through the consumption of contaminated food, through the respiratory system, or by direct contact with the skin (Lazicka and Orzechowski, 2010). The chemistry and structure of mycotoxins vary (Streit *et al.*, 2013) and when the toxic level is exceeded, mycotoxins in feed can have adverse effects in farm animals such as decreased feed intake and weight gain, immune suppression, fertility disorders, and pathological conditions which may eventually lead to death (Binder *et al.*, 2007; Bryden, 2012).

Ergot alkaloids produced in sclerotia, are responsible for the risk to livestock from exposure to ergot-contaminated feed (Coufal-Majewski *et al*., 2016). Ergot alkaloids are a complex group of compounds which are produced by a group of fungi. They are secondary metabolites from fungal species belonging to the family Clavicipitacaeae (Young *et al*., 2013) which significantly impact livestock health and productivity worldwide (Klotz, 2015a). References to ergot and its deleterious effects on health date back to 1100 BC in China in the Eleusiniam Mysteries of ancient Greece, and in Europe in the Middle Ages (Schiff, 2006). The geographic scope of impact ranges primarily through Europe (di Menna *et al.*, 2012) and North America (Klotz, 2015a). However, due to importation of feedstuff for livestock production, the impact is seen in other countries including Japan, Korea and the United Arab Emirates (Klotz, 2015a). Fungi such as *Claviceps purpurea, C. paspaspali,* and *C. fusiformis* have been long known in history to be a preferential pathogen of livestock and human feeds predominantly infesting feed staples such as wheat, barley, and rye but also rice, maize, sorghum, oats, and millet and are ubiquitous in the temperate regions of the globe (Roberts, 2018). Neotyphodium and Epichloe species exclusively infect forage and turf grasses, such as perennial rye grasses and tall fescues. Young *et al*. (2014) reported that, over 90 % of the tall fescue pastures in the US harbour plants infected with *Neotyphodium coenophialum* and the economic losses due to livestock infections approximate over one billion dollars annually. *Claviceps africana*, first described in 1991, was noted to be more virulent and spreading faster as compared to Indian sorghum ergot (Frederickson *et al*., 1991). *Claviceps africana* (Reed *et al.*, 2011) is a concern for international trade for its preferential pathogenicity

for various sorghum varieties, and has spread from Africa to America, Asia, and Australia (Bandyopadhyay *et al*., 1998; Blaney *et al*., 2000).

2.3.1. Ergot lifecycle

Ergot contamination is a result of fungal infection of cereal grains and is primarily observed as dark, purplish-colored structures on the ears of healthy grains or grasses which are referred to as sclerotia or ergot bodies. The sizes and shape of the sclerotia differ depending on the host plant. These structures are cylindrical with round ends, and tapered at the distal end that replace healthy grains (Coufal-Majewski *et al.*, 2016; Grusie, 2017). The interior is typically gray to white in color, and this distinguishes it from other debris. They appear in some grains four times larger relative to the grain kernel, while in some grains, such as wheat, the sclerotia are the same size as the host grain. The cycle of infestation occurs when ergot bodies fall to the ground to infest cereal crops at time of planting in the next season from planting contaminated seed or from contaminated grasses (Grusie, 2017). The Claviceps life cycle consists of an asexual and sexual phase (Didek-Brumec *et al.*, 1996). Germination (Figure 2.1) is usually initiated after four to eight weeks of temperatures from 0 to 10 °C. An outbreak of ergot infestation typically occurs after a cold winter followed by a wet spring when the ergot bodies produce drumstick-like structures (stroma) (Figure 2.1). The asexual phase is characterized by the development of an ascospore in the ovary of the flower of the host plant. In optimal conditions in the presence of the required soil moisture, spores germinate and are ejected into the air by drumstick-like structures. The ascospores are then able to drift with the wind until they fuse with the stigma of the ovary then replacing a healthy kernel (Schumann and Uppala, 2000).

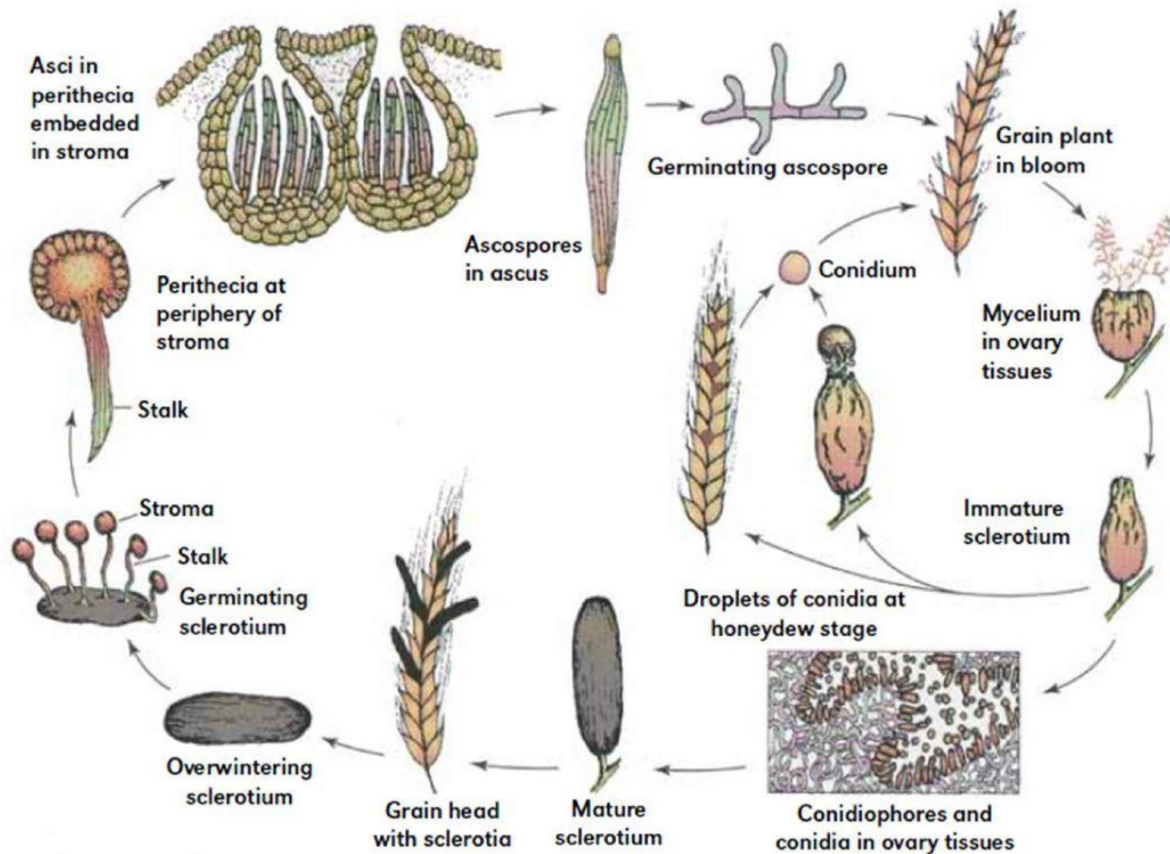

Figure 2.1. The life cycle of Claviceps purpurea (Schumann and Uppala, 2000)

Approximately one week post-infection, the infected flowering plant begins to exude a yellow-white, sweet sticky substance, the honey dew which contains infectious conidia germinated by splashes of rain or even dew. Wind, rain, and insects spread the conidia from one plant to the other distributing infection throughout the field during the flowering period and after two weeks of infection, the spore body develops into the fungal structure, a sclerotium. The sclerotia remain intact during storage of grains in low temperatures which protects the fungus and at this point the sclerotia have developed into a black kernel. This is when the alkaloids are produced, and the ergoty grain is toxic if ingested (Grusie, 2017). Ergot has a short time to infest cereal grain plants hence if the required conditions for their spread and growth are not present, contamination will not occur or may be minimal (Roberts, 2018).

2.3.2. Composition and chemistry of ergot alkaloids

The fungal genera, Claviceps produce the class of compounds referred to as the EAs, and different species of Claviceps produce different alkaloids. Claviceps spp. are not the only fungi which produce EAs. *Neotyphodium ganseunse*, produce EAs, and the main alkaloids present are ergometrine with another lysergic acid derivative called ergine (Zhang *et al*., 2014). The toxic compounds produced in sclerotia, EAs, vary in amount and type depending on cereal species, geography, and environmental conditions during the growing season. For instance, in a comparative study, ergot found in the southern area of Germany had relatively higher concentrations of EA compared to ergot from central and northern Germany (Franzmann *et al.*, 2010). The most prominent EA found in contaminated grains across Canada are ergosine, ergocristine, ergocryptine, ergoconine, ergometrine and ergotamine (Figure 2.2) with ergocristine and ergotamine found in higher concentrations (Grusie *et al.*, 2018). EAs are colourless crystals which are readily soluble in various organic solvents, but nearly insoluble or soluble in water depending on the specific alkaloid (Krska and Crews, 2008). Ergometrine is the alkaloid amongst the other principal alkaloids of ergot which is more soluble in water (Schiff, 2006). Ergot alkaloids exist in 2 conformational forms; R epimers (denoted by an "ine" suffix) and S epimers ("inine''). Under normal physiological conditions, the R epimers are believed to exert biological effects while the S epimers are thought to be less biologically active (Smith and Shappell, 2002; Klotz, 2015b). There is evidence in literature with original reports from the 1970s to 1980s stating inactiveness of the S epimer (Cherewyk *et al*., 2020). A previous study was conducted to examine the influence of baking on EA contents in ergot flour bread, and the authors reported that the EA content was reduced, with a decrease to a much greater extent of the pharmacologically active alkaloids (R epimer) compared to the increase in the less active S epimer (Wolff *et al*., 1988). A feeding trial in mice with diets containing ergot flour showed that the toxic effects on reproduction in mice was reduced with the diet which contained the reduced R epimer. The 2 isomeric forms can undergo epimerization bi-directionally (Komarova and Tolkachev, 2001). The 2 forms have different chemical, physical and biological effects, and both forms are present in naturally contaminated samples (Mavungu *et al*., 2012). However, Cherewyk *et al*. (2020) in a recent in vitro study indicated that the S epimers are biologically active and may have similar negative effects as the R-epimers. A study conducted to determine the influence of pelleting on the EA epimer contents of ergot in flour and ergot in bread showed that contents of EA epimers were reduced by a baking

process with increases in the isomeric S epimer forms and more decreases in the R epimers (Wolff *et al*., 1988). Epimerization can be influenced by heat, humidity, pH, and UV light (Schummer *et al*., 2020). The “ine” epimers convert rapidly into the “inine” epimers in aqueous solutions and can convert back in some aqueous and organic solvents (Mavungu *et al*., 2012). Krska *et al*. (2008) showed that extraction with acid or alkaline buffers favoured epimerization. The R epimers convert back to S epimers under alkaline conditions and also during long storage (Krska *et al.*, 2008). The epimerization can be bidirectional (Merkel *et al*., 2012). Temperature and environmental pH are likely to affect the epimer profile and the rapid occurrence to epimerization (Komarova and Tolkachev, 2001).

