Characterising the impact of ergot alkaloids on digestibility and growth performance of lambs

Stephanie Coufal-Majewski

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Abstract

The decrease to grain yield, quality and livestock performance caused by mycotoxigenic fungi from cereal grains, has caused detrimental losses in the agricultural world for many decades. It is more commonly researched in fescue sources however, the rise in ergot contaminated grain originating from different fungal species producing different ergot alkaloids has seen contaminated grain restricted from human consumption and used as feed for livestock, in order to minimise wastage. Allowable limits for ergot in livestock feed are confusing as they may be determined by proportions of ergot bodies or by total levels of alkaloids where measurements can differ widely in their estimation of toxicity. The research presented in this thesis attempts to understand how the amount of toxin present, the feed source and feed intake can have profound impacts on performance and metabolism in growing lambs to enhance the knowledge base concerning ergot alkaloids in grain and further help determine if the current standards are valid. The first study involved an animal performance component which measured weight gain, feed intake and prolactin concentrations, in addition to a metabolism study where total collection of faeces and urine was used to determine alkaloid digestion in lambs. It was concluded that feeding 433 ppb total alkaloids in pelleted diets significantly decreased serum prolactin concentrations, ADG, NDF and ADF digestibility. In comparison, the second study incorporated alkaloid epimer analysis using electrospray ionisation mass spectrometry, demonstrating how the method choice for alkaloid detection can greatly impact the total alkaloid concentration in a feed sample. Moreover, alkaloid concentrations were increased to that of the recommended dosage to observe the impacts to lamb performance and metabolism. Though, it was clear that ram lambs fed up to 2447 ppb total alkaloids had reduced ADG and prolactin concentrations, we were able to acknowledge the biggest
issues in the progression of ergot alkaloid investigations were surrounded by the methodology selected for alkaloid determination and the interactions amongst alkaloids, particularly between $R$ and $S$ epimers. This thesis used two existing methods of analysing alkaloids and found contrasting results, demonstrating that ergot alkaloid concentration greatly varies depending on the methodology used. Therefore, this thesis reveals that the safe concentration of ergot alkaloid for growing lambs should not exceed 433 ppb, however is greatly dependent on the source of grain ergot, the alkaloid profile present and the methodology used for alkaloid detection.
Declaration

The work described in this thesis was conducted under the supervision of Associate Professor Alexandre V. Chaves, School of Life and Environmental Science, The University of Sydney, Australia and under the co-supervision of Hon. Prof. Tim A. McAllister, (The University of Sydney and Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada) and Dr. Kim Stanford, (Alberta Agriculture and Forestry, Lethbridge, Alberta, Canada.

I declare that the intellectual content of this thesis is the result of my own work, original and is not currently being submitted for any other degree or qualification. Full acknowledgement has been made where the work of others has been cited or used.

Stephanie Coufal-Majewski
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List of Abbreviations

µg: microgram
°C: degrees centigrade
%: percentage

ADF: Acid Detergent Fibre
ADFD: Acid Detergent Fibre Digestibility
ADG: Average Daily Gain
aDM: Analytical Dry Matter
CP: Crude Protein
DM: Dry Matter
DMD: Dry Matter Digestibility
DMI: Dry Matter Intake
DON: deoxynivalenol
EFSA: European Food and Safety Authority
ELISA: enzyme-linked immunosorbent assay
EU: European Union
FC: Feed Conversion
FDA: US Food and Drug Administration
g: gram
GR: Grade Rule
H: High ergot contaminated diet
h: hour
HPLC: High Performance Liquid Chromatography
kg: kilogram
L: litre
L: Low ergot contaminated diet
LC: liquid chromatography
LC-MS: Liquid chromatography mass spectrometry
ln: natural log
LOD: limit of detection
LW: Live Weight
M: molar
MC: Mash control
mg: milligram
MH: Mash high
min: minute
ML: Mash low
MS: mass spectrometry
N: Nitrogen
n.d.: no date
NDF: Neutral detergent fibre
NDFD: Neutral detergent fibre digestibility
ng: nanogram
NIR: near infrared spectroscopy
OM: Organic matter
OMD: Organic matter digestibility
P: Pellet
PH: Pelleted high contaminated diet (Chapter 4)
PL: Pelleted low contaminated diet (Chapter 4)
**ppb:** parts per billion

**ppm:** parts per million

**SEM:** Standard Error of the Mean

**UV:** ultraviolet

**VFA:** volatile fatty acids
List of Publications and Poster Presentations

Peer-reviewed articles


Conference poster presentations

Lamb feeding digestibility and performance experiments were conducted at the Lethbridge Research and Development Centre (LRDC) of Agriculture and Agri-Food Canada, Alberta, between May and September of 2015 and June and September of 2016. Protocols for these experiments were approved by the LRDC Animal Care Committee according to the guidelines of the Canadian Council on Animal Care (2009).
Chapter 1 – General Introduction

1.1 General Background

Mycotoxins are toxic secondary metabolites of fungi that thrive on a variety of plants under a wide range of climatic conditions. As a result of this diversity, livestock are often exposed to feed containing more than one mycotoxin (Songsermsakul and Razzazi-Fazeli, 2008; Fink-Gremmels, 2016). Mycotoxins on grain were first documented in the fifth century AD for their negative impacts on human health, but today mycotoxin contaminated grain is often deemed unfit for human consumption and designated as feed for livestock. One of the main groups of mycotoxins of concern are the ergot alkaloids as grain screenings which are frequently used as feed which often contain ergot bodies (Nicholson, 2007).

Fungal infection occurs when an ergot sclerotium infects a floret of flowering grasses or cereal, imitating normal ovary development and producing honeydew, which is often further dispersed to neighboring crops by insects. The sclerotium then hardens with each kernel containing different profiles and concentrations of ergot alkaloids as the ergot body matures (Panaccione, 2005). Once mature, the kernel drops to the ground and remains dormant until ideal conditions (either the onset of spring, cool periods or in wet, rainy conditions) trigger germination and further contamination (Cowan and Blakley, 2014). Additionally, with extreme changes in weather, especially in cool and moist environments and repeated cereal grain monocultures, ergot can further spread (Cowan and Blakley, 2014). When cereal crops are harvested for livestock consumption, grain kernels contaminated with ergot bodies are frequently present resulting in exposure of livestock to the alkaloids. Commonly, the first detectable symptom of ergotism in ruminants is feed refusal, with which can be either established in as little as a few hours while other symptoms such as reduced growth rate may require months to become observable (Belser-
Ehrlich et al., 2012). Depending on the type and concentration of alkaloids present, a range of physiological responses can be observed, often accounting for the frequent misdiagnosis of the condition. Moreover, symptoms often resemble other conditions such as foot rot as alkaloids can cause vasoconstriction (Strickland et al., 1993), which can lead to lameness, frostbite in the extremities and respiratory disease often leading to misdiagnosis (Wegulo and Carlson, 2011; Cowan and Blakley, 2014).

Reports on the frequency of ergotism in livestock vary from year to year, and are often related to temperature, rainfall patterns and geographical region. The condition often presents as 4 different syndromes; nervous/convulsive ergotism, gangrenous ergotism, enteroergotism and hyperthermic ergotism. In sheep, symptoms often consist of gastrointestinal inflammation, gangrene on the tips of ears, low concentrations of prolactin (causing agalactia) and reduced fertility (Evans et al., 2003).

Although there are no reports on the economic loss from ergotism, fescue toxicosis is estimated to cause more than $1 billion USD per year in production losses on the equine and small ruminant industries in the United States (Strickland et al., 2011), with as much of 20% of the wheat in Canada contaminated with ergot in 2011 (Edwards et al., 2011) and 50% of wheat in Alberta being contaminated in 2013 (Tittlemier et al., 2015).

Whilst ergot alkaloids present in grain and tall fescue are of major concern in North America, one of the first documented outbreaks of ergot toxicity in cattle occurred during the early 1980’s in Australia. Furthermore, with the majority of Australia incorporating pastoral agricultural systems, animals are still exposed to mycotoxins produced by fungi, prevalent in the coastal and tableland regions. In the recent cropping year (2016/17) Australia had a total grain production of
42,333 (‘000 tonnes; Spragg, 2016), with approximately 63% exported, revealing the high potential for ergot contaminated grain to enter livestock systems. With this in mind, the importance of observing animal responses to consuming cereal grain ergot demonstrates the relevance of the research within this thesis to the Australian context.

Different species of *Claviceps* sp. are present in Australian, in combination with less common species such as *Balansia* sp., *Neotyphodium* spp. and *Ephelis* spp. (Belser-Ehrlich et al., 2012). *Claviceps paspali* has been previously identified in Australia, producing alkaloids which cause tremors and staggers in cattle, horses and sheep grazing infected paspalum (Ryley et al., n.d.). Similarly, other species such as *Claviceps cyanodontis*, recently identified in three locations in Queensland, have been found on a variety of grasses which contain toxic alkaloids to livestock (Ryley et al., n.d.). Rye ergot (*Claviceps purpurea*) commonly contaminates grain crops used for livestock feed in Australia, with ergotamine being the predominant alkaloid present in grain screening samples across southern Australia (Blaney et al., 2009). With samples often containing 1000 to 3321 mg/kg total alkaloids, there are many implications for Australian livestock consuming such feed and whether current stockfeed regulations are safe for consumption (Blaney et al., 2009).

Although pastoral systems are more common in Australia, the use of contaminated cereal grains in feedlot systems is of concern, as not only can it negatively impact animal performance and health, but it can potentially contaminate animal products for human consumption. Thus, it is evident that the concern of contaminated cereal grains is not limited to North America, but is in fact a worldwide issue.

1.2 The Current Problem
Previous research has predominantly focused on tall fescue toxicosis and direct administration of ergot alkaloids, which is primarily caused by fungal endophytes (mainly ergovaline and ergotamine) through injection into the blood stream. Although the agronomic and environmental characteristics that lead to the formation of ergot in tall fescue and cereals grains are similar, the types of alkaloids produced by the *Claviceps* spp. differ between these two sources due to the differences in fungal species present (Fink-Gremmels, 2008). With all cereal grains and grasses vulnerable to infection, there is a major gap in understanding the impacts of cereal ergot ingestion on livestock health and production, a fact that is a growing issue as there has been an increase in ergot related toxicity over the last 10 years in Western Canada (Cowan and Blakley, 2014). Few investigations on the impact of direct consumption of ergot contaminated cereal grains in livestock have been conducted, and there has been no research on the impact of these toxins on growing lambs.

Although ruminants have the ability to detoxify ergot alkaloids, it has been speculated (Cowan and Blakley, 2014) that the current regulations for ruminant alkaloid consumption (2-3 ppm; EFSA, 2012) are too high, with symptoms of ergotism possible arising at concentrations that are at least 10-fold lower (100-200 ppb). Although regulations for ergot alkaloid concentrations in animal feed exist, a scientific basis for these recommendations is generally lacking. Consequently, a threshold dose and accurate dose-response data has not yet been established (Craig et al., 2015).

To further complicate this issue, individual sclerotia can have significant differences in their total alkaloid content, with variations between 0.01 and 0.05%, as influenced by geographical region (Krska and Crews, 2008). Thus, frequent testing of grain and monitoring contamination levels in a wide range of geographical locations are needed to further assist in understanding how the ingestion of contaminated feed can have undesirable impacts.
How the feed is processed is another factor that could influence the degree of ergot alkaloid toxicity. Anecdotal observations suggest that the pelleting process may increase the bioavailability of ergot alkaloids in feeds, resulting in a greater potential toxicity. With visual detection of ergot contamination being impossible after grinding and pelleting, chemical analyses are required to determine the extent of ergot contamination in processed livestock feed. These analyses are exceedingly complex and exhibit substantial variability depending on the sampling, extraction and detection methods selected to characterise the alkaloid profiles in contaminated feed.

1.3 Project Objectives

This thesis aims to provide an insight into cereal ergot alkaloids, with a focus on the impacts of increasing ergot alkaloid concentrations and how they can affect the digestibility and performance traits of growing lambs.

The objective of study 1 (Chapter 4) was to use two different diet forms, mash and pellet, to determine whether diet preparation has an impact on alkaloid toxicity. This study evaluated whether increasing concentrations of alkaloids impacts nutrient digestibility, growth performance and carcass traits of both growing ewe and ram lambs by estimating total tract nutrient digestion, body weight gain, feed intake, serum prolactin concentration and measuring carcass traits.

The objective of study 2 (Chapter 5) was to investigate how different methods of alkaloid detection can impact alkaloid recovery of R and S epimers to generate new data on the composition of ergot alkaloids and their epimers in ergot contaminated grain screenings. Different sources of ergot contaminated screenings were evaluated in studies one and two. This study was an extension of the first study in which we increased dietary alkaloid concentrations and evaluated the effects of feeding a different alkaloid profile on metabolism and growth performance of lambs.
Thus, this thesis further discusses the known correlations between the ergot alkaloids and development of ergotism in clinical cases to serve as a starting point for suggesting threshold levels of inclusion of these toxins in lamb diets. The impact of a lack of reliable lab analysis methods for alkaloids is also discussed, which increases the difficulty in defining allowable inclusion levels of these toxins in ruminant diets.
1.5 References


Chapter 2

Literature review: Impacts of cereal ergot in food animal production

2.1 Brief background

Mycotoxigenic fungi have the ability to inhabit grain cereals, leading to decreased grain yield and quality, mycotoxin production and reduced animal performance (Young et al., 1983; Vermeulen et al., 2012). These negative impacts of ergot contamination of grain on the health of humans and animals were first documented during the 5\textsuperscript{th} century AD. Today, grain that is contaminated with ergot is comprehensively regulated for human consumption and thus redirected for use as livestock feed, with low concentrations of alkaloids in the diet (<100 ppb total) reducing the growth efficiency of cattle (Schumann, 2000). Consequently ergot alkaloids continue to be a concern for livestock as allowable limits are less rigorous for feeds and the screenings containing ergot bodies are frequently used as feed (Trenholm et al., 1989).

The study of ergot toxicosis is further complicated due to changes in alkaloid profiles from geographical regions, harvest years, cereal species, variety and genotype, potentially leading to previously uncharacterised alkaloids (Shelby, 1999; Miller and Richardson, 2013). In combination with considerable animal to animal variation and current processing procedures such as pelleting potentially increasing the concentration of toxic \( R \) epimers, the use of binders as a potential avenue to reduce the bioavailability of alkaloids requires further study. Accordingly, unknowns greatly outnumber the knowns for cereal ergot and further study to help better define allowable limits for livestock would be welcome. This review aims to describe the major ergot alkaloids currently identified in grain, how the alkaloids impact livestock and the technologies which can be used to measure alkaloids and reduce their impacts on livestock.

2.2 Ergot and Its Lifecycle
Ergot found in grain crops arises from a parasitic fungus of the genera *Claviceps* with *C. africana*, *C. sorghi*, *C. gigantean* or *C. purpurea*, being members and *C. purpurea* the predominant species (Table 2.1). The term ‘*purpurea*’ originates from its ability to replace kernels in grain with hard purplish ergot bodies (sclerotia) that contain a diversity of alkaloids (Nicholson, 2007; Kraska and Crews, 2008).

Grain field and storage mycotoxins are either moulds that contaminate crops in the field or during post-harvest and storage. These typically originate from *Fusariums* (field mycotoxins) and *Aspergillus* and *Penicillium* (storage mycotoxins), which have become more abundant over the past 5 years in some areas of Canada as environmental conditions favoured growth of mycotoxigenic fungi (Leroux, 2012). For example, as much as 20% of the wheat produced in western Canada in 2011 was infected to some degree by ergot (Canadian Grain Commission, 2011). With climate-change models predicting increased precipitation and prevalence of insects, concentrations of ergot in Canadian cereal grains are likely to increase in the future (Government of Canada, 2014). Susceptibility of grains to ergot (from most to least) is ranked rye (*Secale cereal*), wheat (*Triticum spp.*), triticale (*Triticosecale*), barley (*Hordeum vulgare*), and oats (*Avena sativa*; Canadian Wheat Board, 1999). Rye, an open pollinator is more susceptible to ergot infection, whereas wheat and barley are self-pollinators. Ergot contamination typically reduces yield by 5-10% (rye and wheat respectively), but the reduction in quality grade accounts for the majority of the economic loss associated with contaminated grain (Canadian Wheat Board, 1999). Ergot alkaloids are also produced by the fungus *Neotyphodium coenophialum* in grasses, particularly fescues (Klotz et al., 2006).

The life cycle of ergot has two stages, germination and the honeydew stage (Nicholson, 2007). While germination typically refers to the developmental stage from a seed to plant growth,
ergot germination is defined by drumstick-shaped fruiting structures which develop from the sclerotia (Nicholson, 2007). These structures produce spores known as ascospores, which become wind-borne and easily infect the ovaries of flowering cereals (Nicholson, 2007). Contaminated grain heads can contain multiple ergot sclerotia which often require differing incubation periods to germinate. Generally, the sclerotia of *C. purpurea* require 4 to 8 weeks at 0-10°C to initiate germination, with higher temperatures (>25°C) prolonging germination (Mitchell and Cooke, 1968). The optimal temperature range for germination of ergot in rye is thought to be 18-20°C (Kirchhoff, 1929), although germination in rye has also been documented between 9-15°C (Krebs, 1936). Furthermore, it has been noted that germination can occur without a chilling period, but ergot body formation is enhanced during cool, wet weather, especially during the flowering stage (Kirchhoff, 1929).

The second stage involves the florets oozing a sticky conidia which is spread by insects and in moist environments. Following the honeydew stage, the infected ovary hardens and is replaced by an ergot body which either falls before or during harvest, contaminating the field or the harvested grain (Government of Saskatchewan, 2015). However, if the flowers have been fertilised prior to infection, they become resistant (Krska and Crews, 2008).

### 2.2 Ergot Alkaloids

Although fescue toxicosis have been studied for over 50 years, the alkaloids prevalent in fescue differ from those in grain (Canty et al., 2014) and few studies have investigated the impact of grain ergot on livestock production (Charmley and Trenholm, 2000). Cattle, sheep and swine have a greater tolerance of mycotoxins produced by *Fusarium* spp. such as deoxynivalenol (DON) than for ergot alkaloids (Whitlow and Hagler, 2008; Cowan and Blakley, 2014). The FDA restricts
the levels of DON in grains and grain by-products to 5 ppm for swine and 10 ppm for cattle as greater concentrations can adversely impact weight gain (Aakre et al., 2005).

Concentrations of ergot alkaloids in the sclerotia of *Claviceps* can be as great as 0.75% DM (EFSA, 2012). The concentration and the type of alkaloid produced can vary among fungal species, the type of cereal grain and with environmental conditions, with production being more pronounced in periods of heavy rainfall and with moist soils (Krska and Crews, 2008; Dewell and Ensley, 2014). More than 50 different ergot alkaloids have been identified in grains infected with *Claviceps* spp., which are divided three biogenetically related classes: clavine, simple lysergic acids and peptide alkaloids (Evans et al., 2003; Krska and Crews, 2008, which include subfamilies ergopeptide and ergoline. However, new alkaloids are continually discovered further increasing the complexity of defining the toxicity of ergot (Evans et al., 2003).

The most dominant alkaloids in grain ergot bodies are ergotamine, ergocristine, ergosine, ergocornine and ergocryptine (Evans et al., 2003). In contrast, ergovaline is the most common form of alkaloid present in forages infected by endophytic fungi, followed by ergine (Porter, 1995; Kemp et al., 2007; Fink-Gremmels, 2008). Endophytic fungi produce alkaloid concentrations far lower than those found in the sclerotia of *Claviceps*, accounting for the differences in clinical symptoms between the two forms of toxicosis (Shelby, 1999).

When describing ergot alkaloids, it is essential to identify their chemical structure (Figure 2.1; EFSA, 2012) as the degree of toxicity may be dependent on the nature of the matrix and feed processing technique. The main ergot alkaloids, ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine are structurally similar, differing only in substitutions on C-8 (Government of Canada, 2014). Moreover, alkaloids containing a C9=C10 double bond easily epimerize depending on temperature and pH (Figure 2.2; Komarova and Tolkachev, 2001; Krska
and Crews, 2008) and it is possible that application of heat during pelleting may alter chemical bonds and the chemical composition of feed (Muller, 2007). However, minimal effects on ergot alkaloids have been observed at storage temperatures <5°C (Komarova and Tolkachev, 2001), but prolonged storage at higher temperatures can increase the amount of ergopeptinines which arise from natural right-hand rotation epimerisation (C-8-(S); Krška and Crews, 2008).

Figure 2.1 Chemical structure of ergoline (I), lysergic acid (II) paspalic acid (III) ergopeptines (IV) and lactam ergot alkaloids – ergopeptams (V); figure adapted from Battilani et al. (2009).
2.3 Ergot alkaloid epimers

Ergot alkaloids are typically stable, however they can bi-directionally convert among epimers between the highly toxic ($R$) form and the biologically inactive ($S$) form (Merkel et al., 2012) induced by changing pH conditions and exposure to strong light (Crews, 2015). The activation of “–ines” to “–inines” is rapid in acidic and alkaline solutions, increasing the challenge of ergot removal using extraction and cleaning processes. Avoiding the reactivation of –ines is important as this conversion appears to produce products that are more toxic to livestock (Lampen and Klaffke, 2006; Krska and Crews, 2008).

Furthermore, with diet pH and the influence of the gastrointestinal tract affecting the extent of epimerisation (Merkel et al., 2012), changes in dietary starch content could influence the proportions of some $R$ epimers, potentially increasing the toxicity within a single source of ergot. In addition, heat application has been shown to result in epimerisation, with the extent of

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Figure 2.2 Ergot alkaloids containing C9=C10 double bond readily epimerize at the centre of symmetry C-8; figure adapted from Crews (2015).
epimerisation differing among alkaloid types and the temperature used (Krska et al., 2008; Merkel et al., 2012).

2.4 Determination of ergot and ergot alkaloids

Analytical methods to determine ergot alkaloids should aim to detect major alkaloids in combination with their corresponding biologically active metabolites. While some techniques are more sensitive than others, European Feed Standard Association (EFSA) determined that new validated methods are still required to quantify ergot alkaloids in feed materials to provide more reliable regulatory limits for each individual alkaloid in food and feed (EFSA, 2012). All methods have detection limits, yet information concerning these limits for different alkaloid types is scarce. Similarly, issues surrounding the accuracy of detection continue to be a major issue, with coefficients of variation varying depending on alkaloid concentrations (17% CV for very high concentration; 284% CV for very low concentration; Grusie et al., 2016).

2.4.1 Ergot contamination by visual detection

Grain ergot is typically detected upon visual inspection, with dark sclerotia bodies being up to ten times larger than grain kernels. However, ergot bodies may range in size from a few millimeters to more than 4 cm depending on the size of the host plant (Krska and Crews, 2008). In some cases sclerotia bodies are smaller (Government of Saskatchewan, 2015), increasing the degree of difficulty in detecting them within grain screenings (Cowan and Blakley, 2014). Upon visual inspection, estimates counting 5-20 sclerotia/L grain, or having sclerotia weighing 0.1-0.3% of grain DM is sufficient contamination that the grain should deemed dangerous (Cowan and Blakley, 2014).
2.4.2 Thin-layer chromatography (TLC)

This method uses a plate which is coated in a solid adsorbent (silica gel) in combination with a small amount of the mixed sample to be analysed (Fowler et al., 1972). The method is often used to identify a compound of interest in a mixture, as different components will vary in solubility and therefore migrate and be absorbed at different locations on the plate. Lobo et al. (1981) found that it was difficult to separate the 12 main alkaloids in rye ergot, even using two-dimensional TLC, a result that likely reflects the low sensitivity of the method (Shelby, 1999). Nevertheless, TLC may be valuable for separating individual alkaloids because it only requires a small amount of mixture to identify the compound of interest (Krska and Crews, 2008; Battilani et al., 2009).

2.4.3 Liquid chromatography (LC) and mass spectrometric (MS) detection

Liquid chromatography is often used to analyse ergot alkaloids in combination with mass spectrometric detection for different matrixes in feed and foodstuffs (Battilani et al., 2009). The benefit of this technique is that any known alkaloid can be determined in one run using solvent extraction, separation, detection and quantification (Krska and Crews, 2008; Blakley and Cowan, 2014).

Although only a few studies have used LC-MS-MS to detect ergot alkaloids, this technique incorporates two mass analysers to increase the limit of detection, whilst producing more structural information on the unknown analyte (Downard, 2004; Krska and Crews, 2008). With the capability of individually identifying structurally similar compounds such as those of the same molecular weight, this methodology can be effectively used to identify trace concentrations of alkaloids in feed samples (Downard, 2004). Moreover, Stahl and Naegele (2004) reported that this technique
can be used to reveal unknown ergot derivatives (semi-synthetically derived alkaloids such as lysergic acid diethylamide), emphasising the importance of implementing such chemical analysis for future research. However, issues with the collection of representative samples, variation in kernel size and crop type may produce inaccurate results with this method (Blakley and Cowan, 2014).

Byrd (2012) determined the limits of detection of six ergot alkaloids in wheat and their epimers, in combination with their corresponding retention time (Table 2.2). Moreover, Kraska and Crews (2008) validated the use of LC-MS as a means of reliable detection in determining certain alkaloids, yet today only six alkaloids and their isomers can be accurately identified using this method (Figure 2.3).

**Figure 2.3** Retention times of ergometrine (8.05 min), ergosine (13.26 min), ergotamine (13.79 min), ergocornine (14.68 min), ergocryptine (15.21 min), ergocristine (15.45 min) and ergosinine (15.86 min) from LC-MS consisting a Agilent 1100 HPLC system with Agilent Zorbax Eclipse XDB-C18 narrow bore 2.1x150mm, 5μm HPLC column and a Quattro Ultima Ptm mass spectrometer. The analysis uses mixture of acetonitrile (85%) and 10mM ammonium acetate (15%) as sample extraction solvent and 10mM ammonium acetate as mobile phase A and
acetonitrile as mobile phase B with the same analytic conditions as described by Krška et al. (2008).

2.4.3.1 Liquid chromatography electrospray ionisation-tandem mass spectrometry (LC/ESI-MS/MS)

This is the most prevalent form of ionisation used, which allows for the extraction of trace concentrations of metabolites using a strong electric field to a liquid passing through a capillary tube with a weak flux (Crews, 2015). The analyte undergoes three major processes after being transferred from solution to the gas phase: 1) charged droplets are produced from the capillary tip in which the analyte solution is injected; 2) repeated solvent evaporation (from the charged droplets) which forms a very small charged droplet; 3) gas-phase ion is formed (Banerjee and Mazumdar, 2012). The advantages of this method is that denaturation is not required for proteins to be ionised, with fragile molecules capable of being ionised and detected, granting access to information beyond the molecular weight of the analyte (Banerjee and Mazumdar, 2012).

2.4.4 High-performance liquid chromatography (HPLC)

High-performance liquid chromatography uses a column to pump the sample mixture at great pressure in a solvent with chromatographic packing material, producing excitation wavelengths ranging between 235-250 nm as detected by UV absorption (Scott and Lawrence, 1980; Linde Group, 2015). With the ability to detect compounds at concentrations as low as parts per trillion, HPLC is a common method currently used to identify ergot alkaloids. The most common alkaloids detected using this method are ergometrine, ergotamine, ergocornine, ergocryptine, ergocristine, ergosine and their respective isomers, with the sum of these alkaloids equating to total alkaloid content (Mainka et al., 2005).
Although alkaloid concentrations have been detected as low as 0.02-1.2 µg/kg using multi-analyte LC-MS/MS, extensive epimerisation was noted, affecting the estimation of overall alkaloid content (Krska and Crews, 2008). Sulyok et al. (2007) demonstrated that HPLC could detect concentrations as low as 0.17-2.78 µg/kg without epimerisation, validating the prevalent use of HPLC for determining alkaloid content.

### 2.4.5 Enzyme-linked immunosorbent assay (ELISA)

The ELISA involves combining antibodies with an enzyme-mediated color change (commonly alkaline phosphatase and glucose oxidase) to identify small quantities of targeted substances. The antigen is capable of binding to the specific antibody, which can be identified by a secondary antibody and revealed using fluorogenic substrates (Gan and Patel, 2013). This technique is attractive for ergot screenings in crops, but has difficulty in identifying a marker toxin to serve as a standard to determine the extent of alkaloid contamination (Schnitzius et al., 2001). Furthermore, cross-reactivity can vary substantially depending on the nature of alkaloids being detected (Krska and Crews, 2008).

### 2.4.6 Near infrared spectroscopy (NIR)

The NIR method is used to estimate total ergot alkaloid content, particularly in tall fescue, with calibrations based on measurement obtained through ELISA (Battilani et al., 2009). This method can be employed with both grain and pelleted feeds, however pelleted grain must be ground prior to measurement to improve the accuracy of estimates (Battilani et al., 2009; Vermeulen et al., 2012). A great advantage of NIR is the speed of detection and its ability to analyse both large and small quantities of feeds, thereby avoiding errors associated with
inconsistent sampling (Vermeulen et al., 2012). The system can also make measurements in real time by placing sensors in grain augers or belt systems (100 kg grain can be analysed in 1 h. However, the system is heavily dependent on the establishment of an accurate calibration in which alkaloids have been measured using the sensitive techniques described above. Variation in the types of alkaloids present in grains and feeds may make development of universal calibration equations difficult.

2.4.7 Detection in animal tissues

Alkaloids such as ergocornine can decrease pituitary prolactin release and counteract the stimulatory effect of estrogen on prolactin concentrations, significantly reducing milk production (Fell et al., 1974). Therefore, isolating serum from whole blood and conducting prolactin analysis may be useful in the detection of ergot alkaloids as a low prolactin concentration could be indicative of ergot alkaloid poisoning. Direct detection of ergot alkaloids and/or their derivatives in both blood matrices and liver tissues is as yet, only at a preliminary stage (Cowan and Blakley, 2014). However only 8-(R) isomers have successfully been shown to penetrate through the blood-brain barrier within a few hours, triggering toxic effects and further either interacting with receptors or accumulating in the brain, with ergocristinine (a S isomer) unable to penetrate the barrier (Mulac et al., 2012). Although minimal research has been conducted on ergot alkaloids and blood matrices, Mulac et al. (2012) demonstrates how easily toxic ergot alkaloids can transport and permeate through animal tissue, revealing the major concern in livestock food and therefore the consumption of potentially toxic animal tissues.

Tissue accumulation of ergot alkaloids, while of concern, has been little studied, largely due to a lack of suitable assays. Dairy cattle fed 125 mg ergot alkaloids/kg dietary DM over a 2
week period led to a carry-over of toxins into milk, although less than 10% of ingested ergot alkaloids were detected (Wolff et al., 1995). Similarly, Schumann et al. (2009) discovered a potential for carry-over of ergot alkaloids into the milk of fistulated cows fed 16.3 µg/kg body weight, with approximately 67% of alkaloids recovered in the duodenal ingesta and 24% excreted in faeces, however no alkaloids residues were detected in blood or milk samples. Moreover, ergot alkaloids have been successfully detected in adipose tissues of steers grazing on endophyte infected tall fescue (Realini et al., 2005), and the liver and milk of lactating ewes exposed to endophyte infected ryegrass (Zbib et al., 2015). However, when swine were fed 1 to 10 g ergot/kg body weight, no evidence of ergot alkaloid residues was found in meat (Mainka et al., 2005). Similar to a long-term study observing growing bulls over 230 days consuming 633 mg/kg total ergot alkaloids, with no detectable alkaloids into tissues, nor no recognisable impacts on carcass composition and quality (Schumann et al., 2007). Although several studies have attempted to detect alkaloids in animal tissue, the challenge in sample preparation and analytical capabilities have been a major factor in establishing advancements in this area.

2.5 Feeding ergot-contaminated grain to livestock

2.5.1 Allowable limits

The concentration of ergot alkaloids that are allowable for livestock consumption is presently contentious, as there are several different measurements in the literature which are not interchangeable. The toxicity of ergot alkaloids depends on both the type and the absolute concentration of the individual alkaloid as well as interactions with other mycotoxins that may be present in feed (EFSA, 2012).
Individual countries have established specific tolerances for concentrations of ergot bodies in both cereal grains and animal feed (Table 2.3). Legislation is in place that sets the limits of ergot contamination in cereal grains for the human market at 0.05% in Australia and European Union (EU) and 0-0.05% in North America. The EU and the United States require grains destined for livestock feed to contain less than 0.1 ppm and 0.3 ppm total ergot, respectively. The United Kingdom has a 0.001 ppm tolerance for total ergot in animal feeds. Grain exceeding these limits is banned from entering either the food or feed chain.

In Canada, maximum allowable levels of ergot alkaloids in cattle and swine feed have also been established and are 2-3 and 4-6 ppm, respectively (Belser-Ehrlich et al., 2012). It is also recommended that feed contaminated with 250 ppb ergot alkaloids not be fed to pregnant or lactating animals, due to a greater risk of abortion and agalactia syndrome. In general, 5-10 µg ergot alkaloids/kg body weight represents the general threshold dosage for all livestock (Belser-Ehrlich et al., 2012), yet EFSA recommends doses as low as 0.6-1 µg of ergot alkaloids/kg body weight to avoid their vasoconstrictive effects (EFSA, 2012).

Although legislation establishes tolerances for ergot alkaloids or ergot bodies in livestock feed, in most cases these concentrations have not been established through toxicological studies with livestock (Scott, 2009; Vermeulen et al., 2012; Belser-Ehrlich et al., 2012). For example, dietary concentrations of ergot alkaloids as low as 100-200 ppb (ergovaline) can have adverse impacts on livestock growth, especially livestock suffering from heat stress and interactions among alkaloids can lead to heightened toxicity (Cowan and Blakley, 2014). The concentration of alkaloids in the ergot bodies also varies between 0.01 and 0.21% (EFSA, 2012). The great variation in reported impacts of ergot on animal performance has led to inconsistent recommendations of tolerable limits of ergot across countries (Vermeulen et al., 2012). It is also evident that calves and
horses are the most sensitive to ergotism, with poultry having the greatest tolerance (Table 2.4; Belser-Ehrlich et al., 2012).

2.5.2 Impact of feed processing and grain storage on ergot alkaloids

Unlike other mycotoxins which are capable of forming post-harvest as a result of spoilage during storage, ergot only forms pre-harvest, with concentrations of alkaloids remaining relatively constant during storage (Cowan and Blakley, 2014). However, Krska and Crews (2008) found that extended storage of high-moisture grain that led to aerobic instability resulted in increased ergopeptinines by promoting ergot growth. Despite speculations that alkaloids may degrade over time, ergot stored at 15°C for 12 months still germinated, emphasising the importance of screening techniques to avoid propagation of ergot in grain (Mitchell and Cooke, 1968). Storage temperatures lower than 5°C had little effect on ergot alkaloids (Komarova and Tolkachev, 2001), although high temperature storage has the potential to alter their chemical structure and biological activity.

Pelleted grain screenings are a popular low-cost feed for both sheep and cattle. Anecdotal observations suggest that pelleting or high-temperature processing of feed may increase the bioavailability of ergot alkaloids (Cowan and Blakley, 2014). However, heating of alkaloids has been shown to result in epimerisation of alkaloids from the toxic \((R)\) configuration to the biologically inactive \((S)\) configuration (Merkel et al., 2012), with the degree of epimerisation differing among alkaloid types and affected by the temperature used (Krska et al., 2008). Grain by-products used for ethanol production such as distiller’s grains are frequently fed to livestock, although this product retains and concentrates ergot alkaloids through the production process (Cowan and Blakley, 2014). However, the effects of fermentation on the activity of ergot alkaloids
and potential implication for animal health and productivity have not been fully studied. Further complications arise with grain after being processed and pelleted as ergot is then impossible to visually detect.

2.6 Effects of ergot alkaloids on health and productivity of livestock

2.6.1 Clinical symptoms of ergot poisoning in livestock

Ergot toxicity was first described in the middle ages as a gangrenous outbreak in humans known as “St. Anthony’s fire”, responsible for disfigurement of people and deaths (Shelby, 1999; Fink-Gremmels, 2008). At present, ergot poisoning rarely occurs in humans due to advanced grain processing technology and strict legislation.

Clinical symptoms of ergot poisoning can be manifested in as little as a few hours or may require months to become observable. This variability reflects differences in physiological responses to the type and concentration of alkaloids and accounts for the frequent misdiagnosis of the condition (Belser-Ehrlich et al., 2012). Furthermore, symptoms of ergot toxicosis often resemble other conditions such as foot rot, frostbite and respiratory disease, further complicating diagnosis (Wegulo and Carlson, 2011; Cowan and Blakley, 2014).

Generally, ergot toxicosis is manifested in three forms:

1) **Convulsive:** Convulsions, staggering, muscle spasms and temporary paralysis occur. This condition is often confused with tremors associated with *Claviceps paspali* (which contains great amounts of lysergic acid). This type of poisoning is more common in sheep and horses but seldom seen in cattle (Table 2.5; Evans et al., 2003). Upon slaughter, rigor mortis is never complete, leaving muscles flaccid.
2) **Gangrenous**: This form results in lameness, followed by the loss of extremities such as the ears, tail, hooves, and in severe conditions even limbs (Shelby, 1999). This form results from impaired circulation and blood supply and is most common in cattle and pigs. The condition is more severe under hot or cold conditions where vasoconstriction or vasodilation is necessary for thermoregulation (Dewell and Ensley, 2014). Gangrenous ergotism can require up to 3 months to become clinically obvious, with early symptoms including an elevated respiration rate, gradual weight loss, a reduction in milk production and reduced reproductive performance.

3) **Other**: These symptoms can be less severe and include vomiting (enteroergotism), fever (hyperthermic ergotism) and alterations in endocrine function. Long-term exposure to ergot, intensified during hot and humid conditions, favours hyperthermic ergotism (Heyden, 2014). Heifers injected with ergotamine and ergonovine exhibited a combination of symptoms such as lower skin temperature, heart rate and blood prolactin concentrations, with an increase in respiration rate and blood pressure (Browning and Leite-Browning, 1997). Chronic exposure to alkaloids can result in the greatest economic losses due to decreased reproductive performance and increased abortions (Fink-Gremmels, 2008).

Ergot toxicosis can often be misdiagnosed as other forms of syndromes associated with feed refusal such as those associated with vomitoxin (Scott et al., 1985; Trenholm et al., 1989).

**2.6.2 Effects on health and performance of livestock animals**

Consumption of ergot-contaminated grains can have negative effects on feed intake, growth and reproduction, but factors such as livestock species, age, and the presence of other stressors such as heat or cold can influence the extent of negative health outcomes (Schumann et
al., 2008). Low concentrations of ergot alkaloids (< 2 ppm) in feed can depress animal performance and result in intoxication, especially if feeds are administered for a prolonged period of time.