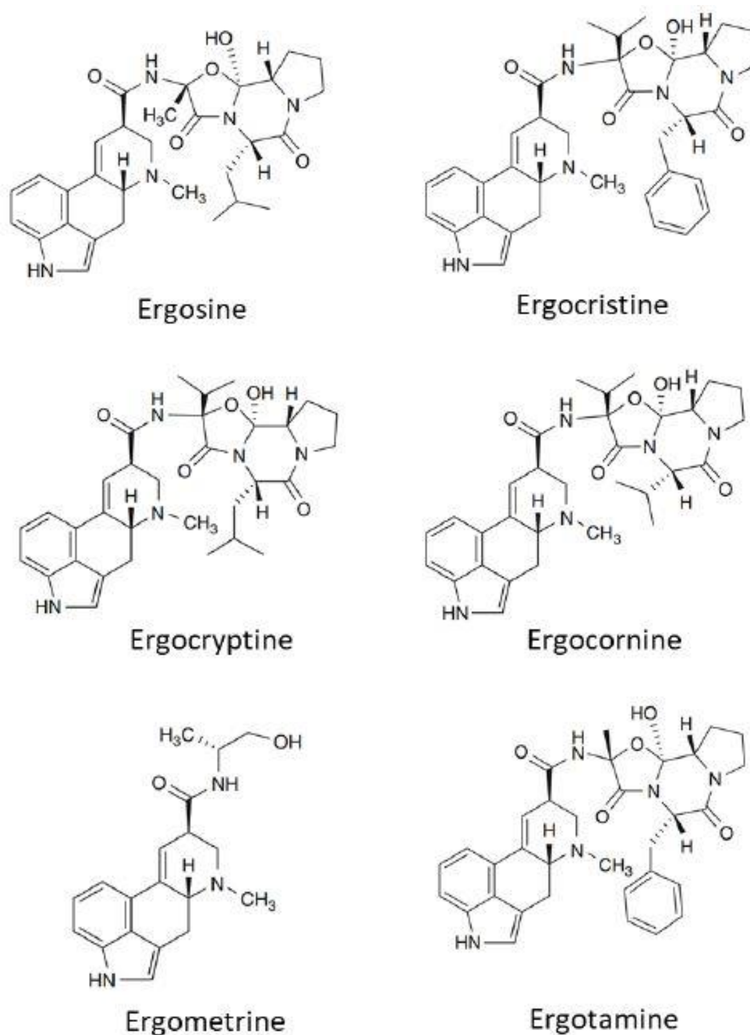

Figure 2.2. Structures of the six ergot alkaloids found in Canadian grains (Grusie, 2017)

2.4 Physiological responses to ergot ingestion

The effects of EAs ingestion vary, largely due to the complex interaction between fungus, animal and the environment which leads to varying alkaloid proportions, availability, and forms (Klotz, 2015a). The symptoms of EAs are related to the similarity in structure of ergoline rings to norepinephrine, dopamine and serotonin and the structural similarity to these biogenic amines that enable the EAs to bind and interact with receptors (Klotz, 2015a; Grusie, 2017). There are four forms of ergotism caused by consumption of ergot infected cereal grains: convulsive, gangrenous, hyperthermic and reproductive (Grusie, 2017). In general, the elucidation of symptoms and forms depends on type and location of the receptor, amount of alkaloid binding to the receptor, animal physiological state and other stressors (Klotz, 2015a). Different EAs have comparable effects due to similarities of the tetracyclic ergoline rings (Figure 2.3A) with the ring structure of the biogenic amine neurotransmitters norepinephrine, dopamine, and serotonin (Figure 2.3B). The ergoline rings give the toxins the receptor binding affinity for neurotransmitter receptors through which the toxic effects are elicited. The EAs specifically bind to monoamine neurotransmitter receptors such as adrenergic, dopaminergic, and serotonergic. The accumulation and activation of adrenergic and serotonin receptors greatly impact species-specific toxicological outcomes through a complex network (Roberts, 2018).

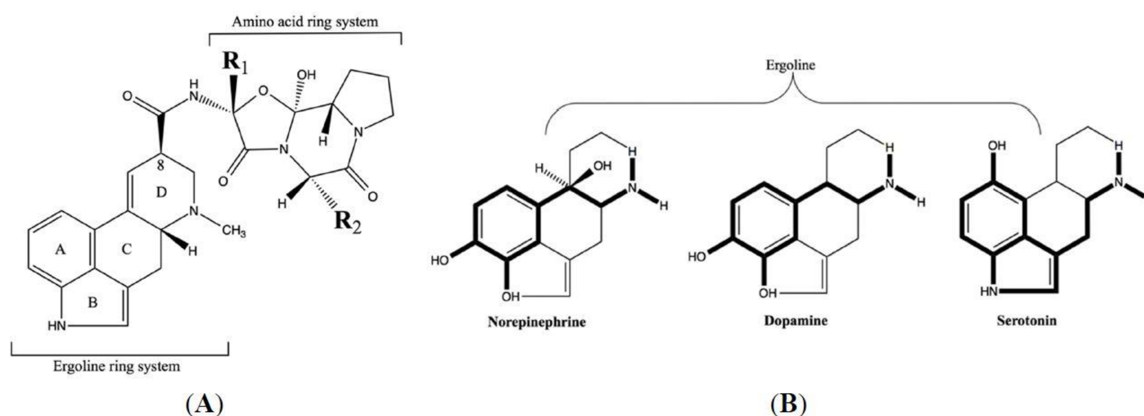

Figure 2.3. (A) The tetracyclic ergoline ring common to all EA that is variously substituted on the C-8 and has an amino acid ring system which varies at the R1 and R2 substituents to create the various ergopeptine alkaloids. (B) The structural similarities between the ergoline ring and the catecholamines norepinephrine, dopamine, and serotonin (Klotz, 2015a)

2.4.1 Gangrenous ergotism

Gangrenous ergotism, (also known as fescue foot or lameness in livestock) is an acute and observable effect of ergot intoxication resulting from vasoconstriction and dysfunction of blood vessels in the extremities (Strickland *et al.*, 2011). This form of ergotism has been referred to as St. Anthony's fire in humans which is explained as an intense burning sensation and cyanosis of the extremities (Klotz, 2015a). Ergotism occurs as a result of the perturbation in the regulatory mechanism that controls blood circulation and supply (Klotz, 2015a). The EA induced vasoconstriction is associated with inhibition and partial agonism of α-adrenergic and serotonin receptors of the D1-dopaminergic receptor. In-vitro evidence shows that EAs, primarily ergocryptine and ergometrine, induce an increase in muscle growth which results in a further decrease in blood flow which damages the endothelial lining to the blood vessels thereby causing swelling, edema, and thrombosis. The collective effect results in pain, lameness, gangrene and even death in affected animals if gangrene is severe (Grusie, 2017). Gangrenous ergotism is more pronounced in cold weather, when capillary circulation in the extremities is restricted (Belser-Ehrlich *et al*., 2013).

2.4.2 Convulsive ergotism

Convulsive ergotism has symptoms comparable to that of gangrenous ergotism at the onset of the disease (Eadie, 2003). Both forms of ergotism share similar symptoms such as reduced feed intake, feed efficiency, and reduced weight gain (Klotz, 2015a). The convulsive form of ergotism, also referred to as nervous ergotism is characterized by tremors, a twisted neck, hallucinations, seizures, fever, and death (Eadie, 2003; Belser- Ehrlich *et al.*, 2013). Convulsive ergotism is not commonly observed but it has been reported in sheep, horses, and carnivores (Grusie, 2017). Most of the clinical responses are dose dependent and vary with the susceptibility of the species.

2.4.3 Hyperthermic ergotism

The effect of environments beyond the thermoneutral range of the animal may be more apparent following the ingestion of ergot contaminated feed. In some parts of the world, this is observed as a shade-seeking behaviour or as hypothermia in cooler weathers. However, in both

conditions, peripheral vasoconstriction results in reduced transfer of heat to the extremities, and an increased core but decreased peripheral body temperature (Roberts, 2018). The hyperthermic effect observed when animals consume grains contaminated by EA include clinical signs such as labored breathing, elevated body temperature, open mouth breathing accompanied by a protruding tongue, increased salivation, and decreased appetite (Carson, 1977; Jessep *et al*., 1987; Ross *et al*., 1989; Burrows and Tyrl, 2012). Hyperthermia is not directly a productive loss, but it influences the overall EA effects on livestock by impacting feed intake and reproduction which leads to a decline in animal productivity (Klotz, 2015a).

2.4.4 Decreased livestock productivity

Previous reviews have documented a decline in the productivity of livestock due to EA contamination, and while the gangrenous or convulsive forms of ergotism occur due to acute exposure, decreased animal productivity is often a result of chronic exposure (Strickland *et al*., 1993; Strickland *et al*., 2011). Reproductive disorders arising from EA exposure have also been documented, primarily in females (Klotz, 2015a) and the primary effects include declines in serum prolactin and progesterone concentrations resulting in the reduction of milk production (Carson, 1977; Burrows and Tyrl, 2012). Clinical signs in pigs that have been observed following the ingestion of EA contaminated feed include feed refusal and decreased performance (Oresanya *et al*., 2003). Studies conducted by Oresanya *et al*. (2003) reported that feeding weanling pigs diets containing 1800 ppb EA resulted in significant reductions in feed intake (13 %) and growth (18 %) while prolactin was reduced by 53 % at the lowest level of EA inclusion of 1040 ppb. Decreased feed intake is the most sensitive indicator of the presence of alkaloids in poultry feed and an exposure of 20wk led to a loss of appetite, increased thirst, diarrhea, vomiting and weakness (Bailey *et al*., 1999: Danicke, 2016). In an unpublished class project conducted at the University of Saskatchewan, there was no effect observed when young chicks were fed diets containing 20 % ergot contaminated wheat screenings of 70000 ppb total alkaloid (final diet 14000 ppb). Decreased feed intake was observed at 21000 ppb; thus, it was concluded that the toxic level is between 14000 and 21000 ppb (Newkirk unpublished). This contrasts with recent work conducted in Europe (Dänicke, 2017) which observed effects on feed intake at 2500 ppb ergot EAs in the diet of young chicks.

2.5 Allowable limits in diets

Several international agencies are attempting to achieve universal standardization of regulatory limits for mycotoxins such as ergot (Charmley and Trenholm, 2000). This has not yet been achieved, possibly due to the differences from reports of impacts of ergot on animal performance and varying legislated standards among countries (Belser-Ehrlich *et al.*, 2013; Vermeulen *et al.*, 2013).

The allowable inclusion for cereal EAs in livestock diets are being re-examined in various countries, but there are complications due to a number of factors (Coufal-Majewski *et al.*, 2017). Specifically, as alkaloid concentrations in ergot bodies varies, existing regulations for inclusion levels of ergot bodies in grain may conflict with maximum allowable EA concentrations in feed. An increasing incidence of ergot contamination in feedstuffs in Canada has been observed and it has been suggested that changes in climate and the increased prevalence of insects may be causing the concentration of ergot to increase in cereals (CFIA, 2015). In a survey of samples submitted to the Canadian Grain Commission Harvest Sample program from 2002 to 2013, both the percent of samples downgraded due to ergot and the content of ergot in samples had increased (Tittlemier *et al.*, 2015). Less than 5 % of the samples submitted were downgraded due to ergot from 2002 to 2010 whereas from 2011 to 2013, 17 % of these samples were downgraded (Tittlemier *et al.*, 2015). The Canadian Feed Inspection Agency (CFIA) has established a recommended tolerance level of EA for swine and poultry diets of 4000-6000 ppb and 6000-9000 ppb, respectively (CFIA, 2015). However, the CFIA recently reviewed the allowable inclusions and suggested that the maximum allowable inclusion to be lowered. The proposed inclusion limits of EAs in diets for various livestock include for weaned piglets (1000 ppb), growing-finishing pigs (2000 ppb), poultry (2000 ppb), cattle (1000 ppb) and horses (1500 ppb) (CFIA 2015).

CHAPTER 3: EFFECT OF FEED PROCESSING ON ERGOT ALKALOID AMOUNTS, AND EPIMER PROFILES

3.1 Introduction

Ergot alkaloids are secondary metabolites of the fungus, *Claviceps purpurea,* and the exposure of livestock to ergot-contaminated feed continues to be a concern for livestock (Coufal-Majewski *et al*., 2016; Grusie, 2017). The primary EAs, ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocornine, are structurally similar, differing only in substitutions on C-8. Alkaloids contain C9=C10 double bonds which epimerize easily, depending on pH and temperature and the application of heat may alter chemical bonds (Coufal-Majewski *et al*., 2016). Visual inspection and removal is the most practical method for reducing ergot, however, in some instances, the dark sclerotia may be smaller than the grains, making sorting based on appearance or size unreliable (Coufal-Majewski *et al.*, 2016). The sclerotia have a specific gravity that is lower than the grain and therefore, a gravity table can be used to separate ergot bodies from the grain. Washing of grains is usually also effective as the sticky honeydew causes the sclerotia to stick to the grain (Bandyopadhyay *et al*., 1998). Other approaches for the detoxification of infected feed include chemical and biological treatments (Kabak *et al*., 2006; Jouany, 2007; Kabak, 2009). Most mycotoxins are stable to the heat employed during normal feed processing; however, some destruction may occur with more extreme conditions and information about their stability in thermal processing and potential inactivation procedures is needed (Kabak, 2009). Thermal processing of EAs can reduce total ergot content (Coufal-Majewski *et al*., 2016). Thermal processing is achieved by the application of heat, water, and high pressure. Heating reduced EA content by 85-100 % and was more effective when heated for long periods of time and at high temperatures (Scott and Lawrence, 1980; Fajardo *et al*., 1995). According to Coufal-Majewski *et al*. (2017), pelleting diets reduced the negative impact of EAs in lambs, and the authors attributed this mitigation to a change in alkaloid profiles. The authors showed that there was 1.9 times more ergosine and 3.0 more times ergotamine when pelleted diets were compared to mash diets. In contrast, pelleted diets contained 2.7 times ergocornine and 1.9 times more ergocristine than mashed diets. Conversely, limited data showed no effect of extrusion on the toxicity of ergot alkaloids for poultry (Newkirk unpublished). Heat treatment may cause epimerization of the R to the S epimer. In a previous study, baking reduced the R epimers of the alkaloid ergometrine was

by 55 % whilst the S epimers was increased by 74 %. Conversely the R epimers of ergosine were reduced by 39 %, with no change in the S configuration (Bryła *et al*., 2019).