2.6.3 Animal growth

Cattle fed diets containing 1.6% ergot (12.7 g ergot intake/d) exhibited a lower average daily gain (0.55 vs. 0.83 kg/d) and lower feed intake (6.36 vs. 10.1 kg/d) as compared to those fed uncontaminated grain (Burfenning, 1994). The study also showed that ergot intake from 1.14 to 8.17 g/d had little effect but at 12.7 g/d significantly decreased feed intake. In contrast, growth rate was linearly decreased with ergot intakes from 0 to 12.7 g/d. This observation suggests that ergot alkaloids have a direct negative impact on energy metabolism and feed efficiency when ergot intake exceeds certain limit.

2.6.4 Reproductive performance

Cattle consuming endophytic fescue have consistently lower prolactin concentrations in plasma, with minimal changes in plasma luteinizing hormone or growth hormone (GH; Porter and Thompson, 1992; Paterson et al., 1995). Prolactin concentrations sharply declined and plateaued in cattle intravenously injected with 7 mg of ergotamine tartrate in saline over 240 minutes (average dosage of ergot alkaloids was 28.8 µg/kg body weight; Browning et al., 1997). This decline in prolactin secretion is due to activation of D2-dopamine receptors in pituitary lactotrophs (Krska and Crews, 2008). Furthermore, lysergic acid derivatives are structurally similar to noradrenaline transmitters including dopamine and serotonin, enabling ergot alkaloids to disrupt the endocrine system (Krska and Crews, 2008).
In contrast, plasma GH concentrations in steers exhibited a transient increase after ergot alkaloid administration (23.8 µg/kg body weight) through i.v. injections (Browning et al., 1997). While ergot derivatives increased human GH concentrations, ergotamine had no impact on GH secretion from rat pituitary cells (Franz, 1978; Fluckiger and del Pozo, 1978). Accordingly, Browning et al. (1997) found that cattle fed endophytic fescue displayed greater GH concentrations compared to steers grazing fescue with low endophyte content. A suppression of luteinizing hormone when ergotamine was injected suggests that this alkaloid alters the activity of the hypothalamic-pituitary-gonadal axis. In contrast, Christopher et al. (1990) demonstrated that tall fescue has suppressive effects on GH secretion in ovariectomised heifers. Consequently with acute exposure to alkaloids, particularly ergotamine or ergonovine, noticeable alterations to plasma concentrations of prolactin, GH and luteinizing hormone become apparent (Browning et al., 1997). Similar endocrine impacts from grain ergot alkaloids are also likely, although have yet to be studied.

2.6.5 Pregnancy rates

The alkaloids that promote vasoconstriction and lead to gangrene can also promote developmental and reproductive toxicity, such as abortions by restricting blood supply to the uterus. Duckett et al. (2014) documented that ewes fed endophyte infected tall fescue seed had shorter gestation lengths (up to 5 day difference), leading to a 2 kg reduction in lamb birth weights. During pregnancy, consumption of ergot alkaloids can impact maternal lipid metabolism, mammary growth and reduce milk production and secretion from the inhibition of prolactin release (Griffith et al., 1978; Duckett et al., 2014). Similarly, compared to cows consuming endophyte-free fescue, Watson et al. (2004) observed a 15% reduction in birth weight of calves delivered
from cows consuming endophyte-infected fescue. However, both occurred under high ambient temperatures, conditions where alkaloid consumption has the greatest impact on reproductive function. As umbilical blood flow increases throughout pregnancy, the vasoconstrictive response to ergot alkaloids can restrict blood flow to the fetus and impair fetal development (Duckett et al., 2014). Moreover, Dyer (1993) also observed that ergovaline induced contraction in the uterus further altering fetal development.

Abortions and premature births have been noted in sows fed grain ergot (Nordskog and Clark, 1945). Similarly, supplementing ewes with 0.1, 0.5 or 0.7% ergot contaminated feed decreased lambing by 20% (Burfening, 1975). However, because there were no data on the type of ergot and the quantities of alkaloids in these studies, it is difficult to determine whether the reduced pregnancy rate was due to ergot or other mycotoxins. In a later study, Burfening (1994) reported that lambing rate increased to 0.87 lambs/ewe when ewes were fed diet containing 0.5% ergot. This was contrasted to the observation that the lambing rate declined from 1.02 to 0.78 lambs/ewe when ewes were fed diets containing 0.1% ergot. This may demonstrate adaptability of the ewes to the toxin throughout pregnancy or differences in relative concentrations of alkaloids in the two studies. Furthermore, lambs fed a 0.5 or 0.7% ergot-contaminated diet demonstrated a greater susceptibility to lameness with 21% of lambs showing signs of impaired movement. However, no abortions were observed in either of the above trials, although reduced body condition from grain ergot ingestion was noted (Burftening, 1975).

Agalactia refers to the absence or failure to secrete milk, displaying irreversible effects for pregnant livestock during late gestation, with greatest susceptibility in sows (Osweiler, 2014). A direct correlation with a decrease in prolactin secretion and the inhibition of milk production was first identified by Zeilmacker and Carlsten (1962) in rats injected with 1 mg of ergocornine, a
condition that could be reversed by continuous administration of prolactin. Similarly, it has been shown that feeding 0.5 to 1.0% ergot to gestating sows impaired udder development (Peace & Shaw. 1967). Yaremcio (2010) proposed that estrogen concentrations in ergot can cause abortions, along with temporary sterility resulting in lowered subsequent conception rates. Hence, even low concentrations of ergot should be avoided in the feed of pregnant or lactating animals, to avoid the risk of underdeveloped neonates and reduced mammary tissue development (Osweiler, 2014). However, even though several studies have showed that prolactin concentrations decrease upon exposure to ergot alkaloids (Fell et al., 1974; Akers et al., 1981), milk production in ewes fed diets containing 0.5 to 0.7% grain ergot did not decrease (Burfening, 1975).

2.6.6 Sperm motility

Some ergot alkaloids can negatively affect sperm and uterine motility in mammals through agonistic interactions with dopaminergic, alpha-adrenergic and serotonergic receptors (Gallagher and Senger, 1989; Wang et al., 2009). Such membrane receptors are involved in the regulation of mammalian sperm function and increases in intracellular cAMP and calcium concentrations can negatively impact the motility of bovine spermatozoa (Wang et al., 2009). Moreover, ingestion of ergot alkaloids by growing bulls depressed growth rate, serum prolactin concentration, scrotal circumference and sperm motility (Looper et al., 2008). Treating sperm with ergonovine (20 mg/mL) resulted in the greatest reduction in sperm motility and the percentage of intact acrosomes as compared to treatment with phenylephrine, oxytocin and norepinephrine (Gallagher and Senger, 1989). Ultimately, sperm motility is affected by grain ergot whereas both cortisol and testosterone concentrations are not impaired when bulls were fed toxic endophyte-infected and novel endophyte-infected feed (Looper et al., 2008). It has been shown that the interaction of ergot
alkaloids with membrane receptors is complex and different alkaloids affect different receptors in different types of tissues (Wang et al., 2009).

### 2.7 Poultry and ergot alkaloids

In addition to noticeable differences in tolerance levels between species, variation in absorption rate and ability to detoxify toxins is extremely diverse. Although poultry is regarded as a group for recommended allowable limits, dependent on species, they can either be quite tolerant or extremely sensitive (ducks) to ergot alkaloids. This demonstrates the need to develop recommended allowable limits of alkaloids for all species of livestock and poultry.

Compared to mammals, poultry appear to have a greater ability to detoxify alkaloids (EFSA, 2012). Mainka et al. (2005) reported that ergot did not cause changes in weight gain of 28-d old chickens fed *ad libitum* with an ergot content of 0, 0.5, 1, 2 and 4 g/kg diet. The same levels of ergot reduced weight gain in piglets. Chickens rapidly turn over epithelial cells (within 48 h) which may explain their rapid detoxification of ergot (Imondi and Bird, 1966; Moran et al., 1982).

However, even for poultry long term exposure to alkaloids may lead to loss of appetite, increased thirst, diarrhoea, vomiting and weakness (Bailey et al., 1999). Similarly, Dänicke (2015) exposed Peking ducks to 4 different diets containing 1, 10, 15 and 20 g ergot/kg diet, respectively. This corresponded to total ergot alkaloid contents of 0.0, 0.6, 7.0, 11.4 and 16.4 mg/kg. He found that feed intake decreased up to 47% with the high ergot diets. While Mainka et al. (2005) identified no adverse effects on weight gain of chickens, Dänicke (2015) observed a significant growth reduction after two weeks, suggesting that existing ergot alkaloid limits for poultry (1 g ergot/kg unground cereal grains in EFSA regulations) may not offer sufficient protection for ducks. Furthermore, Dänicke (2015) detected alkaloid residues in edible tissue (5 g/g) of Peking ducks.
that also had ergonovine in bile (40 ng/g). Thus, the negative performance of ducks when exposed to 0.6 mg/kg of ergot alkaloids indicates that not all species of poultry are equally tolerant of dietary ergot.

2.8 Impact on the plant and animal industries

Most mycotoxins which infect growing crops and stored feed will be detected based on the type of symptoms shown by livestock (Nicholson and McQueen, 1980). However, with ergot displaying broad symptoms such as heat stress, reduced growth and feed refusals, producers are challenged to identify the occurrence of ergot toxicosis before it has already had a negative impact on the economics of livestock production. With no universal standard for the safe concentration of ergot in feed, producers must exercise caution when introducing potentially contaminated feed sources such as grain screenings into their feeding programs.

While some livestock can tolerate greater concentrations of ergot in feed, the potential for residual toxins to remain in tissues of animals could cause detrimental effects to the human population (Young et al., 1983; Wolff et al., 1995; Mainka et al., 2005). More importantly, by-products, such as screenings for livestock feed may be highly contaminated with mycotoxins and moreover have a greater potential of harming livestock (Trenholm et al., 1989). With the prevalence of ergot increasing from 0.01% in 2002 to 0.025% in 2014 in western Canada (Tittlemier, 2014), and, even though quite rare in Australia still observed in temperate grasses across all states (Ryley et al., n.d.) it is evident that monitoring ergot is becoming more essential for the safety of both livestock and humans (Charmley and Trenholm, 2000).

The need to produce cereal varieties that are capable of withstanding ever-changing climatic conditions has seen an increased use of hybrid varieties of rye and perennial rye breeds in
the last 10 years, particularly in European countries such as Germany (Krska and Crews, 2008). However, today with grain cleaning procedures now capable of removing up to 82% of ergot bodies from unprocessed grain (broken ergot sclerotia are less reliably removed as the particle size is similar to the grain), it is evident that improvements are being made, though often at substantial cost to the producer (Krska and Crews, 2008; EFSA, 2012).

The European Food Safety Authority (EFSA, 2012) suggested that in order to successfully reduce the risk of ergotism in livestock, contaminated cereal grains should undergo seed cleaning, in combination with the adoption of certain husbandry measures such as crop rotation and grazing during summer months to reduce the establishment of flower-heads. However, when considering the level of contamination in cereal crops, it is important to determine alkaloid epimers, as these could alter the toxicity of ergot and cause more harm to livestock than anticipated (Lampen et al., 2006; Krska and Crews, 2008).

Economic impacts surrounding reproductive losses and lowered growth performance are detrimental both on a domestic and global basis. Moreover, with no current treatment marketed to improve symptoms of ergot toxicity and the difficulty of diagnoses, the only available response is to remove the contaminated feed from the diet and allow the liver to detoxify consumed alkaloids (Dewell and Ensley, 2014). It is evident that further investigations are needed to develop effective measures to prevent ergot toxicity in livestock and reduce the economic impact of ergot on agricultural commodities.

2.9 Detoxification and absorption of ergot alkaloids

Livestock and poultry have the capacity to detoxify ergot alkaloids in the liver. However, given the diversity of ergot alkaloids, it is impractical to estimate the length of time required for
detoxification and clearance of all alkaloids from the liver. Moubarak et al. (2009) characterised the role of cytochrome P450 3A (CYP3A) subfamily in the metabolism of ergot alkaloids, in beef liver microsomes. Ergotamine was metabolised by CYP3A after 60 min of incubation; however, other alkaloids such as ergocryptine and ergocornine, inhibited CYP3A activity. Eckert et al. (1978) noted that alkaloids are metabolised or excreted based on their molecular weight, determining that alkaloids with a molecular weight < 350 are excreted in the urine and >350 are excreted in the faeces. This is made clear in a study observing the effects of initial and extended exposure of endophyte infected tall fescue on geldings, concluding that exposure time has no impact on the route of elimination, and consequently is determined by molecular weight revealing that the major route of elimination of lysergic and ergovaline was through urinary excretion (Schultz et al., 2006).

If absorption varies among alkaloids, it may be possible that not all ergot alkaloids are harmful to livestock. Schumann et al. (2008) identified that while ruminants have the potential to detoxify mycotoxins in the rumen, microbes are influenced by the passage rate of feed. Increased feed intake reduces feed retention time in the rumen and increases passage rate, impacting digestion and metabolism. Increasing ergovaline in feed from 0, 1.5 to 3 mg/kg diet depressed feed intake, in addition to reducing ruminal and total tract organic matter and neutral detergent fibre (NDF) digestibilities in sheep. This may have lowered the metabolism of ergot alkaloids in the rumen (Hannah et al., 1990; Browning and Leite-Browning, 1997). Westendorf et al. (1993) reported that feeding 945 mg/d ergovaline (16 mg/kg body weight) decreased DM and NDF ruminal digestibilities, while exposing sheep to 2,346 mg/d ergovaline increased DM and NDF ruminal digestibilities, also possibly due to reduced intake and a longer retention time of feed in the rumen.
Absorption of alkaloids, specifically smaller ergot alkaloids such as ergoline and lysergic acid alkaloids, are primarily absorbed over the ruminant foregut epithelium (25% more than the omasum; Hill et al., 2001). Extensive excretion of toxins via the urine was noted in steers exposed to infested tall fescue as measured by ELISA (Stuedemann et al., 1998). In comparison, faecal excretion was limited to 5% of alkaloids fed to sheep, emphasising the high level of absorption that occurs in ruminants (Westendorf et al., 1993) though the degree of absorption and disappearance of each alkaloid in the rumen is still yet to be established. Furthermore, varying differences in liver enzyme function and individual rumen microorganisms will alter an individual animal’s capability of detoxifying alkaloids, leading to varying levels of tolerance (Moubarak and Rosenkrans, 2000; Lodge-Ivey et al., 2006).

Veterinary recommendations suggest that ergotism can be controlled through an immediate change to an ergot-free diet. However, for pregnant livestock and in particular for sows in late gestation (< 1 week prior to parturition), agalactia syndrome cannot be corrected (Osweiler, 2014). Agalactia syndrome from fescue sources can be corrected in horses through administration of dopamine D2 antagonist domperidone (1.1 mg/kg for 10-14 days). In cases where livestock have been clinically diagnosed with peripheral gangrene, the removal of ergot-contaminated feed will not lead to recovery.

2.10 Technologies and practical measures designed to reduce the impact of ergot on livestock

2.10.1 Genetic engineering strategies
It is possible to select for genetic resistance to ergot among grain crops, although genetic engineering strategies and the selection of hybrids naturally-resistant to molds could be a means of controlling ergot in wheat (Rakesh et al., 2002). Though minimal information is known on the role of insects in ergot epidemiology, there is future potential for plants to be selected that deter insects and reduce the spread of mycotoxins (Trenholm et al., 1989; Alderman, 2006).

2.10.2 Development of vaccines and/or alkaloid binders to allow the animal to systemically bind the toxic alkaloids

The development of vaccines against ergot alkaloids is a possible long-term solution. Filipov et al. (1998) observed a greater average daily gain (13.0 g/day) when rabbits were vaccinated with 50 µg lysergol-human serum albumin compared to non-treated rabbits (12.1 g/day). While this study evaluated a vaccine against the effects of alkaloids from tall fescue (total dietary alkaloids 340 ppb), development of a vaccine associated with grain alkaloids should also be possible.

Deoxynivalenone is a mycotoxin causing similar symptoms to ergot in livestock such as reduced feed intake and body weight gain (Trenholm et al., 1989). Although ergot alkaloids and DON differ in chemical structure, studies conducted using DON have relevance for ergot. Young et al. (1987) revealed that feeding swine corn contaminated with 7.2 mg DON/kg resulted in a reduction in feed intake. When corn was treated with sodium bisulfite, impacts of DON decreased 10 fold. Treatment with sodium bisulfite appeared to remove short-term toxic effects on pigs due to the presence of DON in their diet. Accordingly, there is a possibility that chemical treatments could be developed to reduce the toxicity of ergot alkaloids in feed.
It is also plausible that using alkaloid binders will decrease bioavailability of ergot alkaloids. However, studies of alkaloid binders are limited and in the one published study, Friend et al. (1984) evaluated a chemical binding agent, polyvinyl-pyrrolidone (Antitox Vana®) and ammonia carbonate and noted that these binders did not reduce the negative impacts of DON on swine production. Further investigations using alkaloid binders to reduce the toxicity of ergot contaminated grain are required, but care must also be taken to ensure that such binders do not reduce overall nutrient availability (Raymond et al., 2003; Whitlow and Hagler, 2008).

2.10.3 Isolation of anaerobic bacteria to degrade ergot alkaloids before systemic absorption

Anaerobic microbes present in the rumen of sheep and cattle are capable of detoxifying some ergot alkaloids and inoculating other microbes into the rumen might be beneficial in this regard. Anaerobic microbes in the gut of the red wiggler earthworm, *Eisenia fetida*, degraded over 60% of ergovaline, with the flora responsible for this degradation from four major phyla: *Plantomyce*, *Chloroflexi*, *Bacteroides* and *Proteobacteria* (Perumbakkam et al., 2007). Further research to isolate and characterise microorganisms which are capable of detoxifying ergot alkaloids may allow their use as a direct-fed microbial to minimise the impact of feed ergot on animals.

2.10.4 Hydrothermal treatment effects on ergot alkaloid content in contaminated grain

Hydrothermal treatments are often incorporated to improve the digestibility of nutrients and feed value, particularly for non-ruminant species (Mainka et al., 2005). Treating ergot-contaminated grain with steam for 2 min at 95°C at 17% moisture, followed by 5 sec at 120°C at 18% moisture decreased total alkaloid content by 10%, with reductions becoming more marked
with increasing levels of alkaloids (Mainka et al., 2005). This method could be employed during the feed processing stage, to further reduce alkaloids, although impacts on alkaloid toxicity would require investigation prior to use in livestock feeds.

2.10.5 Other on-farm prevention measures

Irrespective of advanced technologies that can be potentially implemented on-farm to minimise negative impacts, ergot toxicosis are mainly controlled by limiting ergot presence at all levels of production including, storage, milling and delivery (Trenholm et al., 1989). Chemical treatments used to clean the grain kernels can be implemented to significantly reduce the toxin level if the ergot contamination is not too severe. Removal of grain dust and lighter, shrivelled kernels through density segregation can also reduce the risk of ergot poisoning (Charmley and Trenholm, 2000; Belser-Ehrlich et al., 2001). Other procedures such as soaking, dehulling, roasting or high velocity air cleaning of grain can be used to remove surface ergot contaminants (EFSA, 2012).

A variety of prevention measures have been identified to help producers minimise ergot establishment and growth in cereal crops (Belser-Ehrich et al., 2012) including:

a) Limiting the number of damaged kernels from birds and insects, as molds thrive on kernels where the pericarp or hull has been compromised.

b) Harvesting grain as soon as practically possible, especially when ergot is visually detected. Areas on-farm highly susceptible to ergot should be harvested as forage prior to the heading stage in order to avoid the formation of ergot bodies.

c) Correctly storing and drying grain. With high moisture content, conditions remain anaerobic, increasing the likelihood of mycotoxin contamination.
d) Rotating crops to avoid the carry-over of molds, as sclerotia are capable of remaining viable prolonged periods. Increasing seedling vigor and using seeds treated with fungicides, will reduce seed-borne inoculum.

2.11 Conclusion

Minimizing the economic loss of producers due to ergot contamination in grains and subsequent ergot toxicosis in livestock is challenging, with government bodies prioritising other mycotoxins such as DON and fumonisins. The diversity of fungal species and ergot alkaloids, their interactions with the surrounding environments for different crops and their varying toxicities in different tissues and/or livestock and poultry add to the complexity of the issue. As the climate is changing to favor ergot-producing fungi in some parts of the world and as regulations for human food become stricter, the frequency of ergot contaminated grains will likely increase in the future. Accordingly, strategies to reduce risks of ergot toxicosis are required to support the livestock industry. Although regulations and recommendations for the ergot alkaloid level in animal feed exist, a scientific basis for these recommendations is generally lacking.

While eliminating the threat of ergot toxicosis in livestock is likely impossible, application of some practical measures including chemical cleaning grain would minimise their impact, but the process is costly and may leave toxic residues. Devising methods to combat toxicosis could be aided by a better understanding of the physiological pathways impacted by ergot alkaloids. Moreover, experiments incorporating individual alkaloids in *in vitro* and *in vivo* animal studies would benefit this effort. Alkaloid binders and the use of antioxidants to lessen the effects of ergot poisoning would be valuable if effective binders could be identified. With grain contamination by ergot increasing annually and globally, effective detection methods are required to distinguish the
concentration of alkaloids present and therefore put measures in place to help reduce the toxicity of alkaloids for livestock.
2.12 References


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Tittlemier, S.A. PowerPoint Presentation, Fusarium, ergot, and mycotoxins in western Canadian grain, Canadian Grain Commission, Canada (2014).


Yaremcio, B. Ergot in Livestock Feed. Ag-Information Centre, Stettler (2010).


**Tables**

**Table 2.1** Species of Claviceps found on grain crops (Krska and Crews, 2008).

<table>
<thead>
<tr>
<th>Claviceps species</th>
<th>Host crops</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. africana</em> and <em>C. sorghi</em></td>
<td>Sorghum</td>
</tr>
<tr>
<td><em>C. gigantean</em></td>
<td>Maize</td>
</tr>
<tr>
<td><em>C. purpurea</em></td>
<td>Barley, Wheat, Rye, Oats</td>
</tr>
</tbody>
</table>
**Table 2.2** Limits of detection (LOD) and retention time of major ergot alkaloids and their epimers in wheat flour (Byrd, 2012).

<table>
<thead>
<tr>
<th>Ergot alkaloid</th>
<th>LOD (µg/g)</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergometrine</td>
<td>0.0034</td>
<td>6.6</td>
</tr>
<tr>
<td>Ergometrinine</td>
<td>0.0017</td>
<td>7.2</td>
</tr>
<tr>
<td>Ergotamine</td>
<td>0.0093</td>
<td>8.2</td>
</tr>
<tr>
<td>Ergotaminine</td>
<td>0.012</td>
<td>9.8</td>
</tr>
<tr>
<td>Ergosine</td>
<td>0.0063</td>
<td>8.1</td>
</tr>
<tr>
<td>Ergosinine</td>
<td>0.0030</td>
<td>9.5</td>
</tr>
<tr>
<td>Ergocristine</td>
<td>0.017</td>
<td>9.1</td>
</tr>
<tr>
<td>Ergocristinine</td>
<td>0.021</td>
<td>10.5</td>
</tr>
<tr>
<td>Ergocryptine</td>
<td>0.0023</td>
<td>9.0</td>
</tr>
<tr>
<td>Ergocryptinine</td>
<td>0.0081</td>
<td>10.4</td>
</tr>
<tr>
<td>Ergocornine</td>
<td>0.0060</td>
<td>8.7</td>
</tr>
<tr>
<td>Ergocorinine</td>
<td>0.0055</td>
<td>10.1</td>
</tr>
</tbody>
</table>
Table 2.3 Allowable levels of ergot contamination (ppm) in cereal grains and feed in various regions of the world (T, triticale; W, wheat; R, rye; B, barley; O, oats; Scott, 2009).

<table>
<thead>
<tr>
<th>Region</th>
<th>Ergot limit in cereal grains for humans (ppm)</th>
<th>Ergot limit in animal feed (ppm)</th>
<th>Other comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia and New Zealand</td>
<td>0.05</td>
<td>N/A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0-0.1% (T)</td>
</tr>
<tr>
<td>Canada</td>
<td>0-0.05</td>
<td>0.10-0.33</td>
<td>Varies with grade of wheat</td>
</tr>
<tr>
<td>European Union</td>
<td>0.05</td>
<td>0.10</td>
<td>-</td>
</tr>
<tr>
<td>Switzerland</td>
<td>0.02</td>
<td>N/A</td>
<td>0.05 limit on cereals destined for milling</td>
</tr>
<tr>
<td>Japan</td>
<td>0.04</td>
<td>N/A</td>
<td>-</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Zero tolerance</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td>United States</td>
<td>0.3 (W, R)</td>
<td>0.3 (W, R)</td>
<td>0.1% (B, O, T)</td>
</tr>
</tbody>
</table>

<sup>a</sup>N/A, not available
Table 2.4 Recommended practical limits for ergot or ergot alkaloids in animal feeds to reduce negative effects on health and performance.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Recommended ergot alkaloids practical limits (ppm; Alltech Canada, 2005)</th>
<th>Maximum tolerance (allowable) level of ergot alkaloids (ppm; Belser-Ehrlich et al., 2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>Piglets/sows/gilts</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Poultry broiler/layer</td>
<td>0.75</td>
<td>1.5</td>
</tr>
<tr>
<td>Dairy/beef cattle</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Calf</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Horses</td>
<td>0.25</td>
<td>0.5</td>
</tr>
</tbody>
</table>
**Table 2.5** Summary of ergot symptoms in mammals (Miller and Richardson, 2013).

<table>
<thead>
<tr>
<th>Form of ergotism</th>
<th>Species</th>
<th>Subfamily</th>
<th>Toxic alkaloid(s)</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convulsive ergotism</td>
<td><em>C. purpurea</em></td>
<td>Ergoline</td>
<td>Ergotoxin, ergometrine, ergotoxin, (lysergic acid amines including lysergic acid, lysergol, ergine)</td>
<td>Writhing, tremors, (torticollis), confusion, hallucinations, tingling sensation underneath the skin (formication) and death.</td>
</tr>
<tr>
<td>Gangrenous ergotism</td>
<td><em>C. purpurea</em></td>
<td>Ergopeptine</td>
<td>(Total dietary concentrations of &gt;100-200 ppm can lead to death) Ergotoxin, ergometrine, ergotoxin, ergovaline, ergocryptine</td>
<td>Vasoconstriction, hot and cold feelings in the extremities, cold skin, spontaneous abortion, heat stress, severe lameness, reduced feed intake, reduced growth rate, agalactia and gangrene. Ergocryptine affects prolactin levels and greatly reduces or</td>
</tr>
<tr>
<td>Condition</td>
<td>Species</td>
<td>Unknown</td>
<td>Secondary Metabolites</td>
<td>Symptoms</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------</td>
<td>------------</td>
<td>-------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Enteroergotism</td>
<td><em>C. fusiformis</em></td>
<td>Unknown</td>
<td>Clavine</td>
<td>Nausea, vomiting, somnolence, and giddiness</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>eliminates milk production for lactation.</td>
</tr>
<tr>
<td>Hyperthermic ergotism</td>
<td><em>A. conphialum</em></td>
<td>Unknown</td>
<td>Ergotamine, ergosine, and agroclavine</td>
<td>Fever, diarrhea, clear nasal discharge, weight loss, labored breathing, increased metabolic rate, excessive salivation and low levels of prolactin.</td>
</tr>
<tr>
<td></td>
<td><em>C. africana</em>, <em>C. cyperi</em>, <em>C. cyperi</em>, <em>C. sorghi</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 3 – Objectives

This thesis aims to provide an insight into cereal ergot alkaloids, with focus on the impacts of increasing ergot alkaloid concentrations and how they can affect the digestibility and performance traits of growing lambs.

The objective of the first study was to use two different diet forms, mash and pellet, to determine whether diet preparation impact of alkaloid toxicity in lambs. This study evaluated whether increasing concentrations up to 433 ppb total alkaloids impacts nutrient digestibility, growth performance and carcass traits of both growing ewe and ram lambs by total collection of urine and faeces, measuring weight, amount of feed intake, prolactin concentration and carcass traits.

The objective of study two aimed to obtain new data on the composition of ergot alkaloids and their epimers from a new source of grain. This study was an extension of the first study in which we increased dietary alkaloid concentrations to the maximum recommended dosage and evaluated the effects of feeding a different alkaloid profile on metabolism and animal performance. Additionally, this study expanded on the significance of alkaloid detection methods.

Thus, this thesis further discusses the known correlations between the ergot alkaloids and development of ergotism to serve as a starting point for suggesting threshold concentrations and metabolism of these toxicants. And also expand on how lack of reliable lab analysis is preventing a conclusion on what the allowable levels of alkaloid concentrations should be.
Effects of pelleting diets containing cereal ergot alkaloids on nutrient digestibility, growth performance and carcass traits of lambs

Abstract
The effects of pelleting feed containing cereal ergot alkaloids was evaluated in performance and nutrient digestibility trials using growing lambs. Defined concentrations of ergot alkaloids [Control (C), no added alkaloids but background concentrations of ~3 ppb; Low (L), ~169 ppb; High (H), ~433 ppb] were achieved by substituting barley grain for ergot-contaminated screenings containing (fed basis) approximately 538 g/kg barley grain, 300 g/kg alfalfa and 160 g/kg canola meal. Diets were fed either as a mash or as a completely pelleted feed. Total alkaloid concentrations did not differ between corresponding mash and pelleted diets, but ergotamine and ergosine were 2-3 times greater in mash feeds, while ergocornine, ergocristine and ergometrine were 2-3 times greater in pelleted diets. The total collection digestibility experiment used 12 ram lambs in a crossover design with 3 experimental periods. Alkaloid dose did not affect digestibility of DM, OM, or CP, but NDF and ADF digestibilities were linearly reduced (P < 0.05) with increasing alkaloid dose. Alkaloid concentrations in faeces depended upon the specific type of alkaloid measured. In preliminary results, ergocristine and ergotamine were the only alkaloids in higher concentrations (P < 0.001) in faeces from lambs fed H as compared to C diets. In the growth experiment, ram and ewe lambs (live weight 24.6±1.08 kg) were randomly assigned to diets, weighed weekly and fed to a slaughter weight of ≥45 kg. Dietary treatments did not affect carcass characteristics, although serum prolactin concentration was linearly reduced (P < 0.001) by increasing alkaloid dosage and was lower (P = 0.01) in lambs fed mash as compared to pelleted diets. Although pelleted diets had total alkaloid concentrations that were similar to mash diets, lambs fed pelleted diets had 60 g/d greater (P < 0.001) ADG than those fed mash diets. For H diets, lambs had lower ADG and feed conversion (P = 0.03) than those fed C or L. Based on the results of this study, pelleting diets reduced negative impacts of ergot alkaloids possibly by changing alkaloid profiles.
Abbreviations: C, control diet with no added alkaloids; L, ~169 ppb added alkaloids; H, ~433 ppb added alkaloids; M, mash diet, P, pelleted diet; MC, mash control; PC, pelleted control; ML, mash diet with ~169 ppb added alkaloids, MH, mash diet with ~433 ppb added alkaloids; PL, pelleted diet with ~169 ppb added alkaloids; PH, pelleted diet with ~433 ppb added alkaloids.

Keywords: alkaloid, ergot, grain, growth, lambs, nutrient digestibility.

1. Introduction

Mycotoxins are toxic secondary metabolites of fungi impacting important agricultural commodities under a wide range of climatic conditions, with livestock often exposed to feed containing more than one mycotoxin (Songsermsakul and Razzazi-Fazeli, 2008; Fink-Gremmels, 2016). In western Canada, contamination of grain with Claviceps purpurea has increased markedly over the past 10 years (Tittlemier et al., 2015), although few studies have evaluated impacts of cereal ergot on livestock. The agronomic characteristics of cereal ergot, Claviceps purpurea, are similar to the ergot of tall fescue, Lolium arundinaceum (Fink-Gremmels, 2008; Lovell, 2013). Alkaloid types differ across species of ergot (Porter and Thompson, 1992), with both type and concentration of alkaloids influencing animal impacts (Klotz, 2015). In Canada, maximum allowable concentrations of ergot alkaloids in feed are the same for cattle and sheep (2-3 ppm; CFIA, 2017) and are at least an order of magnitude greater than the < 0.1 ppm that has been reported to reduce growth rate in cattle (Shelby, 1999; Evans et al., 2003). As well, ergotism may be less severe in sheep than in cattle consuming the same concentration of ergot in the diet (Evans et al., 2003).

Due to concerns based on anecdotal evidence from the feed industry that pelleting increases negative impacts of cereal ergot alkaloids on livestock performance, the primary objective of this
study was to evaluate pelleted and mash diets with increasing concentrations of alkaloids for impacts on growth performance, carcass traits and nutrient digestibility of lambs. A secondary objective was to evaluate the impact of particle size on assay of alkaloids in order to identify and control sources of variation in this methodology.

2. Materials and methods

Lamb feeding and digestibility experiments were conducted at the Lethbridge Research and Development Centre (LRDC) of Agriculture and Agri-Food Canada, between May and September of 2015. Protocols for these experiments were approved by the LRDC Animal Care Committee according to the guidelines of the Canadian Council on Animal Care (2009).

2.1 Source of ergot and ergot alkaloid determination

A single lot of heavily ergot-contaminated barley screenings was obtained from the Canadian Feed Research Centre, North Battleford, SK and used as the source of ergot alkaloids. A representative sample (1 kg) of screenings from a single bag from the same location was analysed for concentrations of six individual alkaloids: ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine by Prairie Diagnostic Services Inc. Saskatoon, SK. Screenings were found to have total alkaloid concentrations of 108.8 ppm and appropriate quantities of screenings were then added to the diets to achieve targeted alkaloid concentrations in treatment diets. Ergot alkaloids in screenings, diets and faeces were determined using the procedures of Krska et al. (2008) in combination with a Romer Labs® extraction and cleanup process. Only metabolically-active R-epimers were quantified. Alkaloids used in this study were determined by comparison to laboratory standards purchased from Romer Labs Inc. (Union, MO) and the procedure had a detection limit of 1.25 ppb.
For alkaloid analyses, ground subsamples (5.0 g) were weighed into 125 mL Erlenmeyer flasks and extracted using 25 mL of acetonitrile (850 g/L) and 10 mM ammonium acetate (150 g/L). Samples were stirred for 10 min at 540 rpm using a multi flask stir plate (CIMARECi, Thermo Scientific, Waltham, MA). The supernatant was filtered through Whatman 41 110 mm filter paper into a 100 mL beaker. A 1 mL subsample of the filtered extract was added to a 16 x 100 mm test tube containing 0.05 g of 40 µm Agilent Bondesil PSA. The test tube was vortexted for 1 min and 0.4 mL transferred to an auto-sampler vial for analysis (Agilent Technologies, Santa Clara, CA). Reagents were filtered through a Millipore system with aqueous ammonium acetate (10 mM) as mobile phase A, and acetonitrile (850 g/L) as mobile phase B. The Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) consisted of an Agilent Zorbax Eclipse XDB-C18 narrow single column (bore 2.1×150 mm, 5 µm pore size), coupled to a Quattro Ultima Pt mass spectrometer (Waters, Milford, MA).

2.2 Treatments and diet preparation

Ergot-contaminated screenings were ground through a 1 mm screen (Wiley® Cutting Mill, Thomas Scientific, Swedesboro, NJ) to ensure uniform distribution of ergot in diets. Diets included Control (C), no added alkaloids; Low (L), average of 169 ppb; High (H) average of 433 ppb in both mash (M) and pelleted (P) forms. Each diet was made in a single batch (1500 kg; Table 1) and used for both growth and digestibility studies. On a monthly basis, a 2 kg sample of feed was collected for subsequent analyses of ergot alkaloids and other feed constituents with a total of 3 composited samples collected over the duration of the study.
To gage the impact of particle size on alkaloid recovery, a second sub-sample of ergot-contaminated screenings was ground either through a 1 mm screen as described above or in a Cyclone Mill (Model 3010-060, UDP Corporation, Fort Collins CO).

2.3 Particle size determination

Three 2.0 mg subsamples from each of two grinding methods (through a 1 mm screen or in a Cyclone Mill) were suspended in 200 µL distilled water. Each sample was vortexed to suspend particles, then centrifuged at 9391 x g for 2 min. The sample material was then carefully re-suspended in distilled water, placed on a microscope slide, covered with an 18×18 coverslip and sealed with acetone.

An Olympus FV1000 confocal laser scanning microscope (Olympus America Inc., Center Valley PA) was used to produce transmitted light images. Due to the wide range of particle sizes, depth scans were required to produce images where all particles were in focus. An overall image of the sample was collected with a 4X objective and subsequent depth images were collected with a 10X objective. Depth images were stacked and scale bars added using Olympus Fluoview software (Version 4.2). Five fields of view were collected for each subsample.

The freeware program ImageJ (Reinking, 2007) was used to analyse the particles, determine area distribution and select binning of area results, followed by manual verification. To compare samples; total numbers of particles (ranging in size from 0 to infinity µm²), total area and area distribution were collected. Due to the wide range of particle sizes it was necessary to collect area distribution (binning) twice; once to collect and separate small particles (sizes 0 to 1000 µm²) and a second determination to collect the remaining large particles (>1000 to infinity µm²).
Binning of particle sizes was also checked manually to ensure that area determinations were collected on all particle sizes.

2.4 Nutrient digestion and metabolism of lambs

Twelve Canadian Arcott ram lambs were used to assess the digestibility of the diets using a crossover experimental design with a $2 \times 3$ factorial layout (two diet forms, pelleted and mash and three concentrations of alkaloids, control, low and high). Each period (total of 3 periods) lasted for 3 weeks, with the first 2 weeks for adaption to diets, to minimise carry-over of alkaloids among treatments and the 3rd week for sample collection. Lambs were randomly assigned to either mash or pelleted C, L or H diets and fed once daily at ad libitum intake during the adaption period and at 950 g/kg of ad libitum during the week of collection. Orts were collected and weighed daily during the adaption period to calculate DMI. Lambs were individually fed in floor pens during the adaption period and then transferred to metabolism crates during sample collection. One week prior to the initial collection period, lambs were shorn and pre-fitted with strap-on canvas faecal collection bags.