The present study was aimed at investigating the effect of various hydrothermal processing treatments on the alkaloid contents and epimer profiles of ergot-infected screenings and their epimers. It was hypothesized that hydrothermal processing will reduce EA amounts and change the epimer profiles in different proportions depending on the severity of processing.

3.2 Materials and methods

3.2.1 Samples

Heavily contaminated screenings were obtained from grain processing plants from different sources by the Canadian Feed Research Centre (CFRC) in North Battleford, SK for the purpose of this study. The contaminated screenings were diluted with clean screenings to form "premixes" (240,000 ppb). The diet samples used for this study were phase 1 and 2 diets containing extruded screenings formulated for grow-finishing pigs and diets containing steam exploded screenings formulated for weanling pigs from 2 swine experiments.

3.2.2 Processing

Processing of heavily contaminated screenings was conducted at the CFRC. Samples were processed by either steam explosion, extrusion or pelleting. Steam explosion is an extreme form of processing with relatively higher temperature and pressure employed compared with moderate processing methods such as extrusion and pelleting. Some samples were treated with water (strained or soaked) prior to steam explosion to assess the effect of processing on soaked or strained screenings. Water, 50 % of the weight of the screenings was used to wash the strained screenings and then the water drained off immediately, and same amount of water added to the soaked screenings stirred together until it was thoroughly mixed and drained prior to steam explosion (197 ˚C) at either 100 or 200 psi for 2 or 5 mins. The Vapor, Hydro Steam Industries (HSI) Vessel (40 L) was used for steam exploding. The screenings were dried at 55 ˚C for three days after steam explosion. The extruded premix was processed at 90 ˚C and 80-100 psi prior to inclusion in the diet. Extrusion equipment used for processing was the UAS-Muyang Extruder (MY56X2 Conditioner Model: SBTZ10). Pelleting equipment used for processing was the UAS-Muyang

Model (MUZL350II). Samples that were pelleted were pelleted at 100 °C (harsh) or at 80 °C (mild).

3.2.3 Analysis of ergot alkaloids

Ground samples were sent to Prairie Diagnostics Services (PDS), Saskatoon, SK, Canada for an ergot panel analysis. All feed samples were inspected for moisture and size and then put in a drying oven at 85 ˚C for approximately 1 day. Samples were reground in an Ultra Centrifugal Mill ZM 200, at 18000 rpm and shaken to be homogenized and subsampled for analysis. All subsamples were sent to the toxicology lab for extraction according to procedures established by Prairie Diagnostics lab. The ergot extraction solvent used was 85 % acetonitrile, 15 % 10 mM ammonium acetate (Grusie *et al*., 2017). Alkaloid and epimer analysis were performed according to established methods of PDS for a complete ergot panel. Samples were analyzed using the Liquid Chromatography Mass Spectrophotometer (LCMS). The limit of identification was 1.25 µg/kg.

3.2.4 Data analysis

A complete statistical analysis was not conducted for this study as there was insufficient replication. However, the percentage difference of triplicate and duplicate technical replications was calculated. This provided an indication of variability due to lab analysis. This was used to aid interpretation of the results. Average variation between duplicates or triplicates was 10-15 %, thus any difference less than 15 % could be a result of analytical variability.

3.3 Results

3.3.1 Effect of processing on total amounts of ergot alkaloids

Figure 3.1 shows the effect of processing on total ergot content (ppb). Treatments included rye screenings that were steam exploded at 200 psi for 2 or 5 mins or were pelleted (mild or harsh), and wheat screenings that were soaked with water for 40 mins or not. Steam explosion, for 2 or 5 mins reduced the content of ergot from between 39-42 %. Mild pelleting had no effect (<15 %) while harsh pelleting reduced the ergot content by 25 % relative to the control (Figure 3.1 a). Steam explosion reduced the ergot content in wheat screenings, regardless of pre-soaking (Figure 3.1b).

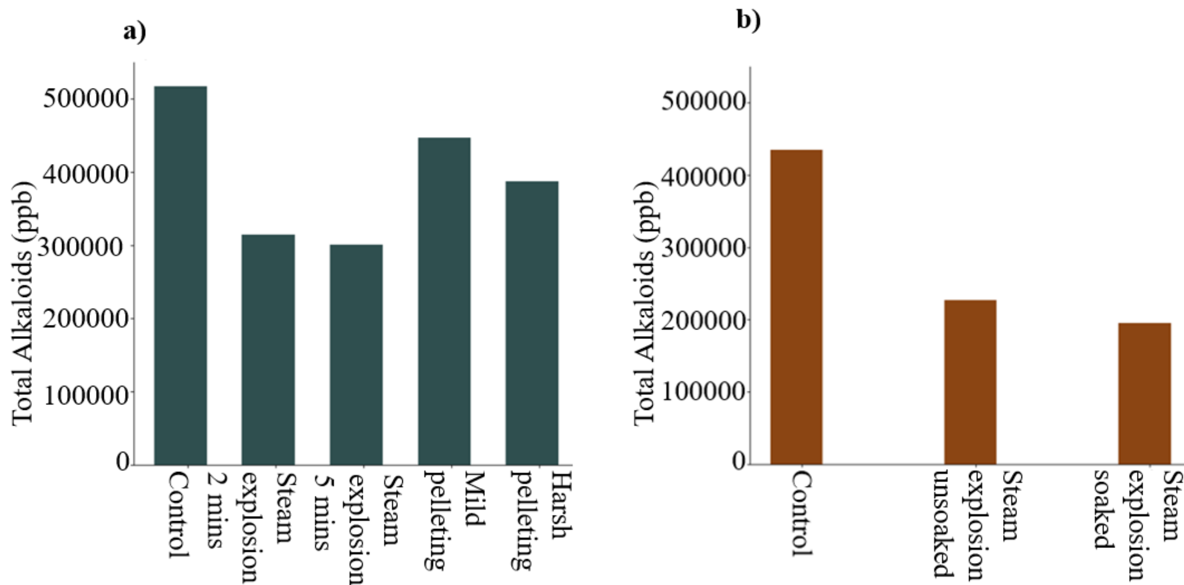

Figure 3.1. Effect of processing on total amount of ergot of a) rye and b) wheat screenings. Treatments included control screenings that were not processed, rye screenings that were steam exploded at 200 psi for 2 or 5 mins and screenings that were pelleted (mild or harsh), and wheat screenings that were soaked in water for 40 mins or not

Figure 3.2 shows the effect of processing on total EA content of diets containing processed screenings (premix). The diets were formulated to contain 4000 ppb. The total EA content was reduced by 96 % in the diet containing the steam exploded premix. However, there was an increase (19 %) in total alkaloid content in the phase 1 diet containing the extruded premix but no effect on the phase 2 diet (<15 %).

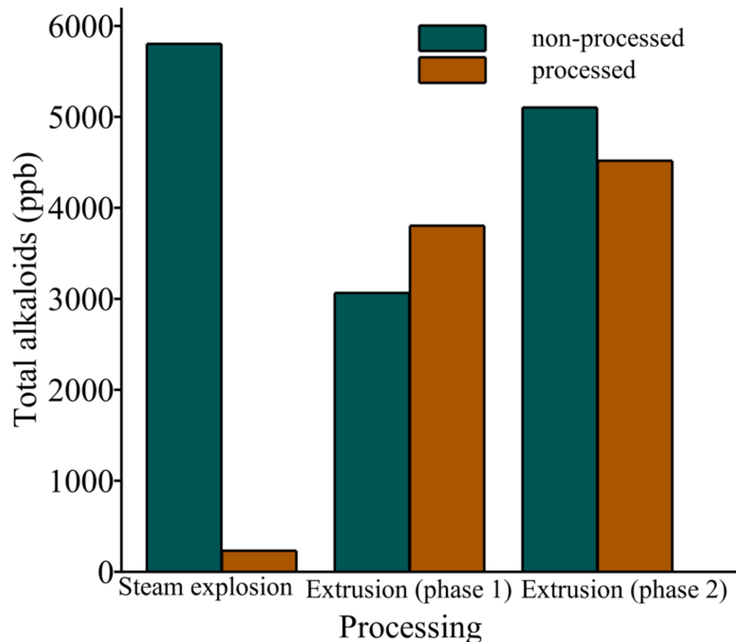

Figure 3.2. Effect of processing on total EA amounts of diets formulated to contain 4000 ppb EAs. Treatments were diets used for 2 swine trials; 4000 ppb EA contaminated diets (phase 1 and 2) containing extruded screenings (premix) that were fed to grow-finish pigs, and a 4000 ppb EA diet containing steam exploded screenings (premix) fed to weanling pigs

3.3.2 Effect of processing on ergot alkaloid profiles

Table 3.1 shows EA profiles of rye and wheat samples which were steam exploded or pelleted. Steam explosion for 2 or 5 mins reduced the ergocornine content of rye screenings between 70-76 %, while mild and harsh pelleting reduced the ergocornine content between 27-30 % relative to the rye control sample. The effect of steam explosion for 2 or 5 mins reduced ergocristine content by less than 15 % (i.e., between 2-10 %) compared to the control screenings. Similarly, ergocristine content of the mild pelleted was less than 15% (i.e., 8 %) but harsh pelleting reduced the ergocristine content by 18 % compared to the control screenings. Also, the ergocryptine content was reduced when rye screenings were either steam exploded (28 % and 42 % for 2 mins and 5 mins respectively) or pelleted (28 % and 51 % for mild and harsh pelleting respectively). The ergometrine content was reduced between 69-78 % when rye screenings were steam exploded for 2 mins or 5 mins and reduced between 25-33 % when mild or harsh pelleted relative to the control screenings. Steam explosion for 2 or 5 mins reduced the ergosine content of rye screenings between 61-69 % whereas mild and harsh pelleting reduced the ergosine content

between 16-22 % compared to the control screenings. The ergotamine content was reduced between 52-60 % when rye screenings were steam exploded for 2 and 5 mins and between 19-25 % when mild or harsh pelleting relative to the control screenings (Table 3.1).

Soaking wheat screenings prior to steam explosion at 200 psi resulted in reduced alkaloid contents relative to the control (Table 3.1). Ergoconine content was reduced by 20 % and 44 % when wheat screenings were unsoaked and soaked respectively. The ergocristine content of the wheat screenings was also reduced by soaking prior to steam explosion; by 31 % and 51 % for the unsoaked and soaked samples respectively. However, regardless of soaking, there was a reduction of the ergocryptine content of wheat screenings between 53-60 % compared to the control screenings. Similarly, ergometrine content of wheat screenings were reduced between 70-78 % when screenings were soaked before steam explosion. Also, the ergosine content was reduced between 52-47 % when screenings were soaked before steam explosion. Lastly, soaking wheat screenings before steam explosion reduced the ergotamine content between 41-55 % when screenings were soaked or not before steam explosion.

Table 3.1. Effect of processing on ergot alkaloid amounts and percentages of heavily contaminated rye and wheat screenings relative to unprocessed

	Rye									Wheat				
		Steam explosion 200 psi				Pelleting					Steam explosion 200 psi			
		2 mins		5 mins		Mild		Harsh			Unsoaked		Soaked	
EA[a] (ppb)	Control	amount	%	amount	%	amount	%	amount	%	Control	amount	%	amount	%
Ergocornine	60920	18440	**-70**	14390	**-76**	44280	**-27**	42950	**-30**	24810	19850	**-20**	13920	**-44**
Ergocristine	183500	165600	-10	180200	-2	197400	8	151300	**-18**	93940	65220	**-31**	45960	**-51**
Ergocryptine	50460	36140	**-28**	29380	**-42**	36120	**-28**	24860	**-51**	159300	64950	**-60**	75630	**-53**
Ergometrine	57790	18170	**-69**	12510	**-78**	38850	**-33**	43130	**-25**	49270	14760	**-70**	10800	**-78**
Ergosine	26300	10330	**-61**	8250	**-69**	22110	**-16**	20420	**-22**	29130	15560	**-47**	14080	**-52**
Ergotamine	139000	66210	**-52**	56150	**-60**	112800	**-19**	104800	**-25**	78480	46660	**-41**	35260	**-55**
Total	517970	314890	**-39**	300880	**-41**	451560	-12	387460	**-25**	434930	227000	**-48**	195650	**-55**

Notes: Samples for rye included a no processed control, screenings that were steam exploded at 200 psi for 2 or 5 minutes and screenings that were pelleted (mild or harsh). Samples for wheat consisted of a non-processed control, screenings that were soaked with water for 40 minutes or not. Temperature for mild and harsh pelleting were 80 ˚C and 100 ˚C respectively. Amount refers to the individual alkaloid content of a processed sample and % denotes the percentage change of an individual alkaloid of a sample compared to its respective control. The controls represent a 100 % of each individual alkaloid amount. Values (in %) with a negative sign denote a reduction in percentage from the respective control. Values (in %) with no sign denote an increase in percentage compared to the respective non-processed sample. Boldened percentage values within a row are more than 15 % different than the non-processed control

[a]EA: Ergot alkaloid

Table 3.2 shows the effect of processing on individual EA contents of diets from 2 swine trials formulated to contain steam exploded or extruded screenings (premix) of 4000 ppb. Treatments were diets used for two swine experiments; 4000 ppb EA contaminated diets (phase 1 and 2) containing extruded screenings (premix) that were fed to grow-finish pigs, and a 4000 ppb EA diet containing steam exploded screenings (premix) fed to weanling pigs. All EAs were reduced between 90-97 % in the diet sample containing the steam exploded screenings relative to the non-steam exploded sample. The ergorconine, ergocristine, ergocryptine, ergosine and ergotamine contents of the phase 1 diets were increased by 30 %, 23 %, 11 % and 6 % respectively in the diet sample containing the extruded screenings relative to respective non-extruded sample. In contrast, the content of ergometrine was a reduced by 26 % in the diet containing the extruded premix. However, the ergorconine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine contents of the phase 2 diets were reduced by 16 %, 6 %, 12, 13 %, 9 % and 10 % respectively in the diet sample containing the extruded screenings relative to the non-extruded sample.