Daily faeces and urine outputs of each lamb were determined by total collections during the last 4 d of the 3rd week in each period. Sulfuric acid (100 ± 50 mL) was placed in urine collection containers each morning, to prevent ammonia volatilization. Faecal collection bags were emptied twice daily at 0800 h and 1500 h prior to the next collection to avoid loss of faeces from bags. Faeces that fell into the crate were included in calculations of total faecal production, but not used as subsamples. Faeces and urine were subsampled daily (150 g/kg and 100 g/kg respectively), composited over each period and stored at -10 °C. A second faecal subsample (approximately 100 g) was collected daily for faecal DM determination.
2.5 Laboratory analyses

Pooled faeces were thawed and dried at 55°C for one week prior to grinding (Cutting Mill SM 100; Retsch Inc., Newtown, PA) through a 1 mm screen. Samples of diet were dried and ground as described for faeces. Both diets and faeces were dried at 105°C for 24 h and hot weighed for determination of analytical DM (Method #967.03; AOAC, 1995). Organic matter was determined by ashing the samples at 550°C for 5 h (Method #942.05; AOAC, 1995). Samples were ball ground (Mixer Mill MM200; Retsch Inc., Newtown, PA; (Method #990.03; AOAC, 1995) prior to N analyses using a 2100 Elemental Combustion Analyser (Carlo Erba Instruments, Milan, Italy). Neutral detergent fibre and ADF were determined following the procedure of Van Soest et al. (1991) using the Ankom 200 fibre analyser (Ankom Technology, NY) using heat stable α-amylase and sodium sulfite in the NDF procedure. Acid detergent fibre was determined according to method 973.18 of AOAC (1995). Crude fat content was determined by ether extraction (AOAC 2005; method 920.39) using a BUCHI Extraction Unit E-816 (Buchi, Flawil, Switzerland). Non-fibrous carbohydrate (g/kg of DM) was calculated as: 1000 – (CP + NDF + crude fat + ash).

Two composite faecal samples from two animals fed either C or H diet in each period were analysed for ergot alkaloid concentration by the commercial laboratory as described above after first grinding each subsample (approximately 300 g) through a 1 mm screen.

Urine samples were thawed overnight and then diluted 1:5 (urine:distilled water), which was then pipetted (50 µL) into a microwell plate, dried at 55 °C overnight and analysed for total N as described above.

2.6 Growth performance of lambs
Forty eight Canadian Arcott lambs (24 ewes and 24 rams; 24.6 ± 1.08 kg) were randomly assigned to one of the six diets prepared above, and fed individually in indoor pens (60.96 × 106.68 × 73.66 cm). Lambs assigned to high dosage alkaloid mash H (MH) and pelleted H (PH) treatments were offered mash L (ML) and pelleted L (PL) diets for 1 week prior to receiving high-alkaloid diets for the remainder of the experiment, to monitor impacts on animal health and performance from ergot alkaloid ingestion as there was limited documented literature evaluating the impacts of ingesting grain ergot alkaloids on growing lambs. Following the first week, lambs received experimental diets for up to 12 weeks. Lambs were fed once daily for ad libitum intake and had free access to water throughout the study. Orts were collected daily before feeding for determination of DMI. Lambs were weighed weekly and weight at the beginning of the experiment was subtracted from weight at the end of the experiment and divided by days on feed to estimate ADG. Feed efficiency was calculated as ADG/DMI. Health of the lambs was closely monitored by barn staff and any morbidities observed were recorded.

2.7 Determination of serum prolactin concentration and carcass characterization

Blood samples were taken from 36 lambs (6 per treatment) when they reached 35 kg BW (average d 21 on trial) and at slaughter (≥45 kg; average d 56 on trial). Blood samples (approximately 6.0 mL) were obtained from the jugular vein into 10.0 mL serum vacutainers and stored at room temperature for 30 min. Blood was centrifuged at 2000 × g for 15 min at 4°C. Serum was pipetted from remaining blood components, and stored in 1.0 mL screw-cap centrifuge tubes in duplicate. Samples were stored at –80 °C until further analysed.

Serum samples were analysed using a double antibody radioimmunoassay with oPrl (100:1; first antibody) and rabbit globulins (500:1; second antibody) and reagents obtained from
the National Hormone and Pituitary Program (NHPP; Harbor-UCLA Med Ctr, Torrance, CA). The standard curve ranged from 0.5 to 32 ng prolactin mL\(^{-1}\), and standards were prepared in 0.5M phosphate buffered saline containing gelatin. Tubes were prepared with the first antibody, vortexed and stored at 4°C overnight. The second antibody solution was added on d 2, and tubes were again vortexed and refrigerated at 4°C overnight. On d 3, all tubes were centrifuged except for the total count tubes for 30 min at 1500 \(\times\) g. Supernatants were carefully decanted to avoid disturbing the pellet. Prolactin was then calculated based on radioactivity of the pellet after analysis using a gamma counter (Titertek Apex Plus, Titertek Instruments Huntsville, AL) based on the calculation program RIAPC (Rawlings et al., 1984) incorporating log-logit calculations to create the standard curve and calculate results.

Lambs were removed from the experiment at ≥45 kg and a subset of 38 (5 to 8 per dietary treatment) was slaughtered at a commercial abattoir (SunGold Meats Ltd., Innisfail, AB). Carcass traits including hot carcass weight, dressing percentage and grade rule measurement (mm) were assessed according to Canadian Food Inspection Agency standards (Government of Canada, 2017).

2.8 Statistical Analyses

Feed efficiency and prolactin data were log transformed prior to analyses, as data were not normally distributed. Dry matter intake, ADG and feed efficiency were analysed as a completely randomized design by 2-way ANOVA using the MIXED procedure of SAS, with diet preparation, alkaloid concentration and their interaction as fixed effects, lambs nested within treatment as a random effect, and week as a repeated measure (SAS Inst. Inc., Cary, NC). Sex was included in the model as a fixed effect for serum prolactin analyses. Initial LW, final LW and carcass data
were analysed using the MIXED procedure without repeated measures. Digestibility calculations were also analysed using the MIXED procedure with period and lamb as random effects. Significance was acknowledged for both studies at $P \leq 0.05$. Linear and quadratic effects were determined by using planned orthogonal polynomial coefficients for each parameter. Only linear responses to increasing concentrations of alkaloids are reported as quadratic contrasts were not significant.

3. Results

3.1 Alkaloid analyses

Analyses of screenings revealed that ergocristine, ergotamine and ergocryptine were the dominant alkaloids (Table 4.2). Analyses of mash and pelleted C diets showed that they contained 3.4 ppb and 2.0 ppb of ergot alkaloids, respectively, consisting mainly of ergometrine and ergocristine. In spiked diets, overall alkaloid concentrations were similar regardless of preparation, averaging 153 ppb for ML, compared to 185 ppb for PL, while MH averaged 434 ppb and PH averaged 432 ppb. Although total concentrations of alkaloids were similar in spiked diets, concentrations of individual alkaloids varied with diet preparation. Mash diets averaged 1.9 times more ergosine and 3.0 times more ergotamine than pelleted diets. In contrast, pelleted diets averaged 2.7 times more ergocornine and ergometrine and 1.9 times more ergocristine than mash diets.

Recovery of individual ergot alkaloids also varied with grinding method as ergometrine recovery was 32 times greater after grinding using the cyclone mill vs. through a 1 mm screen (186.40 vs. 5.68 ppm). Total alkaloid concentrations were 7 times greater in screenings ground
using the cyclone mill (average particle size 94.2 µm) as compared to those ground through a 1mm screen (average particle size 124.1 µm; Table 4.2).

3.2 Nutrient metabolism

Alkaloid content or the form of diet did not affect DM digestibility (DMD), crude protein digestibility (CPD) or organic matter digestibility (OMD), but the digestibility of neutral detergent fibre (NDF) and acid detergent fibre (ADF) was linearly reduced by increasing alkaloid concentration ($P = 0.03$; Table 4.3). Both NDF and ADF digestibility were also higher ($P = 0.04$) in lambs fed mash as compared to pelleted diets.

3.3 Alkaloids in faeces

Concentrations of alkaloids were measured only in faeces from C and H diets as a preliminary evaluation of the utility of this methodology. Total concentration of alkaloids in faeces was higher in faeces from H as compared to C diets ($P \leq 0.001$), but the extent of recovery differed ($P \leq 0.001$) among alkaloid types (Table 4.4). Ergocristine ($P < 0.001$) and ergotamine ($P < 0.001$) were present in greater concentrations in faeces from H as compared to C diets, although no alkaloids were present above the limit of detection in faeces from C diets. Diet preparation had no effect on faecal alkaloid concentration ($P = 0.75$).

3.4 Growth Study

3.4.1 Serum prolactin

As alkaloid concentration of diets increased, serum prolactin concentrations linearly decreased ($P < 0.001$; Table 4.5), with declines in prolactin concentration noted ($P < 0.01$) even for lambs fed L as compared to C diets. Diet preparation also impacted serum prolactin
concentrations, which were greater \((P = 0.01)\) in lambs receiving pelleted than mash diets. Ram lambs had greater serum prolactin concentrations than did ewe lambs \((P < 0.001)\) and interactions among preparation, dose and sex were not significant \((P > 0.30)\).

3.4.2 Growth performance

Initial LW did not differ among treatments and the form of diet had no effect on DMI \((P = 0.76)\), but average daily gain was greater \((P < 0.001)\) in lambs fed pelleted as compared to mash diets (Table 4.6). Lambs receiving MH had a 44.9 g/d lower ADG than those receiving mash C (MC) \((P = 0.05)\), while ADG of PH lambs were 28.2 g/d lower \((P = 0.03)\) than those fed pelleted C (PC). Dietary ergot alkaloid concentration did not affect DMI \((P = 0.71)\), but lambs receiving H diets had lower \((P = 0.03)\) FC and ADG than did lambs receiving L or C diets. Lamb health was closely monitored and no lambs exhibited lameness or other symptoms of alkaloid toxicity. No mortalities occurred and no lambs were removed from the study due to morbidity.

3.4.3 Carcass data

Carcass data were obtained for 38 lambs as 10 lambs mostly receiving mash diets (3 MH, 2 ML, 2 MC, 2 PL, 1 PH), did not reach target weights for slaughter \((> 45 \text{ kg})\) irrespective of alkaloid dose. Diet preparation, alkaloid dose or their interaction had no effect \((P \geq 0.31)\) on hot carcass weight, dressing percentage or other carcass traits (Table 4.6). No livers or carcasses were condemned at the commercial abattoir and no abnormalities were observed in carcass fat depots.

4. Discussion

The majority of studies investigating the impact of ergot alkaloids on ruminants have focused on tall fescue toxins, in which \textit{Neotyphodium coenphialum} is the primary etiological agent
(Burns, 2009). Fewer studies have investigated the impact on ruminants of cereal ergot alkaloids produced primarily by *Claviceps purpurea* (Evans et al., 2003). The types of alkaloids present in cereal ergot differ from those in tall fescue, with diversity of alkaloid profiles contributing to the variety of broad symptoms observed in livestock consuming ergot-contaminated feed such as reduced weight gain, elevated core temperatures and reduced fertility (Panaccione, 2005; Rogers et al., 2011).

The widespread prevalence of cereal ergot in western Canada was demonstrated by the background concentrations of alkaloids present in C diets in the present study. Wheat produced in western Canada in 2013 had on average 0.35 g/kg ergot, with ergocristine and ergocristinine the predominant alkaloids (Tittlemier et al., 2015). Profiles of individual alkaloids in the current study associated with background concentrations of ergot differed from those in the ergot-contaminated screenings that were used to spike alkaloids into the diet. Others have found that the types of alkaloids differ markedly among sources of cereal ergot (Panaccione, 2005), but similar to our results, ergocristine is often present in the greatest concentration (Tittlemeier et al., 2015). Impacts of individual alkaloids on livestock growth performance have yet to be evaluated, although ergocristine had the strongest toxic effects on cultured human primary cells out of all ergot alkaloids tested (Mulac et al., 2012). Consequently, results of the present study would apply only to ergot with similar concentrations and proportions of the specific alkaloids investigated. Since ergot contamination introduces a variety of alkaloids into the diet, future studies should be undertaken evaluating impacts of defined mixtures of alkaloids on the growth performance and health of ruminants.

The ergot alkaloid concentrations used in this experiment were selected based upon clinical cases for ruminants collected throughout western Canada (Osweiler et al., 1985; Cowan and
Blakley, 2014) which reported that cereal alkaloids in dietary concentrations exceeding 100-200 ppb often adversely affect the weight gain of ruminants. In contrast, the Canadian Food Inspection Agency (CFIA) regulations allow concentrations of 2-3 ppm of ergot alkaloids in ruminant diets based on concentrations that cause symptoms of ergot toxicosis such as gangrene of hooves. As growing ruminants would be potential end-users of ergot-contaminated feed, they were selected for use in the present study and fed the minimum concentration of alkaloids which could potentially impact performance of the lambs.

4.1 Alkaloid assay

Twenty-two ergopeptine alkaloids (with corresponding isomers and amino acids) have been described, but HPLC assays are as yet available only for six of the most common alkaloids encountered in western Canada: ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine (Scharld et al., 2006; Songsermsakul and Razzazi-Fazeli, 2008). Ergot alkaloids can bi-directionally convert among epimers, the highly toxic ($R$) form and the biologically-inactive ($S$) form (Merkel et al., 2012), but only $R$ epimers were quantified by the commercial laboratory. As pH of the diet and gastrointestinal tract affects epimerisation of alkaloids (Merkel et al., 2012), changes in dietary starch content could likely alter concentrations of some $R$ epimers compared to that in the present study, potentially changing animal impacts even within a single source of ergot.

Our work showed that the efficiency of alkaloid extraction and detection is greatly dependent on the particle size used in the analysis. Grusie et al. (2016) determined that using a 4 mm as compared to a 1 mm grinding screen reduced extraction of alkaloids and led to a greater likelihood for non-representative results. Values of the present study also demonstrate variation in alkaloid assays due to sample preparation as grinding highly-contaminated screenings in a cyclone
mill increased detection of total alkaloids by 7 times compared to grinding the screenings through a 1 mm screen. This is in spite of the fact that average particle size of the 2 methods differed by only 24%. Consequently, sampling and sample preparation must be standardized when analyzing ergot-contaminated grain, otherwise estimates will vary substantially within and among laboratories, especially when calculating concentrations in bulk samples (Whitaker et al., 2004; Songsermsakul and Razzazi-Fazeli, 2008; Grusie et al., 2016).

4.2 Digestibility study

Carry-over of ergot alkaloids into tissues has been difficult to detect (Dänicke, 2016) and the half-life of ergotamine in plasma has been estimated at 2.5 h (Tfelt-Hansen and Paazlow, 1985) due to rapid metabolism in the liver (Krska and Crews, 2008). However, to avoid potential carry-over of alkaloids between periods of the digestibility study, lambs were adapted to diets for 2 weeks, prior to collection of faeces and urine. A similar 2 week adaptation to diets has previously been used to reduce carry-over effects of ergot alkaloids in metabolism studies (Wolff et al., 1995; Koontz et al., 2015), although physiological effects of ergot alkaloids are complex and additional study is warranted.

4.2.1 Metabolism

Both NDF and ADF digestibility were greater \((P = 0.03)\) in lambs receiving mash as compared to pelleted feeds, likely due to a shorter retention time of pelleted fibre in the rumen (Porter et al., 2007). Alkaloid concentration had a significant impact on both NDF and ADF digestibility, with both decreasing with increasing alkaloid dosage. It has been previously documented that sheep presented with dietary ergovaline in endophyte-infected tall fescue (1.5 and 3.0 ppm) had reduced NDF and cellulose digestibility possibly due to ruminal contractions.
triggered by the presence of ergot alkaloids (Hannah et al., 1990). Alkaloid-induced ruminal contractions may have increased rate of passage of digesta, leading to reduced fibre digestibility in the present study. In contrast, other literature (McLeay et al., 2006; Poole et al., 2009; Koontz et al., 2015) suggests that ergot alkaloids decrease both motility and passage rate in the foregut of ruminants, although these studies evaluated endophyte-derived alkaloids compared to the cereal ergot alkaloids of the present study. A greater impact on digestibility may have been detected if environmental temperatures > 30°C had persisted during the present study as the combination of increased body temperature and a greater reduction in intake could further accentuate the toxicity of ergot alkaloids (Hannah et al., 1990).

4.2.2 Ergot alkaloids in faeces

In the present study, alkaloid determination in faeces was performed only for C and H diets as a preliminary evaluation of feasibility of faecal alkaloid assay. Although previous studies showed that only 50 g/kg of alkaloids from diets containing 1500 ppb ergot alkaloids were recovered in the faeces of sheep (Westendorf et al., 1993), we observed an average 370 g/kg recovery of alkaloids in diets containing 433 ppb ergot alkaloids, with ergocristine and ergotamine the predominant alkaloids present, possibly from the different source and proportions of alkaloids present. The proportions of dominant alkaloids in faeces may differ from those in feed, as routes of excretion are thought to be affected by the molecular weight and polarity of the ergot alkaloid. Glucuronidation of ergot alkaloids and extent of excretion of through the biliary system would also influence faecal excretion of alkaloids (Klotz and Nicol; 2016). Lysergic acid and amides (such as ergometrine) having a small molecular weight (less than 350 Da) are excreted in urine (Eckert et al., 1978; Studemann et al., 1998), although ergometrine was also found in faeces in the present study. Larger alkaloids (500 Da) such as ergopeptines (for example ergotamine) are known to be
excreted in faeces (Schiff, 2006; Ayers et al., 2009), but additional study to determine proportional excretion of alkaloids in faeces and urine are warranted.

4.3 Performance Study

4.3.1 Mash vs. pelleted diets

The reduced concentrations of ergotamine and ergosine in both L and H pelleted as compared to mash diets may have been due to heating during the pelleting process. Of the six major alkaloids evaluated, concentrations of ergotamine and ergosine were substantially reduced after baking at 190°C for 9 to 17 min (Merkel et al., 2012). In the present study it is not known if the lower temperatures used in the pelleting process (90 to 100°C) were responsible for a similar reduction in the concentration of these alkaloids in pelleted feed. Pelleting feed does not appear to reduce overall concentrations of alkaloids (Skoch et al., 1983; Wondra et al., 1995; Lv et al., 2015), with an increase in concentration of ergocornine and ergocristine concentrations in pelleted feed, but changes in concentrations of individual alkaloids may have reduced negative animal impacts, particularly of the PH as compared to the MH diet. However, heating of alkaloids has been shown to result in epimerisation of alkaloids from the toxic \((R)\) configuration to the biologically inactive \((S)\) configuration (Merkel et al., 2012), with the degree of epimerisation differing among alkaloid types and affected by the temperature used (Krska et al., 2008). Perhaps the reduction in concentration of ergotamine and ergosine in pelleted diets was due to formation of \(S\) epimers (ergotaminine and ergosinine) for these alkaloids, although these were not quantified by the commercial laboratory.

Although pelleting increases feed production costs by at least 10% (Jahan et al., 2006), the improvement in ADG noted in the present study would support the use of pelleted complete diets for lambs with returns being even greater if the feed were contaminated with low levels of ergot
alkaloids. The present study does not support anecdotal evidence from the feed industry that pelleting increases symptoms of ergotism such as reduced ADG. It seems more likely that this perception arises from the difficulty in visually detecting ergot bodies in pelleted feed.

4.3.2 Impacts of alkaloid concentrations

Growth has been negatively influenced by ergot alkaloids, with cattle fed ergot-contaminated barley displaying a lower ADG as a result of 16 g/kg ergot in feed (Burfening, 1994). Others have estimated that every 100 g/kg increase in endophyte infestation decreases ADG by 0.045 kg in cattle (Patterson et al., 1995). However, in this study, subtle symptoms of ergotism were noted as decreased ADG of lambs fed H as compared to C and L diets, with a reduction in G:F in lambs fed H diets. Cattle fed diets with 12.7 g ergot bodies/animal/day compared to a control (0 g ergot bodies/animal/day), had decreased ADG suggesting a negative impact of alkaloids on energy metabolism and feed efficiency (Burfening, 1994). In the present study, ergotamine and ergosine were present in the highest concentration in MH diets (3-times and 2-times greater, respectively) as compared to PH diets. It is possible that these alkaloids negatively impacted lamb growth, although the relative toxicities of cereal alkaloids in lambs has not been determined.

Ruminants may be less sensitive to ergot alkaloids than monogastrics due to the capacity of rumen microbes to degrade mycotoxins. However, the degree of microbial degradation of alkaloids may also depend on the type of alkaloid, possibly accounting for the varying symptoms that arise between different alkaloid profiles in feed (Hussein and Brasel, 2001; Schardl et al., 2006). It is also possible that variability in the impact of alkaloids on the growth of lambs fed H diets reflects differences in the detoxification capacity of each lamb’s rumen microbiome. Klotz
(2015) recognised that the broad range of symptoms displayed by ruminants fed ergot was not solely due to alkaloid type and concentration. The degree of enterohepatic recirculation of alkaloids within livestock species or even individuals may also ultimately affect the distribution and concentration of these toxins within tissues. Thus, the variability of responses by livestock to ergot alkaloids and the variation in types of alkaloids present compromises the diagnostic investigation of ergot poisoning and the determination of specific alkaloid or alkaloids responsible for the symptoms (Klotz, 2015).

4.3.3 Prolactin

Prolactin plays a significant role in lactation and reproduction, but also influences immune responses, osmoregulation, growth and angiogenesis (Kelly et al., 1991; Freeman et al., 2000). Exposure to ergot alkaloids can reduce plasma concentrations of prolactin, thereby impacting a combination of dopaminergic pathways and inhibiting prolactin secretion through D2 receptors (Porter and Thompson, 1992; Kordon et al., 1994; Patterson et al., 1995).

Acknowledging that estrogen is a known stimulant of prolactin secretion (Chen and Meites, 1970), we observed a greater decline in prolactin in ewe lambs fed diets containing alkaloids as compared to ram lambs. A short-term inhibition in prolactin secretion was observed when ergonovine was administered at 23.8 µg kg⁻¹ body weight to steers, with differing degrees of inhibition depending on sampling time (Browning et al., 1997). Nasr and Pearson (1975) discovered that various alkaloids had different impacts on prolactin secretion and recovery rates, with ergocornine being undetectable in blood at 1 h post-administration. There was evidence that long-term exposure to ergot alkaloids of lambs fed L and H diets suppressed serum prolactin concentration, an outcome that could potentially impact the future fertility of ewes as a result of
decreased blood flow to reproductive tissues (Browning et al., 1997; Klotz, 2015). The presence of ergovaline in feed (0.8 µg g⁻¹ diet) decreased serum prolactin concentrations during gestation of sheep, with prolactin concentrations remaining depressed after 130 d of gestation (Duckett et al., 2014). Moreover, a reduction in gestation length of 4 d and a 37% reduction in lamb birth weight were some of the negative outcomes associated with the consumption of tall fescue alkaloids by ewes during pregnancy (Duckett et al., 2014).

Serum prolactin concentrations in the present study better predicted lamb performance than did alkaloid concentrations of diets and could potentially be used as a proxy to assess the potential reduction in lamb performance due to dietary ergot alkaloids. More studies using ergot with different proportions of alkaloids need to be conducted to ensure the utility of this assay as a bioindicator of cereal ergot ingestion, as most previous studies have only measured prolactin after exposure to fescue alkaloids (Parés et al., 2016).

4.3.4 Carcass quality

Hot carcass weight, dressing percentage and GR measurement (fat cover) were not affected by dietary ergot alkaloids, or the diet preparation. Pregnant ewes fed endophyte-infested tall fescue had decreased muscle mass and kidney fat (Duckett et al., 2014; Klotz, 2015) and in non-ruminants, abdominal fat decreased significantly when ducks were fed ergot-contaminated feed (Dänicke, 2015). Although ergot alkaloids have been known to impact lipid metabolism, concentrations used in this study did not cause observable changes in fat cover or liver health.

5. Conclusions

An increased prevalence of ergot in western-Canadian cereal grains is related to higher rainfall due to climate change and increases the urgency of re-evaluating acceptable concentrations
of ergot alkaloids in feed. Based on results of the present study, assays for alkaloids first require sample preparation standardization, in conjunction with procedural standardisation prior to the generation of recommendations of allowable concentrations of cereal ergot alkaloids in feeds. Pelleting may have reduced bioavailability of ergot alkaloids although several mechanisms are potentially responsible and would require confirmation in future studies. Alkaloid concentrations averaging 433 ppb for the H dosage impacted growth, NDF and ADF digestibility, as well as prolactin concentrations, although reduction in ADG and serum prolactin were most marked in lambs fed MH. Possibly, the concentration of individual alkaloids such as ergotamine which were altered by pelleting may be more closely related to performance of ruminants than are total alkaloid concentrations.
6. References


Table 4.1 Diet composition (as fed)

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Control(^1)</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mash</td>
<td>Pellet</td>
<td>Mash</td>
</tr>
<tr>
<td>Barley grain</td>
<td>539</td>
<td>539</td>
<td>538</td>
</tr>
<tr>
<td>Alfalfa pellets</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Canola meal</td>
<td>168</td>
<td>168</td>
<td>168</td>
</tr>
<tr>
<td>Canola oil</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sheep Mineral(^2)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Vitamin ADE(^3)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Decco(^4)</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Ergot screenings</td>
<td>0</td>
<td>0</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Dry matter and chemical composition (analytical DM basis g/kg)

<table>
<thead>
<tr>
<th></th>
<th>Control(^1)</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mash</td>
<td>Pellet</td>
<td>Mash</td>
</tr>
<tr>
<td>Dry matter</td>
<td>954.5±4.7</td>
<td>955.7±5.6</td>
<td>954.5±4.4</td>
</tr>
<tr>
<td>Organic matter</td>
<td>923.5±2.3</td>
<td>925.2±1.7</td>
<td>927.2±1.0</td>
</tr>
<tr>
<td>Crude protein</td>
<td>183.2±11.9</td>
<td>181.7±4.0</td>
<td>183.1±3.3</td>
</tr>
<tr>
<td>NDF</td>
<td>342.1±96.5</td>
<td>307.9±65.0</td>
<td>296.3±55.7</td>
</tr>
<tr>
<td>ADF</td>
<td>142.5±13.3</td>
<td>131.0±29.8</td>
<td>137.4±17.0</td>
</tr>
<tr>
<td>Ether extract</td>
<td>30.7±12.97</td>
<td>35.5±3.56</td>
<td>30.9±6.40</td>
</tr>
<tr>
<td>Non-fibrous</td>
<td>367.5</td>
<td>400.1</td>
<td>416.9</td>
</tr>
<tr>
<td>carbohydrates</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Control, no added alkaloids; low ~ 169 ppb; high ~ 433 ppb ergot alkaloids.

\(^2\)(g/kg) salt 926, potassium magnesium sulfate 49.8, zinc sulfate 9.21 magnesium sulfate 8.35 organic iodine 0.14, selenium premix 1.43, cobalt carbonate 0.04, canola oil 3.98.

\(^3\) Vitamin A 10,000,000 IU, vitamin D 1,000,000 IU, vitamin E 10,000 IU/kg

\(^4\) Active ingredient: decoquinate (60 g/kg; Zoetis Kirkland, QC).
Table 4.2 Profile of individual alkaloids (ppb) within experimental diets and screenings and particle size of the two grinding methods

<table>
<thead>
<tr>
<th>Alkaloids (ppb)</th>
<th>Control</th>
<th>Low</th>
<th>High</th>
<th>Screening sample processing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mash</td>
<td>Pellet</td>
<td>Mash</td>
<td>Pellet</td>
</tr>
<tr>
<td>Ergocornine</td>
<td>0</td>
<td>0</td>
<td>6.6±1.3</td>
<td>23.1±5.5</td>
</tr>
<tr>
<td>Ergocristine</td>
<td>0.7±1.0</td>
<td>0</td>
<td>51.9±18.2</td>
<td>96.9±4.8</td>
</tr>
<tr>
<td>Ergocryptine</td>
<td>0</td>
<td>0</td>
<td>33.0±4.6</td>
<td>34.3±11.0</td>
</tr>
<tr>
<td>Ergometrine</td>
<td>2.0±0.7</td>
<td>1.48</td>
<td>2.7±0.4</td>
<td>6.5±2.9</td>
</tr>
<tr>
<td>Ergosine</td>
<td>0</td>
<td>0</td>
<td>10.4±2.3</td>
<td>6.5±1.7</td>
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<tr>
<td>Ergotamine</td>
<td>0.70±1.0</td>
<td>0</td>
<td>48.7±48.7</td>
<td>17.9±1.7</td>
</tr>
<tr>
<td>Total</td>
<td>3.4±2.6</td>
<td>2.0</td>
<td>153.3±20.1</td>
<td>185.0±15.6</td>
</tr>
<tr>
<td>Mean particle size (µm)</td>
<td>NM³</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
</tr>
</tbody>
</table>

1 Based on the average of three samples unless otherwise specified.
2 Based on one sample.
3 Based on average total which may include sample concentration <1.25 ppb (marked as 0 in table).
4 NM, not measured.
Table 4.3 Effect of ergot alkaloids on nutrient digestion (g/kg) in lambs ($n = 12$)

<table>
<thead>
<tr>
<th>Digestibility</th>
<th>Diet</th>
<th>Control$^1$</th>
<th>Low</th>
<th>High</th>
<th>Probability</th>
<th>SEM$^2$</th>
<th>Preparation</th>
<th>Dose</th>
<th>Preparation $\times$ Dose</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mash</td>
<td>Pellet</td>
<td>Mash</td>
<td>Pellet</td>
<td>SEM$^2$</td>
<td>Preparation</td>
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<td></td>
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<tr>
<td>Dry matter</td>
<td></td>
<td>702</td>
<td>706</td>
<td>712</td>
<td>711</td>
<td>10.4</td>
<td>0.21</td>
<td>0.13</td>
<td>0.13</td>
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<td>Organic matter</td>
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<td>721</td>
<td>10.3</td>
<td>0.27</td>
<td>0.25</td>
<td>0.14</td>
</tr>
<tr>
<td>Crude protein</td>
<td></td>
<td>721</td>
<td>698</td>
<td>694</td>
<td>691</td>
<td>15.7</td>
<td>0.40</td>
<td>0.46</td>
<td>0.74</td>
</tr>
<tr>
<td>NDF</td>
<td></td>
<td>521</td>
<td>492</td>
<td>499</td>
<td>439</td>
<td>16.0</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.61</td>
</tr>
<tr>
<td>ADF</td>
<td></td>
<td>303</td>
<td>272</td>
<td>313</td>
<td>289</td>
<td>21.9</td>
<td>0.04</td>
<td>0.03</td>
<td>0.62</td>
</tr>
</tbody>
</table>

$^1$Control, no added alkaloids; low $\sim$169 ppb; high $\sim$ 433 ppb ergot alkaloids.

$^2$SEM: Standard error of mean
Table 4.4 Effect of high alkaloid dose (433 ppb) vs. control (3 ppb) on alkaloid composition (ppb) of pooled faeces from metabolism study

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Control Diet</th>
<th>Probability</th>
<th>High Diet</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mach</td>
<td>Pellet</td>
<td>Mach</td>
<td>Pellet</td>
</tr>
<tr>
<td>Ergocornine</td>
<td>0³</td>
<td>0</td>
<td>10.82±35.70</td>
<td>11.29±24.07</td>
</tr>
<tr>
<td>Ergocristine</td>
<td>0</td>
<td>0</td>
<td>67.70±39.61</td>
<td>68.97±21.46</td>
</tr>
<tr>
<td>Ergocryptine</td>
<td>0</td>
<td>0</td>
<td>14.49±38.72</td>
<td>15.20±21.88</td>
</tr>
<tr>
<td>Ergometrine</td>
<td>0</td>
<td>0</td>
<td>8.55±12.35</td>
<td>12.20±7.54</td>
</tr>
<tr>
<td>Ergosine</td>
<td>0</td>
<td>0</td>
<td>12.55±42.71</td>
<td>13.30±28.76</td>
</tr>
<tr>
<td>Ergotamine</td>
<td>0</td>
<td>0</td>
<td>39.30±46.92</td>
<td>43.00±26.15</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>0</td>
<td>153.41±40.76</td>
<td>164.0±21.37</td>
</tr>
</tbody>
</table>

¹No significant effect of diet form (pellet vs mash) on faecal alkaloids.

²Based on the average of three samples from two animals.

³Alkaloid concentrations with means < limit of detection (1.25 ppb) are recorded as 0.

⁵NA: not applicable.
Table 4.5 Serum prolactin (ng mL\(^{-1}\)) in performance lambs \(n = 36\) from samples collected at LW 35 kg and at market weight (≥ 45 kg).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control(^1)</th>
<th>Low</th>
<th>High</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Mash</td>
<td>Pelleted</td>
<td>Mash</td>
<td>Pelleted</td>
</tr>
<tr>
<td>Rams</td>
<td>3.83±0.47</td>
<td>4.11±0.81</td>
<td>1.47±0.47</td>
<td>2.75±0.47</td>
</tr>
<tr>
<td>Ewes</td>
<td>3.15±0.47</td>
<td>5.95±0.81</td>
<td>3.61±0.47</td>
<td>2.91±0.43</td>
</tr>
</tbody>
</table>

\(^1\)Control, no added alkaloids; low ~169 ppb; high ~433 ppb ergot alkaloids.
Table 4.6 Effect of ergot alkaloid concentration and diet preparation (pelleted or mash) on growth performance and carcasses of lambs receiving experimental diets for 0 to 84 days.

<table>
<thead>
<tr>
<th>Growth characteristics</th>
<th>Control¹</th>
<th>Low</th>
<th>High</th>
<th>SEM</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Preparation</td>
</tr>
<tr>
<td>(n=48)</td>
<td>Mash</td>
<td>Pellet</td>
<td>Mash</td>
<td>Pellet</td>
<td>Mash</td>
</tr>
<tr>
<td>Initial LW² (kg)</td>
<td>25.3</td>
<td>24.9</td>
<td>24.7</td>
<td>25.4</td>
<td>23.4</td>
</tr>
<tr>
<td>Final LW (kg)</td>
<td>49.0</td>
<td>49.9</td>
<td>48.9</td>
<td>49.54</td>
<td>47.88</td>
</tr>
<tr>
<td>DMI (kg d⁻¹)</td>
<td>1.41</td>
<td>1.43</td>
<td>1.31</td>
<td>1.32</td>
<td>1.25</td>
</tr>
<tr>
<td>ADG (g d⁻¹)</td>
<td>323.08</td>
<td>393.82</td>
<td>338.66</td>
<td>359.60</td>
<td>278.42</td>
</tr>
<tr>
<td>Feed conversion (gain:feed)</td>
<td>0.23</td>
<td>0.28</td>
<td>0.26</td>
<td>0.27</td>
<td>0.22</td>
</tr>
<tr>
<td>Hot carcass weight (kg)</td>
<td>24.17</td>
<td>23.08</td>
<td>23.68</td>
<td>23.18</td>
<td>22.87</td>
</tr>
<tr>
<td>Dressing %</td>
<td>48.14</td>
<td>47.57</td>
<td>47.44</td>
<td>47.29</td>
<td>46.45</td>
</tr>
<tr>
<td>Grade rule (mm)</td>
<td>16.40</td>
<td>17.33</td>
<td>20.50</td>
<td>15.67</td>
<td>17.67</td>
</tr>
</tbody>
</table>

¹Control, no added alkaloids; low ~169 ppb; high ~338 ppb ergot alkaloids.

²LW: live weight
Chapter 5

Effects of feeding maximal allowable concentrations of cereal ergot alkaloids on alkaloid recovery and performance traits of ram lambs.
Abstract

Allowable limits for cereal ergot alkaloids in livestock feeds are being re-examined and the objective of this study was to compare nutrient digestibility and growth performance of ram lambs fed increasing alkaloid concentrations including the maximum currently allowable dosage (2-3 ppm). Four pelleted diets were fed: control, no added alkaloids; 930, 1402 and 2447 ppb based on total R and S epimers. Eight ram lambs (30.0 ± 3.08 kg) were used in a 4×4 Latin square design to examine the impact of increasing alkaloid concentration on nutrient digestibility and N metabolism. With the exception of ergocorinine and ergocryptine, the recovery of alkaloids in faeces varied among periods, suggesting that individual lambs may differ in their in ability to metabolise ergocristine, ergotamine, ergometrine and ergosine. Forty-seven ram lambs (LW 30±5.8kg) were randomly assigned to one of the four diets and weighed weekly until they achieved a target slaughter weight of ≥ 45kg (average 9 weeks; range 6-13 weeks). Daily feed intake and refusals, along with weekly weight, and blood samples (collected at three different stages throughout the growth trial: 1) commencement of experiment, 2) when lambs reached ≥ 35 kg, 3) prior to slaughter at ≥45 kg) were used to help calculate the impacts of alkaloid consumption on the animal’s growth rate (average daily gain) and hormone concentration. Intake of DM did not differ (1472.01±2.8g; \( P = 0.91 \)) among treatments. Average daily gain (ADG) of lambs decreased (\( P <0.01 \)) with increasing alkaloid concentration, with lambs fed 2447 ppb (R and S epimers) alkaloids having a lower ADG than (\( P < 0.01 \)) other treatments. Rectal temperatures were higher (\( P = 0.005 \)) in lambs fed alkaloids as compared to controls. The concentration of serum prolactin linearly declined (\( P < 0.01 \)) with increasing dietary alkaloids, with carcass dressing % most pronounced in lambs fed 1402 ppb. Based on the results of this study, we can conclude that feeding
2447 ppb total alkaloids in pelleted diets had negative impacts to growth performance, carcase characteristics and prolactin concentrations of ram lambs.

**Keywords**: alkaloid, epimer, ergot, growth, lambs, nutrient digestibility.

### 5.1 Introduction

To date, 50 ergot alkaloids have been isolated and identified (Evans et al., 2004; Krska and Crews, 2008) and all, with the exception of the tricyclic chanoclavine, are derived from the formation of a tetracyclic ergoline ring (Gerhards et al., 2014). Once ergot alkaloids are synthesized, they can revert back and forth between the toxicogenic $R$ epimer and the relatively harmless $S$ form (Merkel et al., 2012). Factors causing conversion among epimers are as yet poorly defined, although both pH and matrix effects such as dietary composition have been implicated (Malachová et al., 2014).

Allowable limits for cereal ergot alkaloids in livestock diets are currently being re-examined, but are further complicated by factors such as epimerisation. Another complicating factor is that minimal information is available regarding the effects of cereal ergot on the growth or reproductive function of ruminants. In a previous study, we determined that pelleting diets reduced the negative impact of cereal ergot in growing lambs, although the maximum concentration of dietary alkaloids evaluated was only 433 ppb total $R$ epimers (Coufal-Majewski et al., 2017), far lower than the maximum allowable limit of 2000-3000 ppb (CFIA, 2017). Based on our previous findings, we hypothesize that by increasing ergot alkaloid concentrations to this limit, a visible decrease in animal intake and growth performance will be observed, subsequently impacting carcass quality and reproductive abilities. Further, reduced digestibility and N
metabolism, with reduced prolactin concentrations with increasing dosage will follow. Consequently, the objectives of this study were to pellet all diets and evaluate increasing doses of alkaloid concentrations including the maximum allowable limits for sheep (2-3 ppm), evaluate diets for both R and S epimers and observe impacts on animal performance, metabolism of nutrients, carcass characteristics and recovery of individual alkaloids in faeces. Additional factors which have been affected by dietary alkaloids such as serum prolactin concentrations and rectal temperature were also evaluated.