Table 3.2. Effect of processing on individual ergot alkaloids of contaminated screenings incorporated into swine diets formulated to contain 4000 ppb EAs

	(Trial 1)[a]			Trial 2 (Phase 1)[b]			Trial 2 (Phase 2)[c]		
EA[d] (ppb)	Non steam exploded	Steam exploded	% change	Non extruded	Extruded	% change	Non extruded	Extruded	% change
Ergocornine	709	21	**-97**	368	**479**	**30**	709	595	**-16**
Ergocristine	2631	81	**-97**	1373	**1685**	**23**	2199	2060	-6
Ergocryptine	1000	72	**-92**	581	**726**	**25**	1049	914	-12
Ergometrine	218	21	**-90**	149	**110**	**-26**	215	188	-13
Ergosine	321	14	**-96**	179	165	-11	283	257	-9
Ergotamine	923	23	**-97**	415	288	-6	647	577	-10
Total	5802	232	**-96**	3065	**3804**	**19**	5102	4591	-10

Note: Samples are diets which were formulated to contain 4000 ppb ergot alkaloids for pigs in phase 1 growth stage (65-90 kg BW) of 2 swine trials that contained contaminated screenings which were steam exploded or not and extruded or non-extruded. Amount refers to the individual alkaloid content of a processed sample and % change refers to the percentage change of an individual alkaloid of a processed sample compared to its respective non-processed sample. The non-processed samples represent a 100 % of each individual alkaloid amount. Values (in %) with a negative sign denote a reduction in percentage from the respective non-processed sample. Values (in %) with no sign denote an increase in percentage compared to the respective non-processed sample. Boldened percentage values within a row are more than 15 % different than the non-processed sample

[a] (Trial 1): Diets to contain a premix that was steam exploded or not formulated for weanling pigs

[b] (Phase 1): refers to phase 1 diets containing a premix that was extruded or not formulated for growing pigs of BW between 65-90 kg

[c] (Phase 2): refers to phase 1 diets containing a premix that was extruded or not formulated for growing pigs of BW between 91-125 kg

[d]EA: Ergot alkaloid

Figure 3.3 shows the effect of processing on EA profiles. The diets were formulated to contain 4000 ppb EA. Treatments included diet samples from 2 swine trials; a 4000 ppb EA diet containing screenings (premix) that were steam exploded or not fed to weanling pigs (trial 1), and 4000 ppb EA contaminated diets containing screenings (premix) that were extruded or not (phase 1 and 2) and fed to grow-finish pigs (trial 2). The change in EA profile was similar regardless of processing.

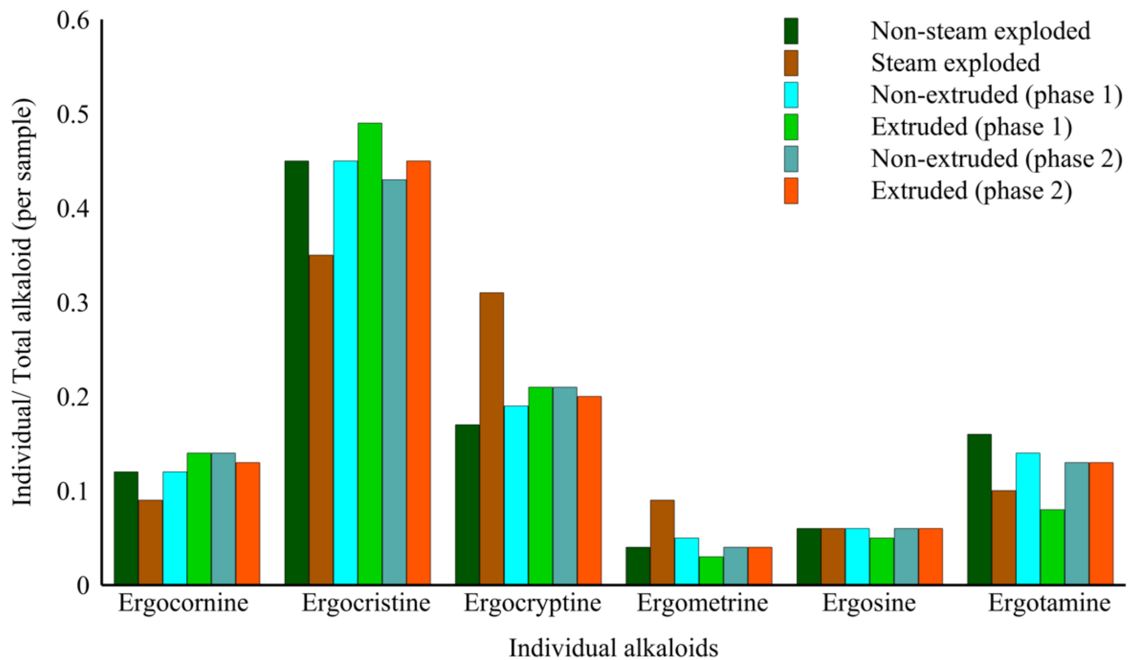

Figure 3.3. Effect of processing on EA profiles of contaminated screenings incorporated into swine diets formulated to contain 4000 ppb EA. Treatments were diets used for 2 swine trials; a 4000 ppb EA diet containing screenings (premix) that were steam exploded or not fed to weanling pigs (trial 1), and 4000 ppb EA contaminated diets containing screenings (premix) that were extruded or not (phase 1 and 2) and fed to grow-finish pigs (trial 2)

3.3.3 Effect of processing on ergot alkaloid epimer profile

Table 3.3 shows the effect of processing on EA epimer contents of wheat screenings that were pre-treated with water or not before steam explosion at 100 or 200 psi. No pre-treatment refers to no prior water treatment of samples before steam explosion at 100 or 200 psi, while for the "strained", the screenings were first rinsed with water and then drained immediately before steam explosion at 100 or 200 psi. Straining samples before steam explosion at 100 or 200 psi resulted in

a decrease in the amount of all epimers compared with the samples that had no prior water treatment, except for ergotamine where there was no effect on the amount of S epimers of the strained samples before steam explosion at 100 psi relative to the samples that had no prior water treatment. The epimer ratios were changed slightly for ergocornine, ergocristine, ergocryptine, ergosine and ergotamine for screenings that were steam exploded at both 100 and 200 psi. The only exception was ergometrine.

Table 3.3. Effect of processing on ergot alkaloid epimer (R and S) contents of contaminated wheat screenings that were treated with water or not before steam exploded at 100 or 200 psi

		Steam explosion at 100 psi		Steam explosion at 200 psi	
EA[a]	Epimer (ppb)	No pre-treatment	Strained	No pre-treatment	Strained
Egorconine	R	**655**	**189**	**2262**	**270**
Egorconinine	S	**1714**	**420**	**4833**	**587**
	R:S	**0.38**	**0.45**	0.46	0.46
Ergocristine	R	**1844**	**723**	**8224**	**752**
Ergocristinine	S	**4558**	**1653**	**14399**	**1490**
	R:S	0.40	0.44	0.57	0.50
Ergocryptine	R	**1513**	**448**	**4157**	**672**
Ergocryptinine	S	**2989**	**700**	**6632**	**1063**
	R:S	**0.50**	**0.64**	0.63	0.63
Ergometrine	R	**316**	**187**	**893**	**162**
Ergometrinine	S	**485**	**133**	**805**	**109**
	R:S	**0.65**	**1.41**	**1.10**	**1.49**
Ergosine	R	**358**	**159**	**1238**	**194**
Ergosinine	S	**267**	**127**	**752**	**138**
	R:S	1.34	1.25	**1.65**	**1.41**
Ergotamine	R	**242**	**306**	**3513**	**374**
Ergotaminine	S	197	218	**2557**	**244**
	R:S	**1.22**	**1.40**	**1.37**	**1.53**
Total	R	**4928**	**-2012**	**20287**	**2424**
	S	**10210**	**-3251**	**29978**	**3631**
	R:S	**0.48**	**0.61**	0.68	0.67

Note: Samples were wheat screenings that were treated with water or not (i.e., no prior water treatment or strained) before steam explosion at 100 or 200 psi. No pre-treatment refers to no prior water treatment of samples before steam explosion at 100 or 200 psi and strained refers to prior treatment of only washing screenings with water and then drained again before steam explosion at 100 or 200 psi. Boldened values within a row are more than 15 % different with respect to the no pre-treatment with water samples

[a]EA: Ergot alkaloid

Table 3.4 shows the effect of processing on EA epimers (R and S) of 4000 ppb EA diets containing premixes (contaminated wheat screening diluted with clean wheat screenings). Treatments include diets which contained premixes that were or were not processed by steam explosion or extrusion. Overall, a reduction in the amounts of both R and S epimers for alkaloids was observed for the diet which contained the steam exploded premix relative to the non steam exploded. The R epimers of ergocristine, ergocryptine and ergometrine but not ergoconine, ergosine and ergotamine were reduced in phase 1 diets which contained the extruded premix, however, there was a consistent increase in the amounts of S epimers of all EAs. The R epimers of ergocornine, ergocristine, ergometrine, ergosine and ergotamine but not ergocryptine were reduced in the phase 2 diets which also contained extruded premix whereas there was an increase in S epimers of ergocornine, ergocristine, ergocryptine and ergosine but no change of ergometrine and ergotamine. There was a change in epimer ratios for ergometrine and ergosine in the diets containing the steam exploded premix relative to the non-steam exploded but only a slight change in epimer ratios for ergocornine, ergocristine ergocryptine and ergotamine. However, there were changes in ratio for all alkaloids in the diets containing the extruded premix compared to the non-extruded. There were more consistent increases of the S epimers than the reductions of R epimers diets containing the extruded screenings compared to the diets containing non-extruded screenings.

Table 3.4. Effect of processing on ergot alkaloid epimer (R and S) contents in diets from 2 swine trials containing 4000 ppb ergot alkaloids

Alkaloid	Epimer (ppb)	Trial 1[a] Non Steam exploded	Trial 1[a] Steam Exploded	Trial 2 (phase 1)[b] Non Extruded	Trial 2 (phase 1)[b] Extruded	Trial 2 (phase 2)[c] Non Extruded	Trial 2 (phase 2)[c] Extruded
Ergorconine	R	**427**	**12**	221	204	366	**239**
Ergorconinine	S	**282**	**9**	**147**	**275**	**343**	**356**
	R:S	**1.54**	**1.30**	**1.50**	**0.74**	**1.06**	**0.67**
Ergocristine	R	**1733**	**51**	**864**	**746**	1237	**910**
Ergocristinine	S	**898**	**30**	**509**	**939**	**962**	**1150**
	R:S	**1.93**	**1.70**	**1.69**	**0.79**	**1.28**	**0.79**
Ergocryptine	R	**673**	**44**	**364**	**323**	583	**406**
Ergocryptinine	S	**330**	**28**	**217**	**403**	**466**	**508**
	R:S	**2.03**	**1.57**	**1.68**	**0.80**	**1.25**	**0.79**
Ergometrine	R	**143**	**7**	**109**	**110**	152	**121**
Ergometrinine	S	**76**	**13**	**40**	**56**	63	67
	R:S	**1.88**	**0.54**	**2.73**	**1.96**	**2.51**	**1.12**
Ergosine	R	**231**	**8**	122	123	166	**136**
Ergosinine	S	**90**	**9**	**57**	**92**	**117**	**121**
	R:S	**2.56**	**0.80**	**2.10**	**1.34**	1.41	1.12
Ergotamine	R	**652**	**16**	293	308	**378**	**291**
Ergotaminine	S	**277**	**7**	**122**	**226**	**269**	**286**
	R:S	2.35	2.29	**2.40**	**1.36**	1.40	1.01
Total	R	**3859**	**138**	**1973**	**1814**	**2882**	**2103**
	S	**1951**	**96**	**1092**	**1991**	**2220**	**2488**
	R:S	**1.98**	**1.43**	**1.80**	**0.91**	**1.29**	**0.84**

Note: Samples are diets which were formulated to contain 4000 ppb ergot alkaloids for pigs in phase 1 growth stage (65-90 kg BW) of 2 swine trials that contained screenings (premix) which were steam exploded or not and extruded or non-extruded. Boldened percentage values within a row are more than 15 % different from the respective diet which contained either a non steam exploded or non extruded premix

[a] (Trial 1): Diets to contain a premix that was steam exploded or not formulated for weanling pigs

[b] (Phase 1): refers to phase 1 diets containing a premix that was extruded or not formulated for growing pigs of BW between 65-90 kg

[c] (Phase 2): refers to phase 1 diets containing a premix that was extruded or not formulated for growing pigs of BW between 91-125 kg

3.4 Discussion

The increase in ergot infestation worldwide has led to an interest for a more detailed understanding of EAs and strategies aimed at reducing the impact of ergot toxicity (Thompson, 2016). There is some evidence that EA amounts may be reduced by grain processing (Mainka *et al*., 2005). Studies by Coufal-Majewski *et al*. (2017) showed that hydrothermal processing i.e., pelleting of ergot contaminated grains reduced toxicity but there was no information on whether the change in alkaloid concentrations with pelleting reduced toxicity. The objective of this study was to investigate the effects of various hydrothermal processing on the EA contents and epimers of ergot infected wheat and rye screenings.