5.2 Materials and methods

All experiments were conducted between June and September 2016 at the Lethbridge Research and Development Centre (LRDC) of Agriculture and Agri-Food Canada. Protocols were reviewed and approved by the LRDC Animal Care Committee according to the guidelines of the Canadian Council on Animal Care (2009).

5.2.1 Ergot source and alkaloid determination

A 1.0-kg representative sample of barley screenings from a single bag obtained from the Canadian Feed Research Centre, North Battleford, SK was analysed for six individual alkaloids: ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine by Prairie Diagnostic Service Inc. (PDS) Saskatoon, SK. Screenings had an average alkaloid concentration of 888 ppm, which was then used to balance alkaloid concentrations of treatment diets. Analysis of alkaloids by PDS determined only R epimers using HPLC analyses previously described (Coufal-Majewski et al., 2017). Briefly, ground subsamples were extracted using an acetonitrile and ammonium acetate solution, with a 1.0-mL subsample of the filtered supernatant vortexed and transferred to an auto-sampler vial for analysis (Agilent Technologies, Santa Clara, CA).
Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) was used to determine alkaloid concentrations.

Similarly, the same feed samples were analysed for concentrations of thirteen ergot alkaloids (i.e., chanoclavine, ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine and ergotaminine) by Biomin Research Center (Tulln, Austria). Alkaloids were determined by HPLC-MS/MS (Malachová et al., 2014), based on retention time and intensity ratio as compared to verified standards (within 2.5% relativity and 30% relativity, respectively; Biomin Laboratory). In brief, a 500 g sample of feed or faeces was sub-sampled (5.0 g), ground through a 1-mm screen and stored in 20 mL of extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) overnight in the dark at room temperature, to avoid alkaloid degradation and allow equilibrium to establish between analytes and matrix (Malachová et al., 2014). Samples were then extracted for 90 min and centrifuged for 2 min at 1509 x g, with 350 µL aliquots diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). Following mixing, 5 µL of extracted alkaloids (in solution) was injected into an Agilent 1290 HPLC (Agilent Technologies Inc, Waldbronn, Germany) coupled to an Applied Biosystems 5500 QTrap mass spectrometer, in the Selected Reaction Monitoring (SRM) mode incorporating ESI (electrospray ionisation) monitoring modes. The method was performed and validated according to SANCO criteria (Malachová et al., 2014) and repeatability was assessed based on analysis of 5 replicates with a residual standard deviation < 20% for all alkaloids considered as being acceptable.

5.2.2 Treatments and diet preparation
To ensure uniform distribution of ergot in diets, a Wiley® Cutting Mill (Thomas Scientific, Swedesboro, NJ) was used to grind contaminated screenings through a 1-mm screen. Four pelleted diets were made, each in a single batch (2500 kg; Table 5.1) and used for both growth and digestibility studies. Dietary treatments had ergot alkaloid concentrations as followed: 34 ppb (no added alkaloids), 930 ppb, 1402 ppb and 2447 ppb.

5.2.3 Nutrient digestion and metabolism of lambs

5.2.3.1 Animals and treatments

Eight Canadian Arcott × Rideau Arcott ram lambs were selected based on similar initial body weight (30.0 ± 3.08 kg), to determine the digestibility of diets in a 4×4 Latin square experiment. Each period lasted for 3 weeks, with the first 2 weeks for adaption and the 3rd week for sample collection (Stuedemann et al., 1998). Lambs were randomly assigned to diet (n=2 lambs/treatment), fed once daily, and restricted to 95% of ad libitum intake during the week of total collection. Orts were collected and weighed daily during the adaption period to determine intake and the health of lambs was monitored daily. One week prior to the first collection period, lambs were shorn and pre-fitted with strap-on canvas faecal collection bags. Lambs were individually fed in crates with transparent panels to allow observation of other lambs and to minimise stress during the collection period.

5.2.3.2 Faecal and urine sampling

Faeces and urine were collected daily throughout the last 4 d of the 3rd week in each period. Sulfuric acid (4M, 100 mL) was placed in urine collection buckets each morning to prevent ammonia volatilization. Faecal collection bags were emptied once daily at 0800 h. Faeces that fell into the crate were collected and included in estimates of total faecal production, but were not
included in subsamples. Sub-samples of faeces (15%) and urine (10%) were collected on each collection day, with a second faecal sub-sample used to calculate daily faecal DM. Sub-sampled urine was pooled by lamb within period, mixed and further divided into two 80 mL urine containers and stored at -10 °C prior to analyses. Similarly, sub-sampled faeces were pooled by lamb within each period for further analyses.

5.2.4 Laboratory analyses

5.2.4.1 Diet, faeces and urine samples

As a measure of bioavailability of ergot alkaloids, faeces were collected to determine percentage recovery of each alkaloid. Pooled faeces were thawed and dried at 55 °C for one week prior to grinding. Both faecal and diet samples were ground through a 1 mm screen (Cutting Mill SM 100; Retsch Inc., Newtown, PA). Samples were prepared and analysed for analytical DM, OM, crude protein, NDF, ADF and crude fat content as described previously (Coufal-Majewski et al., 2017). Briefly, diet and faecal samples were further dried (105°C for 24 h) to estimate dry matter content; ashed at 550°C for 5 h to estimate OM and ball ground (Mixer Mill MM200; Retsch Inc., Newtown, PA; Method #990.03; AOAC, 1995) prior to analysis for N using a 2100 Elemental Combustion Analyser (Carlo Erba Instruments, Milan, Italy). Urine was freeze dried and thawed prior to analysis. Samples were diluted (1:5; urine:water) and 50 µL of sample was pipetted into sample cups prior to drying overnight at 55 °C. Dried urine samples were arranged together with the wheat standard samples and sample cups compressed prior to analysis using the combustion analyser (Stanford et al., 1996). Known concentrations of finely ground spring wheat standard sample were measured (1, 2, 4, 6, 8, 10 mg) and used to produce the standard curve \( y = 0.0045x + 0.0041 \), where \( x = N \) area produced from combustion analysis. This equation was used
to predict the amount of urine N (mg). As the amount of urine sample used is known, it is possible to calculate N % in the urine from this analysis.

The protocol of Van Soest et al. (1991) using an Ankom 200 fibre analyser (Ankom Technology, NY) was used to estimate NDF (with the addition of amylase and sodium sulfite) and ADF (Method 973.18 of AOAC, 1995) concentration in the DM with both expressed exclusive of residual ash. Crude fat content of the samples was determined using ether extraction following AOAC method 920.39 (2005). Composite faecal samples (approximately 400 g) from lambs fed the same diet over the four periods in the digestibility trial were ground through a 1-mm screen and analysed for alkaloid epimers.

5.2.5 Performance study

5.2.5.1 Animals and experimental design

Forty seven Canadian Arcott × Rideau Arcott ram lambs (30 ± 5.8 kg), were randomized by weight and then assigned to one of four diets and housed in individual pens (61 × 107 cm) bedded with wood chips from June to September 2016. Lambs had ad libitum access to their diets (Table 1) and water throughout the experiment. Feed deliveries were recorded daily, with daily refusals collected and weighed for determination of daily DM intake (DMI). Individual LW was recorded at weekly intervals and ADG was determined by dividing weight gain (initial LW – final LW) by the number of days in the study. Feed efficiency was calculated as the ratio between DMI and ADG (g DMI/g of LW gain). Lamb health was carefully monitored by barn staff and ambient temperature in the barn housing the lambs was recorded three times daily throughout the experimental period. Rectal temperature was recorded once at week 10 (0800 h) using a digital thermometer.
5.2.5.2 Sample collection and slaughter

Blood samples were collected from 36 lambs three times during the course of trial 1: 1) prior to commencing experimental diets; 2) after reaching 35 kg live weight and 3) after reaching slaughter weight (≥45 kg). Serum from blood was prepared by centrifugation (2000 × g for 15 min at 4°C), prior to separation of serum by pipette and storage in 1.0 mL screw-cap tubes in duplicate at –80 °C. Prolactin concentrations were determined from serum samples using a double antibody radioimmunoassay with oPrl (100:1; first antibody) and rabbit globulins (500:1; second antibody) and reagents obtained from the National Hormone and Pituitary Program (NHPP; Harbor-UCLA Med Ctr, Torrance, CA) as outlined by Coufal-Majewski et al. (2017).

Lambs were removed from the experiment upon reaching ≥45 kg and slaughtered at a commercial abattoir (n = 38; SunGold Meats Ltd., Innisfail, AB, Canada). Carcass traits were assessed according to Canadian Food Inspection Agency standards (Government of Canada. 2017).

5.2.6 Statistical analyses

Dry matter intake, ADG and feed efficiency were analysed as a completely randomized design by 2-way ANOVA using the MIXED procedure of SAS, with alkaloid concentration as a fixed effect, lamb within treatment as a random effect and week as a repeated measure (SAS Inst. Inc., Cary, NC). The minimum values of AIC (Akaike’s Information Criterion) were used to select the covariance structure which were estimated as N*ln(error-sum-or-squares/N) + 2*(number of independent variable parameters including the intercept term), where N=the number of observations (Akaike, 1974; Liang & Zou, 2008). Prolactin data was log transformed prior to analyses, as data were not normally distributed over the three sampling points. Initial LW, final
LW and carcass data were analysed using the MIXED procedure without repeated measures. Digestibility and N metabolism data were also analysed using the MIXED procedure with period and lamb as random effects without repeated measures. Significance was acknowledged for both studies at $P \leq 0.05$. Linear and quadratic effects were determined by using planned orthogonal polynomial coefficients for each parameter when type 3 tests of fixed effects were $\leq 0.05$. Only linear responses to increasing concentrations of alkaloids were reported as quadratic contrasts were not significant.

5.3 Results

5.3.1 Dietary ergot alkaloid content

Total alkaloids ($R + S$ epimers) in diets 1 to 4 were 34.0, 930, 1402, and 2447 ppb respectively (Biomin; Table 5.2). Limits of detection (LOD) using this methodology ranged from 0.1 to 4 ppb for the majority of alkaloids, with the exception of ergosine, where LOD was 0.9-19 ppb (Biomin. 2016). The $R$ epimer ergocristine was the dominant alkaloid followed by ergometrine, with ergocristinine, the dominant $S$ epimer in all diets. For comparison purposed only, PDS results of total alkaloids in diets 1 to 4 were 68, 467, 710 and 1007 ppb respectively (Table 5.3). The dominant alkaloid across all treatments was ergocristine.

5.3.2 Effects of alkaloid concentration on nutrient digestibility and recovery of alkaloids in faeces

Nutrient digestibility was not impacted by dose nor period of alkaloids fed to lambs ($P \geq 0.20$; Table 5.4). Similarly, no differences were observed in urinary nitrogen intake, excretion or N retention (Table 5.5).
Recoveries of the majority of alkaloids in faeces increased with increasing alkaloid concentration (Table 5.6) with the exception of ergometrine and its S isomer, which declined ($P < 0.001$) with increasing alkaloid concentration. For all alkaloids, with the exception of ergocornine and ergocryptine, recovery varied by period ($P < 0.05$). Ergocornine was consistently the most recoverable alkaloid (average of 92.7%), while ergometrine and ergotaminine were the least with an average of 74.1% and 73.9%, respectively.

5.3.3 Effects of ergot alkaloids on growth performance of lambs

Initial and final LW, DMI and feed conversion efficiency did not differ among treatment groups ($P \geq 0.18$; Table 5.7). Increasing alkaloids linearly decreased ADG ($P < 0.01$), with ADG being lower ($P < 0.01$) for lambs consuming 2447 ppb compared to all other diets. Compared to the control, rectal temperatures were 0.33°C higher ($P < 0.01$) in lambs fed diets with added alkaloids and serum prolactin concentrations linearly declined with increasing alkaloid concentration ($P < 0.01$; Table 5.7). Clinical symptoms associated with alkaloid toxicosis were not evident as lameness or tissue necrosis was not observed. No mortalities occurred, although one lamb from the growth trial was moved to the digestibility trial to replace a lamb with chronic diarrhea after the first collection period.

Alkaloid dosage had no impact on hot carcass weight ($P = 0.45$; Table 5.7). Dressing percentage was lower for lambs receiving 1420 ppb alkaloids as compared to those receiving control and 2447 ppb diets ($P = 0.02$). No livers or carcasses were condemned at the commercial abattoir and no abnormalities in fat cover were observed.

5.4 Discussion

5.4.1.1 Alkaloid profiles
Similar to previous studies of cereal ergot (Young and Chen, 1982, Kraska et al., 2008), both laboratories found ergocristine (alone or with its $S$ epimer) was the predominant alkaloid in all diets. With the exception of ergocristine, there was some degree of variation in proportions of alkaloids across treatments. Ergometrine, an alkaloid commonly used to stimulate uterine contractions in humans (Blaney et al., 2000) was the second-most predominant alkaloid in diets analysed by Biomin, followed by ergocryptine and its epimer. In analyses of PDS, ergocryptine was consistently the second-most prevalent alkaloid detected, with ergometrine consistently the least prevalent alkaloid detected, while in analyses of Biomin, ergocryptine was third or fourth in prevalence. It is important to note that for the current study the results from Biomin were used to analyse and conclude all impacts of nutrient and performance effects on ram lambs, as both $R$ and $S$ epimers were detected and hence each treatment has been identified using these alkaloid concentrations. Thus, the inclusion of PDS results is exclusively to gauge the vast variability between laboratory results using the same sample but different method of detection.

Comparing analyses of both labs, a pronounced variation was also present in alkaloid concentration, with total $R$ epimers in diet 4 detected by Biomin 178% greater than those reported by PDS, likely due to sampling and the different methods used for analysis. Consequently, comparison of alkaloid data across studies using different methods for alkaloid analysis would be difficult. Grusie et al. (2016) found that coefficients of variation for ergot alkaloids in feed varied markedly depending on alkaloid concentration (17% CV for very high concentration ($\geq$ 80000 ppb; 284% CV for very low concentration $\leq$ 35 ppb, which can be similarly observed in diet 1 results from PDS). Thus it is evident that methodology to analyse alkaloid concentrations requires standardization as a 24% reduction in particle size of samples prior to extraction of alkaloids resulted in a 7-fold increase in concentrations of alkaloids detected in our previous study (Coufal-
Majewski et al., 2017). Within the current study, we ensured that each sample analysed had the exact same particle size (done by grinding through a 1mm screen), to reduce extraction inaccuracies, however consistency with detection methodology is essential to minimise the range of concentration values produced from the same sample.

Methods used for measuring other mycotoxins such as aflatoxins and fumonisins have greater accuracy, but the procedures for ergot alkaloids are relatively new and possible matrix and extraction effects on the sensitivity and the form of epimerisation of ergot alkaloids is not well defined (Malchová et al., 2014). Similar to the present study, most labs currently use liquid chromatography–mass spectrometry (LC/MS) to produce structural information on the analyte based on the fragment pattern and identifying analytes on the basis of molecular weight (Downard, 2004). However Biomin used LC/ESI-MS/MS, extracting trace concentrations of metabolites using the mass of protonated molecular ions from extracted ion chromatograms (EICs; Crews, 2015; Bandu et al., 2015). Crews (2015) identified the importance of having standards where the sample extract itself acts as the matrix for the calibration standards. They also noted that the recoveries of ergometrine and its epimer, ergometrinine, were the most influenced by sample preparation procedures. In the current study, the comparison of alkaloid concentrations using multiple methodologies is novel, emphasising the importance of consistency throughout all alkaloid analysis.

5.4.1.2 Alkaloids and their epimers

Ergot alkaloids are relatively stable, however epimerisation can be induced in high or low pH conditions and when exposed to strong light (Crews, 2015), indicating that storage conditions (best below – 20 °C), handling and analysis need to be consistent to avoid altering alkaloid
chemistry. Whilst it is understood that ergot alkaloids produce a positive charge in acid solution and are neutral in alkali (Crews, 2015), actual mechanisms leading to conversion between epimeric forms are unknown (Andrae et al., 2014). In addition, epimerisation can be bi-directional in ergopeptides (which includes ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine), either forming toxic C8-(R) isomers or more inert C8-(S) isomers referred to as ergopeptinines (Frach and Blaschke, 1998). In the present study, concentration of S epimers averaged approximately 1/3 that of R epimers across all diets, although the ratio of R/S epimers varied among alkaloids. For example, the S epimer, ergometrinine was only 1% of the concentration of ergometrine, minimizing potential increases in toxicity of this alkaloid through additional R epimerisation. In contrast, if conversion to the R epimer was favored for ergocorninine or ergocristinine, toxicity of these alkaloids could increase by 76-81%. Further investigation of factors causing conversions among epimers is important to better define the toxicity of these alkaloids to livestock.

5.4.1.3 Nutrient digestibility and alkaloid recovery in faeces

According to Smith (1992), ruminal microorganisms are capable of detoxifying plant-derived chemicals, thus speculating that ruminants have an increased tolerance to toxins such as ergot alkaloids compared to monogastrics such as swine. Ayers et al. (2009) noted that steers have a greater capacity to digest fibre from ergot-contaminated tall fescue sources (specifically ergot alkaloids associated with vasoconstriction such as ergonovine, ergovaline and lysergic acid) than do non-ruminants. As microbial digestion may enhance ruminal ergot alkaloid solubility through the metabolism of ergovaline alkaloids to lysergic acid (DeLorme et al., 2007; Strickland et al., 2011), it is unknown whether alkaloid solubility is strictly a function of particle size reduction or a function of conversion from low-soluble alkaloid species to alkaloids with higher solubility (such
as the conversion from ergovaline to lysergic acid). By increasing the amount of soluble content processed by the rumen, less toxic alkaloids will thus be digested, reducing the potential negative impacts of ingesting a great concentration of toxic ergot alkaloids (DeLorme et al., 2007). Moyer et al. (1993) found that less than 50% of ergovaline was soluble in in vitro ruminal fermentations, with almost 100% of the soluble components being transformed into an ergot alkaloid derivative such as lysergic acid, within 46 h. However, as diets increased in concentration of ergot from 0 ppb to 47 ppb, ruminal motility has been observed to decrease in steers (Ayers et al., 2009), potentially decreasing digestibility of nutrients due to insufficient mixing of feed and reduced exposure of feeds to digestive enzymes. This is not the case in the current study, possibly due to the greater digestibility of grain diets over pasture feed.

The current study used a 2 week adaption phase between each digestibility collection period to reduce carry-over effects of alkaloids. Within 96 h after steers switched from grazing endophyte-infected tall fescue to endophyte-free tall fescue, Stuedemann et al. (1998) alkaloids were no longer detectable in urine. Acknowledging that alkaloids have the ability to suppress prolactin production, Aiken et al. (2001) showed prolactin stabilization after 3 d removal from ergot-contaminated feed. These studies used similar withdrawal periods, suggesting that the clearance phase chosen (2 weeks) was adequate for animals to overcome most of the toxic effects of the previously ingested diet. However, carry-over of alkaloids in tissues appears to vary depending on the lipophilicity of individual alkaloids (Dänicke, 2016).

Literature has suggested that increasing ergot content in feed reduces nitrogen (N) intake and digestibility in livestock fed endophyte-infected hay (Friend and MacIntyre, 1970; Matthews et al., 2005). However, these observations are likely due to reduced intake (Koontz, 2013), as faecal N and urinary N concentrations remained the same. Additionally, Koontz (2013) also noted
that ruminal volatile fatty acid (VFA) were altered by alkaloid ingestion, which could cause a reduction in nutrient absorption and blood flow (Foote et al., 2013). This indicated that observed reductions in weight gain and productivity may primarily be a function of reduced DMI and not secondary effects of alkaloid toxicity as a result of ingestion. Moreover, ingestion of ergot alkaloids could potentially reduce the blood flow to the absorptive surface of the gut of ruminants, which would trigger a decrease in VFA absorption, thereby impairing productivity (Bergman, 1990; Foote et al., 2013).