Samples obtained for this study were heavily contaminated screenings, and diets formulated to contain 4000 ppb EA using these screenings. Prior to incorporation into the diets, the screenings were diluted with uncontaminated screenings to obtain a premix which was subjected to the processing treatments. Screenings and premixes were processed by steam explosion, extrusion, or pelleting and analyzed for EA and EA epimer content. Steam explosion for 2 or 5 mins reduced the ergot content of rye screenings between 39 and 42 %. Mild pelleting at 80˚C had no effect (<15 %) but increasing the pelleting (harsh) temperature to 100 ˚C resulted in a 25 % reduction of EA content relative to the unprocessed control. It is possible that the greater reduction of total ergot amounts for the steam exploded samples was due to the extremely high temperature employed via pressurized steam compared to the pelleted samples. The results of the present study indicated that the ergot content in the diet was reduced more when the premixes were steam exploded but there was an increase in the total EA content of the phase 1 diet which contained the extruded screenings compared to the non processed. The processing methods used in this thesis affected the EA contents in the samples differently. Compared to all the processing methods that have been used in research on feed materials, steam explosion is the application of a relatively higher-temperature pressurized steam for a short period followed by a sudden decompression of the cereal grain (Yu *et al*., 2012). Due to the severe nature of conditions employed during steam explosion, it has been shown to decrease neutral detergent fibre and acid detergent fibre (Liu *et al*., 2019). Steam explosion was used as a comparable methodology to test the effect of the most extreme form of processing on ergot-contaminated screenings. It is commonly used in the bioprocessing industries to disrupt lignocellulosic materials to make cellulose available for further enzymatic treatment (Ziegler-Devin *et al*., 2021). The current study

indicated that the overall EA content of the contaminated wheat and rye screenings was reduced more when samples were steam exploded than pelleting. Pelleting involves the application of a relatively lower temperature (about 90 ˚C), moisture and pressure but its effect will be less and not a severe combination of factors compared to steam explosion (Sánchez and Cardona, 2008). Coufal-Majewski *et al*. (2017) reported that hydrothermal processing i.e., pelleting does not reduce overall concentrations of EAs but observed changes in concentrations of individual alkaloids. In the current study, although ergot was not completely eliminated, there was a relatively greater reduction when screenings were steam exploded compared to the harsh and mild pelleted screenings. Similar to studies by Coufal-Majewski *et al*. (2017), individual EA amounts changed differently by all processing methods compared to their respective control samples in the present study. However, the levels of reduction were not consistent, which corresponds to previous studies conducted by Friedmann and Dao *et al*. (1990). The authors baked muffins from contaminated flour with different heating temperatures of baking and autoclaving and reported that alkaloid concentrations varied when subjected to different processing conditions indicating different relative susceptibilities of individual alkaloids to heat damage and initial alkaloid levels (Friedmann and Dao *et al*., 1990). Extrusion is a processing method where materials are conditioned with high temperature and pressure (Al-Marzooqi and Wiseman, 2009). EA analysis of the diets indicated that more contaminated screenings went into the phase 1 diet than phase 2. The observed increase of 19 % in total EA amount of the phase 1 diet containing the extruded premix in the present study was primarily due to the increases in ergocornine, ergocristine and ergocryptine content which were present in higher amounts of the diet containing the extruded premix compared to the diet containing the non-extruded premix. The change in profile of EAs in the diets were similar regardless of processing.

The addition of water can soften grains and increase the efficiency of processing methods (Matumba *et al*., 2015). In the present study, water was added as a pre-treatment to some screenings prior to steam explosion to test if the addition of water to the grain before steam explosion would affect the EA profile. Our results indicate that the effect of steam explosion was higher with the samples that were strained compared to the non pre-treated screenings and this could be attributed to the addition of water that facilitated the steam explosion processing method. At high pressure, steam penetrates into materials through diffusion and when the pressure suddenly releases, creates a shear force to cause the material to explode (Rahman *et al*., 2019). The addition

of water might have facilitated the steam explosion effectiveness. The epimer (R and S) amounts were reduced for all individual EAs when screenings were strained prior to steam explosion at 100 or 200 psi compared the with non pre-treated with water samples. The results of the present study indicated that there was a greater change in epimer ratio of ergometrine and slight changes of the other 5 EAs . According to Schiff (2006), ergometrine is the only alkaloid among the other principal alkaloids which is soluble in water. However, epimers of the other 5 EAs of the samples pre-teated with water before steam explosion were also reduced relative to the samples that were non pre-treated with water before steam explosion. A plausible explanation is that although the remaining 5 EAs are insoluble in water, the addition of water enhanced the effect of steam explosion.

Changes in epimer ratio (R:S) for diets containing the processed screenings were influenced by the epimer(s) that were increased or decreased. Results of this study indicate that, change in epimer profile was not consistent for steam explosion and extrusion for the diets containing 4000 ppb processed contaminated screenings (premix). A decrease in the R:S ratio was observed for the samples containing the screenings that were steam exploded due to a decrease in both R and S epimers. The increase in epimer amounts with extrusion observed was primarily due to an increase in the S epimers which was as a result of diet mixing. There was more of an increase of the S epimers of the diets containing extruded premixes compared to the reduction of the R epimers. Bryła *et al*. (2019) performed a study to assess the changes in R and S epimer contents during the production of bread. It was concluded in the study that epimerization of the R to S epimers was enhanced by baking. The authors reported that the concentration of R epimers decreased with a simultaneous increase in the S epimers and concluded that processing (baking) may contribute to a reduction in toxicity (Bryła *et al*., 2019). Similarly, increases in the S epimer and decreases in R epimer contents in the diets which contained the extruded premixes was observed in the present study. Changes in the epimer profile of the diet containing the extruded premix was observed. The results from this study shows that the impact of processing method employed on screenings affected the EA and epimer contents. The effects on EA content and EA epimer profile of ergot contaminated screenings depended on the hydrothermal processing method utilized.

3.5 Conclusion

Hydrothermal processing affected the EA amounts and their epimers differently and was related to the severity of processing. The effects on the epimer ratios in the diets and screenings depended on conditions (i.e., water addition) and epimers impacted by the processing method utilized. The extreme conditions employed in steam explosion had greater effects compared to extrusion.

CHAPTER 4: EFFECT OF EXTRUSION ON TOXICITY OF ERGOT ALKALOID WHEAT SCREENINGS WHEN INCLUDED IN DIETS FOR GROWING PIGS

4.1 Introduction

Ergot alkaloids are secondary metabolites produced by the fungus *Claviceps purpurea*. The fungus infects cereal grains (Mulac *et al*., 2012). The exposure of livestock, including ruminants, to ergot contaminated feed can cause reduced performance, reproductive problems and interfere with prolactin production, all which may have economic implications for livestock farmers (Coufal-Majeski *et al*., 2016). Ergot contamination may be increasing in Western Canada. Less than 5 % of the cereal samples submitted from 2002 to 2010 to the Canadian Grain Commission Harvest Sample (CGCHS) program were downgraded due to the content of ergot, while from 2011 to 2013, 17 % of the samples were downgraded (Tittlemier *et al*., 2015). EAs exist as 2 epimers; R (ines) and S (inines) (Mainka *et al*., 2005). There is some evidence that concentrations of ergot contaminated grains may be reduced by processing (Coufal-Majewski *et al*., 2016). According to Coufal-Majewski *et al*. (2017) pelleting reduced the negative impacts of EA on livestock performance. The authors showed that pelleting diets reduced the negative impacts of EA in lambs possibly due to changes in alkaloid profiles. Extrusion was chosen for the processing method to be utilized to process wheat screenings because changes in EA contents and epimer ratios were observed in chapter 3 of this thesis. The overall objective of this study was to determine whether extrusion of ergot contaminated wheat screenings would reduce toxicity, estimated as effects on growth performance, nutrient digestibility, and serum prolactin. It was hypothesized that extrusion of ergot contaminated grains would mitigate ergot toxicity measured as feed intake, growth, and prolactin output.

4.2 Materials and Methods

Animal protocols used in this study were reviewed and approved by the Animal Research Ethics Board at the University of Saskatchewan (AUP20130054) and followed principles established by the Canadian Council on Animal Care Guidelines for Humane Animal Use (CCAC, 2009). Two experiments were conducted, a growth performance and a nutrient digestibility trial.

4.2.1 Experiment 1: Growth performance trial

4.2.1.1 Animals, experimental housing, and design

A total of 160 grow-finisher pigs (Camborough Plus females x C337 sires; PIC Canada Ltd., Winnepeg, MB, Canada) consisting of 80 barrows and 80 gilts with an initial body weight (BW) range of 65 ± 4 kg were used in a 56d growth performance trial at the Prairie Swine Centre Inc. (Saskatoon, SK). Pigs were housed in groups of 5 per pen in environmentally controlled rooms. The selection of pigs (male and female) was done such that the average and standard deviation of BW was consistent among the pens. The pens were randomly assigned to 1 of the 4 dietary treatments in 2 rooms (blocks) in a randomized complete block design (RCBD). There were 16 pens with 8 pens assigned for males and females each, in each block. There were 4 pens per treatment in each block (n=8 pens per treatment). Block 1 began a week earlier than block 2.

Floors of the pens were fully slatted concrete over shallow manure pits. Gates were made of polyvinyl chloride (PVC) and concrete partitions divided pens. In each of the pens, there was a nipple drinker at the back and a single feeder situated at the top-right corner. The rooms were washed, sanitized and manure pits were emptied, a week before the beginning of the first block. An environmental controller (Phason, Winnepeg, MB) controlled ventilation and temperature which was set at 15 °C and relative humidity at ~40 %. Temperature of the room was checked and recorded daily. Rooms were kept on automated timers with 11 h light 13 h dark cycle.

4.2.1.2 Treatment diets

Pigs were fed a commercial diet until they were selected and placed in assigned pens for the growth performance trial. Experimental diets were prepared at the Canadian Feed Research Centre, North Battleford, SK. There were four treatments arranged in a 2 × 2 factorial with main effects of ergot level, (0 or 4000 ppb) and processing (extrusion or not). The ergot levels were achieved by formulating a "clean" premix (that contained only clean wheat screenings) and a diluted premix (that contained contaminated clean wheat screenings diluted with contaminated wheat screenings) of ergot levels of 0 and 240,000 ppb respectively. Premixes were extruded at 90 ˚C with 80-100 psi pressure. Extrusion equipment used for processing was the UAS-Muyang Extruder (MY56X2 Conditioner Model: SBTZ10). Diets (Table 4.1) were formulated for phase 1 to be fed to 60 to 90 kg BW pigs and phase 2 to be fed to 90 to 120 kg BW pigs. Phase 1 and phase 2 diets were similar, the only difference was reduced amino acids in phase 2. Diets were typical Western Canadian wheat-barley-soybean meal diets formulated to be isonitrogenous and

isoenergetic to meet or exceed the recommended nutrient requirements for growing pigs in each phase (NRC, 2012). Feed and water were provided *ad libitum*. Pens were checked daily to ensure that pigs had access to feed and ensure pig well being and health.

Table 4.1. Mycotoxin content of ergot-contaminated wheat screenings used to formulate premixes incorporated into experimental diets

Mycotoxin	Content (ppb)
Total ergot	438500
Deoxynivalenol	258.1
HT-2 toxin	39.5
Fumonisin B1	20.5
Fumonisin B2	5.67
Aflatoxin B1	4.05
3+15-acetyldeoxynivalenol	<16
Diacetoxyscirpenol	<16
Nivalenol	<16
T-2 toxin	<4
Zearalenone	<4
α-Zearalenol	<4
β-Zearalenol	<4
Ochratoxin A	<0.4

Table 4.2. Ingredient and nutrient composition (as-fed basis) of the diets used in a 2 × 2 factorial arrangement (EA and extrusion) for experiment 1 and 2 in phase 1 (60-90 kg BW)[a]

	0 ppb EA[b]		4000 ppb EA[c]	
Extruded	No	Yes	No	Yes
Ingredient (%)				
Wheat	50.00	50.00	50.00	50.00
Clean non-extruded[d]	1.67	0.00	0.00	0.00
Clean extruded[e]	0.00	0.00	1.67	0.00
Ergot non-extruded[f]	0.00	1.67	0.00	0.00
Ergot extruded[g]	0.00	0.00	0.00	1.67
Wheat screenings	8.33	8.33	8.33	8.33
Barley	27.86	27.86	27.86	27.86
Soybean meal	8.74	8.74	8.74	8.74
Canola oil	0.89	0.89	0.89	0.89
Dicalcium Phosphate	0.33	0.33	0.33	0.33
Vitamin/Mineral premix[h]	0.20	0.20	0.20	0.20
Limestone	1.19	1.19	1.19	1.19
Salt	0.20	0.20	0.20	0.20
L-Lysine-HCl[i]	0.36	0.36	0.36	0.36
DL-Methionine	0.02	0.02	0.02	0.02
L-Threonine	0.21	0.21	0.21	0.21
Calculated Nutrient Content				
Net Energy, kcal/kg	2394	2394	2394	2394
Crude protein, %	16.04	16.04	16.04	16.04
SID[j] Lysine, %	0.78	0.78	0.78	0.78
Calcium, %	0.56	0.56	0.56	0.56
STTD[k] phosphorus, %	0.46	0.46	0.46	0.46
Analyzed Nutrient Content				
Dry matter, %	86.55	88.42	86.54	86.42
Gross Energy, kcal/kg	3846	3922	3918	3916
Crude protein, %	15.07	15.58	15.68	16.30
Acid detergent fibre, %	5.51	4.73	4.78	4.79
Neutral detergent fibre, %	12.07	12.50	11.52	13.12

[a](60-90 kg): Weight range of pigs on phase 1 diets
[b]0 ppb EA: diet formulated to contain 0 ppb EA with only clean wheat screenings (premix)
[c]4000 ppb EA: diet formulated to contain 4000 ppb EA with clean and ergot contaminated wheat screenings (premix)
[d] Clean non-extruded: Premix containing clean wheat screenings which were non-extruded
[e] Clean extruded: Premix containing clean wheat screenings which were extruded
[f] Ergot non-extruded: Premix containing diluted (clean and contaminated) ergot wheat screening which were non-extruded
[g]Ergot extruded: Premix containing diluted (clean and contaminated) ergot wheat screening which were extruded
[h] Supplied per kg of complete diet: vitamin A, 8000 IU; vitamin D, 1500 IU; vitamin E, 30 IU; menadione, 2.5 mg; vitamin B12, 0.025 mg; thiamine, 1.00 mg; biotin, 0.10 mg; niacin, 20 mg; riboflavin, 4 mg; pantothenate; 12 mg; folic acid, 0.50 mg; pyridoxine, 2.0 mg; Fe, 100 mg; Zn, 100 mg; Mg, 40 mg; Cu, 15 mg; Se, 0.30 mg; and I, 1mg
[i]HCl: hydrochloric acid
[j]SID: standardized ileal digestibility
[k]STTD, standardized total tract digestibility

4.2.1.3 Sample and data collection

Blood samples were taken via the cranial vena cava from an average BW pig from each pen on d 7 and 56 of each block into a 5.5 ml vacutainer (BD, Mississauga, ON, Canada). The serum

tubes had no anticoagulant. Collected whole blood samples were put in racks at room temperature and sent to the lab to be centrifuged at 2500 × g for 15 mins, and then serum samples were stored at -20 °C prior to further analysis.

Pigs were weighed individually each week for the first 4 wks and biweekly for the remaining 4wks of the 56d period. Feed disappearance was calculated every weigh day by deducting the remaining feed in the feeder from the amount provided. Average daily feed intake (ADFI) was calculated by dividing the feed disappearance by the number of days between consecutive weigh days. Average daily gain (ADG) per pig was calculated by dividing the total weight gain by the number of days between 2 consecutive weigh days. Gain: Feed (G: F) was calculated by dividing the ADG by the ADFI.

4.2.2 Experiment 2: Digestibility trial

4.2.2.1 Experimental housing and design

A total of 32 growing pigs (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada); 16 barrows and 16 gilts with an initial weight of 75 ± 5 kg were used in this study at the Prairie Swine Centre, Inc. (Saskatoon, Canada). Pigs were individually housed in metabolic crates (1.5 × 1.5 m) in an environmentally controlled room. The room was maintained at 16-18 ˚C, humidity at ~40 % and a 12-12 h light-dark photoperiod. The crates had 0.9 m polyvinyl chloride walls, with plexiglass windows between crates (0.3 × 0.3 m) to allow pigs to see each other and serve as an enrichment. Pigs had access to a single space dry feeder and a bowl drinker. Each metabolic crate had a urine collection tray underneath. Considering the BW, pigs were assigned to one of 4 dietary treatments with 16 pigs per block over two blocks providing an n of 8 pigs per treatment in an RCBD. The pigs were adapted for 7 days to the diets containing celite (4 days in grow finish room and 3 days after moving into the metabolic room) prior to fecal and urine collection. Individual BW of 75 ± 5 kg was recorded following adaptation of the pigs in the metabolic room. The dietary treatments were arranged as a 2×2 factorial in an RCBD with main factors of ergot level, (0 or 4000 ppb) and processing (extruded or not).

4.2.2.2 Experimental diets

The same diet used for the phase 1 performance experiment was used for the digestibility experiment. Diets were formulated to meet or exceed NRC (2012) recommendations for 60 to 120

kg BW pigs (Table 4.1). The diets were isonitrogenous and isoenergetic and a source of acid insoluble ash (AIA), 0.4 % celite (celite 545, Celite Corporation, Lompoc CA, USA) was used as an indigestible marker to allow estimation of nutrient digestibility. Feed was offered to provide 3 x maintenance energy requirement (197 kcal ME/kg x $BW^{0.60}$, NRC 2012). Feed allocated was divided into two equal amounts and fed to the pigs twice daily at 0600 h and 1400 h. Clean water was provided *ad libitum* throughout the experiment.

4.2.2.3 Sample collection

During the digestibility study, there was a 7 d adaptation to the experimental diets followed by a 4 d collection of fecal and urine samples. Fresh fecal grab samples were collected daily for each pig and frozen immediately at -20 ^{o}C. At the end of the experiment, fecal samples were thawed, pooled for each pig, and homogenised. Subsamples were taken and stored at -20 ^{o}C. Urine samples were collected quantitatively for 4 d with urine jars placed under the metabolism crates for each 24 h period. A funnel with glass wool was placed into the opening of each jar to reduce contamination of the urine. At the end of each day (24 h), total urine was weighed, and a 5 % (by weight) subsample per pig was retained. At the end of each block, the subsamples were pooled within pig per collection period, and an aliquot was stored at -20 ^{o}C until further analysis.

4.2.3 Analytical procedures

4.2.3.1 Analysis of feed, fecal, and urine samples

Fecal samples were dried in a forced-air draft oven (Despatch V-3) at 55 ^{o}C for 3d before grinding in a centrifugal mill (ZM 100, RETSCH GmbH & Co. Rheinische Straße, 35 Germany) through a 1 mm sieve. Analysis of dry matter (DM), acid insoluble ash (AIA), crude protein, gross energy (GE) and urine were conducted in the General Nutrition Lab at the Department of Animal Science, University of Saskatchewan, whereas the acid detergent fibre (ADF), neutral detergent fibre (NDF), and starch analysis were done at Central Testing Lab Inc (Winnipeg, MB). The dry matter (DM) content of the feces and diets was measured in duplicate by drying at 135 ^{o}C in an airflow-type oven for 2 hrs by method 930.15 (Association of Official Analytical Chemists (AOAC) 2007). The AIA content of the fecal and diet samples was measured in duplicate and triplicates respectively, according to methods of van Keulen and Young (1997). The GE content of the feces and feed was analyzed by bomb calorimeter (6400 automatic Isoperibol system, Parr

Instruments Company Illinois, USA). Nitrogen content of the feces, feed and urine samples was analyzed by combustion with an automatic analyzer (LECO FP 528; MI, USA; Method 990.03; AOAC 2007). The NDF and ADF contents of the feces were determined according to AOAC 991.43 (AOAC, 2007) using an ANKOM 200 fibre analyzer (ANKOM Technology, Macedon NY).

4.2.3.2 Ergot alkaloid analysis

Ergot alkaloid analysis of screenings and diets were performed as described previously in Chapter 3 section 3.4.3.

4.2.3.3 Prolactin

Serum samples, packed in dry ice were shipped to the Agriculture Canada research station lab at Lennoxville, QC. Samples arrived within 24h. Samples were analyzed for prolactin, a sensitive indicator of ergot toxicity using a radioimmunoassay (RIA) according to Robert *et al.* (1989) except 400 μL of serum sample was used. The average recovery calculated by addition of various doses of radioinert prolactin to 50 μL of a pooled sample was 94.8 %. Sensitivity of the assay was 1.5 ng/mL. The intra- and inter- assay CV were 3.62 % and 6.65 %, respectively.

4.2.4 Statistical analysis

Statistical analyses were conducted using the PROC MIXED procedure of SAS (version 9.4, SAS Institute, Inc. Cary, NC). The univariate procedure of SAS was used to verify the normality of the data. All data analyzed in this study were normally distributed (Shapiro-Wilk test; $P>0.05$). Data from the digestibility and growth performance (weekly, phases and overall, for ADFI, ADG and gain to feed (G: F)) study were analyzed using analysis of variance (ANOVA) for RCBD with treatments arrangement as a 2×2 factorial. Data were analyzed with ergot level (n=2; fixed effect), extrusion (n=2; fixed effect), and block (n=2) as a random effect and interactions included in the model. Significance was determined at $P<0.05$, and a tendency towards significance was defined as $0.05 \leq P<0.10$. Data for prolactin at d 7 and 56 were analysed with ergot level (n=2; fixed effect), extrusion (n=2; fixed effect), Sex (n=2; fixed effect) and block (n=2) as a random effect and interactions included in the model. Days were analyzed independently.

4.3 Results

4.3.1 Ergot alkaloid levels in diets

Prior to the beginning of the performance and digestibility experiments, a mixture (premix) containing contaminated wheat screenings diluted with clean wheat screenings was formulated and then analyzed for ergot content (Table 4.2). The experimental diets (phase 1 and 2) that were formulated were also analyzed for EA contents. The total EA content of the 0 ppb diet containing the extruded premix was reduced compared to the non extruded control whereas the total EA content of the 4000 ppb diets containing the extruded premix was increased relative to the diet containing the non extruded premix (Tables 4.3 and 4.4).

Table 4.3. Ergot alkaloid content of premixes that were incorporated into experimental diets

	0 ppb EA[a]		4000 ppb EA[a]	
Extruded	No	Yes	No	Yes
Alkaloid (ppb)				
Ergocornine	127	47	17286	18948
Ergocorninine	86	80	17006	9457
Ergocristine	371	268	70118	76670
Ergocristinine	226	383	62622	30288
Ergocryptine	201	115	28422	31207
Ergocryptinine	108	125	27494	14728
Ergometrine	61	33	8787	7946
Ergometrinine	19	23	3598	2344
Ergosine	78	37	8697	8721
Ergosinine	51	48	6527	3311
Ergotamine	89	77	1344	21438
Ergotaminine	66	113	15953	7000
Total	1483	1349	267854	232058

Note: 0 ppb EA refers to diet formulated to contain 0 ppb EA with only clean wheat screenings (premix) and incorporated into the diet. 4000 ppb EA refers to diet formulated to contain 4000 ppb EA with clean and ergot contaminated wheat screenings (premix) and incorporated into the diet. Alkaloids include individual R or 'ine'-epimers, and S or 'inine'-epimers
[a]EA: Ergot alkaloid
ppb: parts per billion

Table 4.4. Ergot alkaloid content of phase 1diets used for the performance and nutrient digestibility experiment

	0 ppb EA[a]		4000 ppb EA[a]	
Extruded	No	Yes	No	Yes
Alkaloid (ppb)				
Ergocornine	2	3	221	204
Ergocorninine	1	3	147	275
Ergocristine	70	11	864	746
Ergocristinine	30	11	509	939
Ergocryptine	6	5	364	323
Ergocryptinine	3	5	217	403
Ergometrine	16	3	109	110
Ergometrinine	4	ND[b]	40	56
Ergosine	2	2	122	123
Ergosinine	1	2	57	92
Ergotamine	22	5	293	307
Ergotaminine	10	3	122	226
Total	167	53	3065	3804

Note: 0 ppb EA refers to diet formulated to contain 0 ppb EA with only clean wheat screenings (premix) and incorporated into the diet. 4000 ppb EA refers to diet formulated to contain 4000 ppb EA with clean and ergot contaminated wheat screenings (premix) and incorporated into the diet. Alkaloids include individual R or 'ine'-epimers, and S or 'inine'-epimers

[a]EA: Ergot alkaloid

[b]ND: Not detected

ppb: parts per billion

Table 4.5. Ergot alkaloid content of phase 2 diets used for the performance experiment

	0 ppb EA[a]		4000 ppb EA[a]	
Extruded	No	Yes	No	Yes
Alkaloid (ppb)				
Ergocornine	71	11	366	239
Ergocorninine	8	15	343	356
Ergocristine	62	57	1237	910
Ergocristinine	63	55	962	1150
Ergocryptine	8	1	583	406
Ergocryptinine	15	23	466	508
Ergometrine	5	5	152	121
Ergometrinine	2	2	63	67
Ergosine	9	8	166	136
Ergosinine	7	7	117	121
Ergotamine	17	15	378	291
Ergotaminine	15	ND	269	286
Total	282	199	5102	4591

Note: 0 ppb EA refers to diet formulated to contain 0 ppb EA with only clean wheat screenings (premix) and incorporated into the diet. 4000 ppb EA refers to diet formulated to contain 4000 ppb EA with clean and ergot contaminated wheat screenings (premix) and incorporated into the diet. Alkaloids include individual R or 'ine'-epimers, and S or 'inine'-epimers
[a]EA: Ergot alkaloid
[b]ND: Not detected
ppb: parts per billion

4.3.2 Growth performance

Most pigs remained healthy throughout the experiment and consumed their feed. Only five pigs were culled in total. Three pigs in total were culled from block 1. One pig was culled on d13 of the experiment (treatment diet: 0 ppb and no extrusion). Two pigs were culled in weeks 5-6: one pig on d40 (treatment diet: 4000 ppb and extrusion) and the other on d42 (treatment diet: 0 ppb and no extrusion). Only one pig was culled in block 2 (treatment diet: 4000 ppb and no extrusion). Growth performance results, including BW, ADFI, ADG and G: F are presented in Table 4.6. There was no effect of ergot by extrusion interactions on any of the growth or feed intake measurements (P>0.10). Pigs that consumed the diet formulated to contain 0 ppb EA had higher BW on d14, d21,

d28 and d42 compared to pigs that consumed the diet formulated to contain 4000 ppb EA (P<0.05). Pigs fed diets containing 4000 ppb EA had reduced ADG on d0-7 (P=0.008), d22-28 (P=0.03), phase 1 (P=0.001) and overall period (P=0.001). Pigs fed contaminated diets (4000 ppb EA) had a reduced G: F on d 0-7 (P=0.03), and d22-28 (P=0.03).

Table 4.6. Effect of on performance of growing pigs fed diets containing ergot levels (0 and 4000 ppb) which were extruded or not in experiment 1

Parameter		Ergot		Extruded		Pooled SEM	*P*-value		
		0 ppb	4000 ppb	No	Yes		Ergot	Extrusion	Ergot × Extrusion
BW, kg									
	Initial	65.94	65.82	65.87	65.9	1.54	0.84	0.97	0.58
	d7	72.38	70.89	71.66	71.61	1.53	0.06	0.94	0.92
	d14	79.50	77.64	78.73	78.41	1.63	0.03	0.70	0.96
	d21	88.10	85.89	87.30	86.60	1.18	0.03	0.55	0.79
	d28	96.47	93.03	94.91	94.60	1.03	0.002	0.76	0.88
	d42	112.56	109.55	111.33	110.77	1.15	0.01	0.62	0.32
	d56	124.98	123.14	124.41	123.71	1.54	0.11	0.55	0.51
ADG, kg/d									
	phase 1	1.07	0.95	1.03	1.00	0.03	0.001	0.36	0.28
	phase 2	1.08	1.05	1.07	1.07	0.03	0.33	0.90	0.23
	overall	1.08	1.00	1.05	1.03	0.02	0.001	0.36	0.92
	d0-7	0.80	0.61	0.70	0.71	0.05	0.008	0.75	0.26
	d8-14	1.20	1.08	1.18	1.10	0.06	0.05	0.20	0.86
	d15-21	1.21	1.17	1.21	1.18	0.08	0.45	0.55	0.50
	d22-28	1.19	1.02	1.09	1.12	0.07	0.03	0.67	0.39
	d29-42	1.15	1.16	1.18	1.16	0.03	0.80	0.27	0.07
	d43-56	0.95	0.97	0.95	0.93	0.06	0.22	0.76	0.75
ADFI, kg/d									
	phase 1	2.61	2.47	2.53	2.54	0.14	0.25	0.91	0.94
	phase 2	3.16	3.04	3.08	3.13	0.11	0.16	0.52	0.17
	overall	2.88	2.76	2.80	2.84	0.11	0.13	0.68	0.54
	d0-7	2.10	1.91	1.98	2.03	0.10	0.09	0.65	0.50
	d8-14	2.63	2.64	2.62	2.65	0.21	0.98	0.86	0.63
	d15-21	3.10	2.86	2.99	2.96	0.15	0.12	0.79	0.86
	d22-28	3.44	3.47	3.43	3.48	0.29	0.77	0.73	0.32
	d29-42	3.13	2.89	3.00	3.01	0.12	0.06	0.96	0.76
	d43-56	2.98	2.78	2.79	2.89	0.14	0.39	0.47	0.30

G: F									
	phase 1	0.41	0.38	0.40	0.38	0.01	0.06	0.40	0.30
	phase 2	0.34	0.35	0.35	0.34	0.01	0.51	0.39	0.72
	overall	0.38	0.36	0.38	0.36	0.01	0.21	0.21	0.32
	d0-7	0.38	0.32	0.35	0.35	0.03	0.03	1.00	0.36
	d8-14	0.46	0.42	0.46	0.42	0.04	0.13	0.13	0.46
	d15-21	0.40	0.41	0.40	0.40	0.02	0.53	0.82	0.82
	d22-28	0.35	0.30	0.32	0.33	0.02	0.03	0.84	0.75
	d29-42	0.37	0.41	0.40	0.38	0.07	0.07	0.40	0.40
	d43-56	0.31	0.33	0.34	0.32	0.02	0.10	0.44	0.24

Note: 0 ppb refers to diet formulated to contain 0 ppb EA with only clean wheat screenings (premix) and incorporated into the diet. 4000 ppb refers to diet formulated to contain 4000 ppb EA with clean and ergot contaminated wheat screenings (premix) and incorporated into the diet. Values are least-squares means (n=8 pens/treatment). Phase 1 refers to pigs in growth stage 60-90 kg and phase 2, 91-125 kg BW

ppb: parts per billion

SEM: standard error of mean

4.3.3 Prolactin

Results from the prolactin analysis are presented in Table 4.6. There was no effect of extrusion or sex on prolactin concentration (*P*>0.10). Prolactin concentration was reduced for pigs that consumed the 4000 ppb EA diets on both d 7 and d 56 (*P*<0.05). No interactive effects were observed for ergot, sex, and/or extrusion (*P*>0.10).

Table 4.7. Effect of ergot (0 and 4000 ppb), extrusion, and sex on serum prolactin (ng/mL) of growing pigs

	Ergot		Processing		Sex			*P*-value		
Day	0 ppb	4000 ppb	Yes	No	Male	Female	Pooled SEM	Ergot	Extrusion	Ergot × Extrusion
7	1.67	0.57	1.17	1.07	1.11	1.13	0.16	<0.001	0.49	0.99
56	0.71	0.43	0.52	0.62	0.62	0.52	0.11	0.03	0.38	0.40

Notes: 0 ppb refers to diet formulated to contain 0 ppb EA with only clean wheat screenings (premix) and incorporated into the diet. 4000 ppb refers to diet formulated to contain 4000 ppb EA with clean and ergot contaminated wheat screenings (premix) and incorporated into the diet. Values are least-squares means (n=8 pens/treatment). There was no interaction of extrusion × sex, ergot × sex or extrusion × ergot × sex (P>0.05)

SEM: standard error of mean

4.3.4 Nutrient digestibility

All pigs remained healthy throughout the experiment and consumed their allocated feed readily during the digestibility study period. Results of the effect of ergot and extrusion on nutrient digestibility are presented in Table 4.7. Extrusion of contaminated screenings in the diets reduced N intake (g/d) ($P<0.01$). Pigs that consumed ergot contaminated diets had greater fecal N output (g/d) ($P=0.03$). There were no interactions of ergot by extrusion ($P>0.10$).

Table 4.8. Effect on nutrient digestibility of growing pigs fed diets containing ergot levels (0 and 4000 ppb) which were extruded or not in experiment 2

	Ergot		Extrusion			*P*-value		
Item	0 ppb	4000 ppb	No	Yes	Pooled SEM	Ergot	Extrusion	Ergot × Extrusion
N intake, g/d	64.39	65.67	63.60	66.45	0.85	0.08	<0.01	0.30
ATTD[b] of N[a], %	85.14	83.70	84.49	84.33	0.51	0.06	0.82	0.48
Fecal N output, g/d	9.60	10.70	10.42	9.86	0.34	0.03	0.25	0.31
Urine N output, g/d	5.03	5.00	3.91	6.12	0.73	0.97	0.02	0.26
N retention, g/d	49.83	49.91	49.83	49.92	0.78	0.94	0.94	0.23
N retention, % of intake	77.34	76.19	78.44	75.09	1.15	0.49	0.05	0.49

Note: 0 ppb refers to diet formulated to contain 0 ppb EA with only clean wheat screenings (premix) and incorporated into the diet. 4000 ppb EA refers to diet formulated to contain 4000 ppb EA with clean and ergot contaminated wheat screenings (premix) and incorporated into the diet. Values are least-squares means (n=8 pens/treatment)

[a] SEM: standard error of mean

[b] N: nitrogen

[c] ATTD: apparent total tract digestibility

4.4 Discussion

Unlike other mycotoxins such as deoxynivalenol or zearalenone which are of concern only to swine and poultry, ergot affects all classes of livestock, including ruminants (Coufal-Majewski *et al*., 2016). It has been suggested that hydrothermal processing may reduce the negative impacts of the EAs (Bryła *et al*., 2019). For example, Coufal-Majewski *et al*. (2017) showed that pelleting diets reduced the negative impacts of EA in lambs. Earlier studies have implied that epimers differ in toxicity (Smith and Shappell, 2002; Klotz, 2015b) and more increases was observed in the S epimers compared to the reduction of the R epimers of EAs in diets containing extruded screenings. The objective of the present study was to determine if extrusion of contaminated wheat screenings reduces toxicity when included in diets of growing pigs. Toxicity was estimated as effects on growth performance, nutrient digestibility, and serum prolactin. Diets for the current study were formulated to contain processed or unprocessed wheat screenings, included in the diet to attain ergot levels of 0 or 4000 ppb.

4.4.1 Performance of pigs fed diets containing extruded ergot contaminated wheat screenings

Ergotism is associated with poor performance (Oresanya *et al*., 2003) and the negative impacts due to EA exposure on animal productivity have been well documented (Klotz, 2015a). Clinical signs following the ingestion of EA infected diets include reduced feed intake and decreased weight gain (Oresanya *et al*., 2003; Coufal-Majewski *et al*., 2016). Comparable to previous studies conducted with weanling pigs fed up to 1800 ppb EA (Oresanya *et al*., 2003) the classical signs of ergotism, including those affecting the nervous system were not observed in the present study (Oresanya *et al*., 2003). The present study was a 56d experiment, and pigs were assigned to the experimental diets at 65 kg BW. In the first week of exposure when the pigs consumed the 4000 ppb EA diet, a tendency for reduction in feed intake was observed. A negative reaction to the gustatory and aromatic properties of EAs in 17 kg BW pigs fed diets containing 1100 ppb was observed in a study done by Whittemore *et al*. (1977). It is possible that there was reduced palatability observed as a reduction in feed intake in the present study for the 4000 ppb EA diet in the first couple of weeks, however, pigs apparently adapted. The effect on ADFI with initial exposure was reflected in ADG and BW. In the present study, there was a numerical decrease in N retention expressed as a % of intake when pigs were fed the 4000 ppb EA diet compared to the 0 ppb EA diet. The reduction in feed intake and digestibility decreases nutrients

available for animal growth (Hannah *et al*., 1990). Grenier and Applegate (2013) reported that reduced BW is apparently the consequence of decreased feed intake, but there is also a close association between performance and nutrient digestibility, which suggests that reduced nutrient digestibility may have influenced the decrease in ADG overall. Studies in this thesis aimed to examine the effect of extrusion on ergot contaminated wheat. We observed no ergot by extrusion interactions. There is some evidence that concentrations of ergot contaminated grains may be reduced by processing (Coufal-Majewski *et al*., 2016). Coufal-Majewski *et al*. (2017) reported that pelleting reduced the negative impacts of EAs by altering the EA contents and profiles. A previous study examined changes in contents of R and S epimers in baked bread (Bryła *et al*., 2019). It was hypothesized that the reduction in negative effects (toxicity) would occur through changes in EAs and epimer ratios when ergot contaminated screenings were extruded, however, there was no effect of change in epimer ratios on the performance of pigs. Extruding wheat screenings before incorporation into the diets changed EA contents and epimer profiles. However, there was no evidence that extruding wheat screenings mitigated EA toxicity, hence negative effects (performance) of ergot on performance of growing pigs fed the 4000 ppb EA contaminated diets as was observed as shown in our results. Perhaps, even though extrusion resulted in increases of S epimers which has been stated as biologically inactive (Wolff *et al*., 1988) than the reduction of the R epimers, it did not result in any effect to mitigate toxicity estimated as a reduction in the performance of pigs indicating that both epimers have similar toxicity. Extrusion did not mitigate the negative effects on the growth performance of pigs fed ergot contaminated diets.

4.4.2 Effects on serum prolactin of pigs fed diets containing processed ergot contaminated wheat screenings

Decreases in serum prolactin via EA actions on dopaminergic pathways have been associated with and used as an indicator of EA exposure in livestock such as cattle and swine (Oresanya *et al*., 2003; Klotz, 2015a). Exposure to EA may result in decreased concentration of prolactin (Coufal-Majewski *et al*., 2017). In the present study, extrusion did not mitigate the effect of ergot on prolactin in pigs fed 4000 ppb EA diets containing extruded screenings. Our results showed that there was a decline in prolactin levels by 66 % and 39 % after d7 and d56 after exposure, respectively, when the pigs were fed 4000 ppb EA diets. The greater decline of serum prolactin in the present study in the first week of exposure was perhaps because the pigs had just

been introduced to the ergot-contaminated diet and there was a greater response compared to the later measurement of prolactin on d56. Perhaps Similarly, there was a suppression of serum prolactin in during gestation of sheep with prolactin concentrations remaining reduced after 130d of gestation but lesser than in the first few weeks (Duckett *et al*., 2014; Coufal-Majewski *et al*., 2017). Serum samples were collected during the performance trial and were analyzed for serum prolactin. The reduction in palatability observed as a decrease in feed intake for the 4000 ppb EA diet in the first few weeks and a later adaptation to the diets in the consequent weeks in the performance trial could be a plausible explanation to the declines in serum prolactin in the present study. We observed changes in the epimer profile with extrusion, however, this effect on the epimer profile did not mitigate toxicity estimated as effects on serum prolactin. There was no effect of ergot by extrusion interaction effect on prolactin in pigs. The consumption of ergot contaminated grains reduces prolactin levels (Oresanya *et al*., 2003), and there is evidence that processing may decrease the toxicity of EAs. The change in EA and epimer profile of diets containing extruded screenings was influenced more by the increase of the S than the reduction of the R epimers, and this did not result to any effect on reducing toxicity defined as reducing the negative effects on serum prolactin in the present study. Effects of ergot were observed however, there was no effect of ergot by extrusion interaction on prolactin in pigs.

4.4.3 Nutrient digestibility of pigs fed diets containing extruded ergot contaminated screenings wheat screenings

In the current study, there were differences in CP contents of the diets and thus, N retention on a % basis of intake was accounted for. Diets were formulated to be isonitrogenous however, analysis indicated that one of the diets had an increased CP content. The increased N intake (g/d) when pigs consumed the diet containing the 4000 ppb EA results from increased CP content of the diet containing the extruded premix, and not changes in voluntary feed intake as the pigs were limit fed. Studies done by Friend and MacIntyre (1970) showed that N retention was reduced with the inclusion of EA in pig diets and our results showed that there was a tendency for reduction of % ATTD of N in diets with EA contamination. This is consistent with a study by Whittemore *et al*. (1976) indicating that ergot ingestion would reduce the efficiency of N utilization. The observation of a significant effect on fecal N output for the 4000 ppb EA diet compared to the control in the present study was due to the increased N content of the diet. Processing treatments

for e.g., extrusion, may affect the uptake of nutrients and perhaps alter digestibility (Araba and Dale, 1990; Nursten *et al*., 2005). In the present study, only the screenings were extruded and not the whole diet. There was no effect of ergot on N-digestibility but only a main effect of extrusion. It is difficult therefore to explain why N digestibility was affected when less than 2 % of the diet was extruded. Although our results showed an increase of N intake with extrusion, there was no ergot by extrusion interaction. This implies that extrusion did not mitigate the effect of ergot on N digestibility as hypothesized.

4.5 Conclusion

Extrusion of wheat screenings before incorporation into the diets in the current study did not mitigate the negative effects of EA in swine diets. The prolactin result indicates that care should be taken to ensure EA contaminated feed is not consumed by sows.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSION

The Food and Agricultural Organization (FAO) has estimated that at least 25 % of grain produced worldwide is contaminated with mycotoxins, and that mycotoxins such as EAs result in decreased grain yields (Eskola *et al*., 2020) and reduce animal performance when fed to livestock (Coufal-Majewski *et al*., 2016). Studies have investigated the effects of grain processing as a potential strategy for mycotoxin reduction (Fandohan *et al*., 2005, Mainka *et al*., 2005). Ergot alkaloid contents in grains may be reduced by processing (Mainka *et al*., 2005; Coufal-Majewski *et al*., 2017). Previous studies have reported that grain processing enhanced epimerization of the alkaloids, which resulted in the transformation of R epimers into S epimers (Merkel *et al*., 2012). The studies in this thesis aimed to determine the effect of hydrothermal processing on EA amounts and their epimer profiles, and to assess the effect of processing ergot contaminated wheat screenings on toxicity when included in diets of growing swine. Toxicity was measured as effects on growth and feed intake, nutrient digestibility, and serum prolactin. It was hypothesized that hydrothermal processing would reduce EA amounts, change the alkaloid and epimer profile and the extent of these changes would be related to the severity of the processing. It was further hypothesized that the effects of EA on growth, feed intake, nutrient digestibility, and serum prolactin in pigs will be mitigated when the contaminated feed has been subjected to processing.

The samples used were heavily contaminated wheat and rye screenings, or diets formulated to contain 4000 ppb EA with the inclusion of the screenings that were processed or not. Screenings were processed by pelleting, extrusion, or steam explosion. Total EA content was reduced more when screenings were steam exploded than when the screenings were pelleted or extruded. Steam explosion uses high-temperature (180-240 ˚C) pressurized steam for a brief period before the rapid release of pressure in an explosive decompression manner which opens up the fibres and increases the surface area of the grain (Yu *et al*., 2012; Marques *et al*., 2020). Steam explosion reduced EAs more compared to pelleting or extrusion. Although pelleting also utilizes moisture, heat ((mild (80 ˚C) or harsh (90-100 ˚C)), and pressure, its effect was less compared to steam explosion because of the application of a relatively lower temperature and pressure. In the current study, we attribute the greater extent of reduction steam explosion had on the total amount of ergot in the screenings and diets to the severe nature of high temperature employed via pressurized steam compared to the pelleted samples. With the evidence from literature of hydrothermal processing as a technology or practical measure to decrease EA contents in contaminated grains that can be fed to livestock

(Coufal-Majewski *et al*., 2016), we expected the EA contents of diets containing the contaminated premix to be reduced with extrusion. Extrusion is a processing technique where materials are conditioned with a relatively higher temperature (100 ˚C) and pressure (Al-Marzooqi and Wiseman, 2009) compared with pelleting. Coufal-Majewski *et al*. (2017) reported that pelleting does not eliminate the total content of EAs but observed changes of a decrease or increase in individual alkaloid concentrations. In the present study, EAs were reduced consistently with steam explosion. Conversely, an increase in the amount of EA was observed in the phase 1 diet used for the performance trial containing the extruded premix relative to its control which was not likely due to processing. EA analysis of the diets indicated that more contaminated screenings went into the phase 1 diet than phase 2 which possibly explains the differences in EA contents of the 2 phases of diets containing the extruded premix. Although the EA contents were altered, this change did not correlate to a reduction in toxicity of diets containing premixes that were extruded.

The EA contents of samples were reduced when screenings were strained prior to steam explosion compared to samples that were not pre-treated with water. Prior hydration might enhance processing since the grains were pre-treated to an extent and increased the effect or reaction with heat and pressure (Sfayhi-Terras *et al*., 2021). The addition of water to the screenings softened the screenings which facilitated the efficiency of steam explosion and thus reduced the EA contents more compared to the samples with no water addition (Miraji *et al*., 2021). This implies that water treatment of screenings before processing enhances the effect of the processing method utilized to reduce the content of EAs in ergot-contaminated screenings.

Extrusion was chosen for the method to process the contaminated screenings to be incorporated in the diets for the growth and digestibility experiments because we observed some changes with the epimer ratios which was then used to test the hypothesis that extrusion would reduce toxicity in pigs fed diets containing extruded contaminated screenings. Diets for the study were 0 or 4000 ppb EA diets containing premixes which were extruded or not. Four thousand ppb EA had a modest effect on growing pigs with no effect of extrusion or its interaction with ergot on ADFI, ADG, BW and feed efficiency. Toxicity was not reduced in diets of pigs containing extruded ergot contaminated wheat screenings, and hence the negative effects from EA exposure on livestock productivity including reduced feed intake and weight gain was observed in the present study. Although extrusion altered the EA epimer profile, it did not reduce the EA toxicity measured in terms of performance, serum prolactin or nutrient digestibility. There was no effect

on the amount of R epimers in the phase 1 diet but the amount of R epimers of the phase 2 diet were reduced. Previous studies done by Bryła *et al*. (2019) examined changes in the contents of R and S epimers in baking bread. Similar to our results, the authors indicated that there were changes in EA concentration and an influence of epimerization from the R to S epimers. Changes in epimer ratios (R:S) and profile was observed, but this did not mitigate the effect of ergot observed in pigs that consumed the ergot-contaminated diet. The reduction of the R epimers in the phase 2 diet did not translate into decreased toxicity when pigs were fed the phase 2 4000 ppb EA diets containing the extruded screenings in the performance experiment. The study in the current thesis indicates that the increase in S epimers of the screenings which were extruded did not result in reduced toxicity and perhaps the S epimer may have equivalent biological activity as the R epimer. Although several reports in literature have indicated that the R epimer is biologically active, while the S epimer is biologically inactive (Wolff *et al*., 1988), it is difficult to find data supporting the reports of differential effects of the epimers (R and S). Weber (1980) formulated a hypothesis on the basis of the capacity of molecular interaction (biological activity) of an agent with a specified receptor. To formulate this hypothesis, he used data from a personal communication with Sturmer (1970) as evidence to back the hypothesis of differential biological effects of the epimers. However, a recent study by Cherewyk *et al*. (2020) examined the vasoactive potential (contractile response) of 4 pure S-ergot alkaloid epimers in an *in vitro* arterial bath system and showed that the S epimers had biological activity, which contradicted the hypothesis by Weber (1980). Studies in the present thesis indicate that hydrothermal processing altered EA contents and epimer profiles. The change in epimer ratios (R:S) and epimer profile with extrusion of ergot-contaminated screenings incorporated into the diets, however, did not mitigate the negative effects (toxicity). Hence, extrusion is not recommended as a solution to mitigate toxicity of ergot-contaminated grains. Further studies using diluted pure epimers (R and S) either independently or in combination, is required to evaluate the changes in alkaloid and epimer profiles, and quantify ergot amounts to assess the effects when incorporated in feed to be consumed by livestock to support the finding from this research. The information from the present research provides farmers and nutritionists an idea of the importance of testing for ergot alkaloid amounts. The findings from this study indicate that there is the need to consider measuring both epimers in analysis and consideration of amounts, in estimating the toxicity of contamination prior to feeding ergot contaminated grains to livestock.

Conclusion

The hydrothermal processing method utilized affected the EA contents of ergot-contaminated screenings differently. Consumption of diets contaminated with 4000 ppb EA reduced performance, prolactin levels and nutrient digestibility in growing pigs. Although extrusion altered the epimer profile, it did not mitigate the toxicity (negative effects) of ergot-contaminated screenings. Extrusion is not recommended as a solution to mitigate toxicity of ergot-contaminated grains.

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