In the current study, no impacts on N consumption, excretion or retention were observed and there was no difference in feed intake. Measuring microbial populations over time across treatments could have determined whether the rumen can adapt to ergot contaminated feed based on changes in microbes present. When N in feed is increased, urinary excretion and N retention in sheep rises (Siddons et al., 1985). In the current study, regardless of sufficient balancing of nutrient needs of the host and the inclusion of ergot alkaloids, there was no observed decline in DMI, and hence no impact to N retention. Similarly, Flores et al. (2007) observed no practical difference in digestibility between novel and wild-type endophyte infected tall fescue in dairy heifers, indicating that the differences in nutrient flux and metabolism are most likely due to indirect effects of the alkaloids, as opposed to alterations in digestion. It is important to note that both studies, along with the current study, were performed within the thermoneutral zones and the most adverse effects of ergot alkaloids are typically observed during periods of heat stress (Stuedemann and Hoveland, 1998). Thus changes to nutrient influx may occur within elevated environmental conditions where blood may be diverted from the digestive tract to the outer extremities.

Approximately 83%, 82% and 85% of alkaloids were recovered in faeces from diets containing added alkaloids (930 ppb, 1402 ppb and 2447 ppb, respectively), with an average of
16.5% of alkaloids retained. On the contrary, Dänicke (2016) found approximately 11% of ingested cereal ergot alkaloids were recovered from the excreta of chickens, unlike the current study were up to 85% of alkaloids were recovered in faeces. Recovery of ergometrine and ergometринine in faeces decreased with increasing dietary concentrations in contrast to that of other alkaloids which either remained constant regardless of concentration or tended to increase with increasing dietary concentrations, possibly as more is lost within the urine with increasing concentrations.

Mechanisms for metabolism of ergot alkaloids likely differ across livestock species (Thompson, 2016). In general, individuals within the same species such as ruminants, are also known to differ in their tolerance of ergot alkaloids (Klotz, 2015). In the present study, with the exception of ergocornine and ergocryptine, recovery of alkaloids significantly varied with period, suggesting that the metabolism of alkaloids may have differed among individual lambs. Stuedemann et al. (1998) found increased ergot alkaloid concentrations in the faecal and urinary excrements, suggesting that animals can adapt to alkaloids in their diet overtime by developing more efficient methods of excretion, further suggesting that perhaps the lambs adapted in the present study to concentrations in diets, and hence very few effects were observed amongst the pool of lambs.

Previous studies (Matthews et al., 2005) have observed that increasing concentrations of ergot in the diet decreased nitrogen retention of steers by approximately 38% (fed 120 ppb), although in both Coufal-Majewski et al. (2017) and the current study, digestibility of CP did not differ in lambs fed ergot contaminated diets. Metabolic responses to dietary ergot are undoubtedly complex, as NDF digestibility by lambs decreased at 433 ppb (Coufal-Majewski et al., 2017), with no impact on NDF nor ADF digestibility noted in the current study. The proportion of S epimers
present for each alkaloid was not determined for the study of Coufal-Majewski et al. (2017) and sources of contaminated screenings differed between studies, demonstrating the difficulty in determining the concentration of alkaloids fed across different studies. In the study of Coufal-Majewski et al. (2017), the maximum concentration where no adverse effects were noted on nutrient digestibility of pelleted diets was 185 ppb R epimers as compared to 1790 ppb R epimers in the present study. Further investigations on the impacts of individual alkaloids from a variety of sources would be useful to determine the lower limit at which each alkaloid has a negative impact on digestibility, growth performance, and animal health.

5.4.2 Effects of ergot alkaloid concentrations on growth performance

Although Coufal-Majewski et al. (2017) found reduced ADG in lambs receiving diets containing 433 ppb total alkaloids, ADG in the present study was reduced compared to controls only for lambs fed 2447 ppb alkaloids. All diets in the present study were pelleted which may have helped to moderate alkaloid impacts on performance as reductions in ADG in the study of Coufal-Majewski et al. (2017) were more pronounced with mash diets. However, as previously discussed, different sources of ergot were used in each study with differing alkaloid profiles. The previous study by Coufal-Majewski et al. (2017) analysed only R epimers, with the current study producing concentrations both involving only R epimers (PDS results) and results inclusive of both R and S epimers (Biomin results). The diet containing 2447 ppb alkaloids reduced ADG by 27% compared to that of the control diet and was the only concentration to significantly impact growth. These results are surprising as DMI remained the same across dietary treatments, with consideration that a reduction in feed intake is often an initial symptom of exposure to ergot (Klotz, 2015).
Ergot alkaloids have been known to impact lipid metabolism in sheep and other livestock, with increasing concentrations causing observable changes in fat cover and liver health (Duckett et al., 2014; Dänicke, 2015). In the current study, the dressing percentage was reduced for lambs fed 1402 ppb compared to other dietary treatments, possibly because of differing proportions of individual alkaloids, with approximately 2 times as much ergocornine present in the 1402 ppb diet as compared to the 2447 diet, relative to total alkaloid concentrations. The only measure of ergot significantly impacting lambs fed 2447 ppb as compared to 1402 ppb was observed in a reduction to ADG. In contrast, dressing percentage was significantly reduced for lambs fed 1402 ppb as compared to the 2447 ppb diet. Possibly, ratios of alkaloids present within the 1402 ppb diet may also influence animal performance once threshold concentrations are surpassed interfering with metabolism and hence negatively impacting carcase traits. However, knowledge associated with the interactions among alkaloids have not been investigated in detail. Lambs presented with ergot-contaminated diets had a dressing percentage < 48%, a value that is below the optimal range for lambs to receive maximum market value at the abattoir (48-57.9%; SunGold Meats, n.d.). This indicates that upon sale there may have been a marginal reduction in overall revenue from feeding lambs diets contaminated with ergot.

Currently, it is difficult to determine whether reduction in animal performance such as reduced prolactin and ADG are caused by a single alkaloid or are the effects of multiple alkaloids. A preliminary step towards understanding the impact of multiple alkaloids would be to determine the rate of absorption and metabolism of individual alkaloids, although the metabolism of multiple alkaloids may trigger synergistic or additive effects, further obscuring impacts of individual alkaloids. However, this would be very difficult, as purification of sufficient quantities to actually be able to conduct this procedure would present a major challenge.
5.4.3 Effects of ergot on blood prolactin and rectal temperature

Depending on the alkaloid, ergot alkaloids can act as either an agonist or antagonist to the dopamine receptor (Reifel et al., 1983). Administration of ergocristine, a dopamine agonist can instantaneously block release of prolactin (Shin, 1979; Reifel et al., 1983). Ergocristine was the most prevalent alkaloid in feed in the present study, which may explain the significant linear reduction in serum prolactin concentration with increasing alkaloid concentration in the diet. Similarly, Coufal-Majewski et al. (2017) observed a decline in serum prolactin concentrations of lambs even at dietary alkaloid concentrations as low as 170 ppb. Rams grazing endophyte-infected pastures have also exhibited reduced serum prolactin concentrations and testicle weight, but one year after alkaloid exposure, the fertility of rams was unaffected (Emile et al., 2000).

Rectal temperatures recorded for control lambs were within a normal range (between 38.5 to 39.9°C; Cunningham, 2011), but lambs receiving diets with added alkaloids exhibited slightly higher rectal temperatures. Gadberry et al. (2003) found that dietary ergot alkaloids increased susceptibility of sheep to regulate core body temperature, increasing with increasing concentrations of alkaloids present. Previous literature concluded that in order for ergot toxicosis to occur, ambient temperatures typically need to exceed 31°C (Hannah et al., 1990; Hemken et al., 1981; Matthews et al., 2005). Although the average daily ambient temperature never exceeded 27.4°C in the current study, elevated rectal temperature above normal for sheep was detected. This suggests that alkaloid ingestion could further negatively impact core body temperature stabilization and possibly impact animal performance if ambient air temperatures are high, however the use of an infrared camera would be necessary to make such conclusions. Since the animal is most likely attempting to repel infection, reductions in performance may be noticeable. It would be however beneficial for future studies to address impacts of temperatures above or
below the thermo-neutral zone on toxicity of ergot alkaloids, in combination with weekly rectal temperature readings, to detect any differences in core body temperature throughout the experiment.

5.5 Conclusion

In the present study, symptoms of alkaloid toxicosis such as a linear decrease in both ADG and serum prolactin with increasing dietary alkaloids were demonstrated in lambs receiving ergot contaminated diets, although in the case of carcass dressing %, negative impacts of alkaloids were most pronounced for lambs receiving the 1402 ppb diet. Based on these results we accept our hypotheses that increasing ergot concentrations to the maximum allowable limits for cereal ergot alkaloids in ruminant feeds observed a reduction in prolactin concentrations. However our hypothesis that reduced digestibility and N metabolism would be observed at these concentrations was denied. Further complications were addressed in the method of detection, demonstrating the need for a standard method to be established prior to carrying out any further investigations on the allowable limits of ergot alkaloids in livestock feeds, with further understanding on the causes of epimerisation of alkaloids essential. Thus, based on the current study and previous literature, concentrations of alkaloids present in diets appear to be poor predictors of animal performance, as changes can be observed dependent on the method of detection. It is therefore important to recognise the challenges in reliable quantification of ergot alkaloids and identifying those allowable limits that can be included in lamb diets without adverse impact on productivity.
5.6 References


Table 5.1 Diet composition (as fed)

<table>
<thead>
<tr>
<th>Ingredients, %</th>
<th>Diets (alkaloid concentration)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>34 ppb¹</td>
<td>930 ppb</td>
<td>1402 ppb</td>
<td>2447 ppb</td>
</tr>
<tr>
<td>Barley grain</td>
<td>53.93</td>
<td>53.84</td>
<td>53.76</td>
<td>53.69</td>
</tr>
<tr>
<td>Alfalfa pellets (18% CP)</td>
<td>26.96</td>
<td>26.96</td>
<td>26.97</td>
<td>26.97</td>
</tr>
<tr>
<td>Canola meal</td>
<td>16.78</td>
<td>16.78</td>
<td>16.78</td>
<td>16.78</td>
</tr>
<tr>
<td>Canola oil</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sheep Mineral²</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin ADE³</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Deccox⁴</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
</tr>
<tr>
<td>Ergot screening</td>
<td>0</td>
<td>0.09</td>
<td>0.15</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Dry matter and chemical composition (analytical DM basis)

|                                |            |            |            |            |
|                                | Dry matter, % | Organic matter | Crude protein | Neutral detergent fibre |
|                                | 90.8±2.9    | 92.2±0.3    | 18.3±3.8    | 23.9±1.3 |
|                                | 91.0±3.2    | 92.5±0.4    | 18.8±4.3    | 25.0±2.0 |
|                                | 90.7±4.0    | 92.4±0.5    | 19.0±4.1    | 23.7±5.3 |
|                                | 90.8±3.3    | 92.9±0.5    | 19.2±3.9    | 26.8±3.7 |

|                                | Acid detergent fibre | Ether extract | Non fibrous carbohydrates |
|                                | 12.7±0.4       | 3.3±8.9      | 46.7        |
|                                | 12.8±3.9       | 3.6±4.1      | 45.2        |
|                                | 12.5±4.0       | 3.8±4.0      | 46.0        |
|                                | 13.0±6.5       | 3.5±7.2      | 43.3        |

¹no added alkaloids
²Sheep mineral constituents (%): salt 92.6, potassium magnesium sulfate 4.979, zinc sulfate 0.921 magnesium sulfate 0.835 organic iodine 0.014, 1% selenium premix 0.143, cobalt carbonate 0.004, canola oil 0.398.
³Vitamin ADE constituants: vitamin A 10,000,000 IU, vitamin D 1,000,000 IU, vitamin E 10,000 IU/kg
⁴Deccox active ingredient: decoquinate (6%; Zoetis Kirkland, QC)
Table 5.2 Alkaloid profile analysed by Biomin including the concentration (ppb) of each alkaloid (R and S epimer) within experimental diets.

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>34 ppb&lt;sup&gt;1&lt;/sup&gt;</th>
<th>930 ppb</th>
<th>1402 ppb</th>
<th>2447 ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R epimer</td>
<td>S epimer</td>
<td>R epimer</td>
<td>S epimer</td>
</tr>
<tr>
<td>Chanoclavine</td>
<td>2</td>
<td>NA&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4</td>
<td>NA</td>
</tr>
<tr>
<td>Ergocornine</td>
<td>1</td>
<td>1</td>
<td>60</td>
<td>51</td>
</tr>
<tr>
<td>Ergocristine</td>
<td>12</td>
<td>7</td>
<td>219</td>
<td>112</td>
</tr>
<tr>
<td>Ergocryptine</td>
<td>1</td>
<td>2</td>
<td>90</td>
<td>26.</td>
</tr>
<tr>
<td>Ergometrine</td>
<td>ND&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2</td>
<td>209</td>
<td>3</td>
</tr>
<tr>
<td>Ergosine</td>
<td>ND</td>
<td>ND</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>Ergotamine</td>
<td>7</td>
<td>1</td>
<td>85</td>
<td>22</td>
</tr>
<tr>
<td>Total epimer</td>
<td>22</td>
<td>12</td>
<td>695</td>
<td>235</td>
</tr>
<tr>
<td>Total alkaloids</td>
<td>34</td>
<td></td>
<td>930</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>no added alkaloids  
<sup>2</sup>NA, not measured  
<sup>3</sup>ND, not detected
Table 5.3 Alkaloid profile including the concentration (ppb) of each alkaloid (R epimer) within experimental diets analysed by PDS.

<table>
<thead>
<tr>
<th>Ergot analysis (ppb)</th>
<th>Initial screenings</th>
<th>Diet 1 (control)</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergocornine</td>
<td>11,670</td>
<td>121±141</td>
<td>41±60</td>
<td>66±29</td>
<td>89.±45</td>
</tr>
<tr>
<td>Ergocristine</td>
<td>58,650</td>
<td>365±49</td>
<td>286±20</td>
<td>423±10</td>
<td>607±15</td>
</tr>
<tr>
<td>Ergocryptine</td>
<td>23,680</td>
<td>12±121</td>
<td>80±13</td>
<td>132±10</td>
<td>183±8</td>
</tr>
<tr>
<td>Ergometrine</td>
<td>5,680</td>
<td>2±141</td>
<td>5±141</td>
<td>5±95</td>
<td>7±80</td>
</tr>
<tr>
<td>Ergosine</td>
<td>7,243</td>
<td>11±141</td>
<td>13±10</td>
<td>24±5</td>
<td>34±6</td>
</tr>
<tr>
<td>Ergotamine</td>
<td>15,440</td>
<td>5±75</td>
<td>41±6</td>
<td>59±18</td>
<td>86±3</td>
</tr>
<tr>
<td>Total</td>
<td>122,365</td>
<td>68±70(^2)</td>
<td>467±5</td>
<td>710±6</td>
<td>1007±5</td>
</tr>
</tbody>
</table>

\(^1\) Results based on 3 collections.

\(^2\) Range from 14 to 129 ppb
Table 5.4 Effect of ergot alkaloids on dry matter and nutrient digestibility in lambs ($n = 8$).

<table>
<thead>
<tr>
<th>Alkaloid concentration (ppb)</th>
<th>P-value*</th>
<th>SEM$^2$</th>
<th>Dose</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>34$^1$</td>
<td>930</td>
<td>1402</td>
<td>2447</td>
<td></td>
</tr>
<tr>
<td>Dry matter digestibility</td>
<td>71.5</td>
<td>72.2</td>
<td>70.1</td>
<td>71.9</td>
</tr>
<tr>
<td>Organic matter digestibility</td>
<td>72.9</td>
<td>73.3</td>
<td>71.5</td>
<td>73.3</td>
</tr>
<tr>
<td>Crude protein digestibility</td>
<td>73.7</td>
<td>75.3</td>
<td>72.8</td>
<td>73.1</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>38.4</td>
<td>39.7</td>
<td>34.5</td>
<td>39.4</td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>23.9</td>
<td>25.7</td>
<td>21.9</td>
<td>25.7</td>
</tr>
</tbody>
</table>

$^1$no added alkaloids

$^2$SEM: Standard error of means

*linear and quadratic contrasts not included as there was no statistical significance
Table 5.5 Urinary nitrogen, excretion and retention by ram lambs consuming control or ergot alkaloid contaminated grain.

<table>
<thead>
<tr>
<th>Alkaloid concentration (ppb)</th>
<th>SEM</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>34^1</td>
<td>930</td>
<td>1402</td>
</tr>
<tr>
<td>N intake, g</td>
<td>42.9</td>
<td>45.5</td>
</tr>
<tr>
<td>N digested, g</td>
<td>31.0</td>
<td>33.7</td>
</tr>
<tr>
<td>N retained, g</td>
<td>13.3</td>
<td>14.4</td>
</tr>
<tr>
<td>N output, g</td>
<td>18.4</td>
<td>20.2</td>
</tr>
<tr>
<td>% of N retained (intake)</td>
<td>31.5</td>
<td>33.0</td>
</tr>
<tr>
<td>% of N retained (digested)</td>
<td>43.7</td>
<td>44.2</td>
</tr>
</tbody>
</table>

^1 no added alkaloids

^2 SEM: Standard error of means

* linear and quadratic contrasts not included as there was no statistical significance
Table 5.6 Recovery (in %) of individual alkaloids in faeces of lambs.

<table>
<thead>
<tr>
<th>Alkaloid concentration</th>
<th>34 ppb&lt;sup&gt;1&lt;/sup&gt;</th>
<th>930 ppb</th>
<th>1402 ppb</th>
<th>2447 ppb</th>
<th>SEM</th>
<th>P-value</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dose</td>
<td>Period</td>
</tr>
<tr>
<td>Chanoclavin</td>
<td>83.5</td>
<td>89.5</td>
<td>88.8</td>
<td>90.9</td>
<td>0.28</td>
<td>&lt; 0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Ergocornine</td>
<td>100.0</td>
<td>89.2</td>
<td>88.8</td>
<td>89.2</td>
<td>0.20</td>
<td>&lt; 0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>Ergocorninine</td>
<td>86.1</td>
<td>93.5</td>
<td>92.6</td>
<td>92.6</td>
<td>0.22</td>
<td>&lt; 0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Ergocristine</td>
<td>50.6</td>
<td>82.6</td>
<td>81.3</td>
<td>88.3</td>
<td>0.69</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Ergocristinine</td>
<td>66.5</td>
<td>82.7</td>
<td>83.2</td>
<td>88.2</td>
<td>0.51</td>
<td>&lt; 0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Ergocryptine</td>
<td>ND</td>
<td>90.8</td>
<td>89.4</td>
<td>90.4</td>
<td>0.63</td>
<td>&lt; 0.01</td>
<td>0.14</td>
</tr>
<tr>
<td>Ergocryptinine</td>
<td>81.6</td>
<td>87.9</td>
<td>84.9</td>
<td>87.7</td>
<td>0.34</td>
<td>&lt; 0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Ergometrine</td>
<td>ND</td>
<td>78.4</td>
<td>73.3</td>
<td>70.5</td>
<td>0.63</td>
<td>&lt; 0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Ergometrinine</td>
<td>90.1</td>
<td>84.4</td>
<td>82.2</td>
<td>82.1</td>
<td>0.35</td>
<td>&lt; 0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Ergosine</td>
<td>ND</td>
<td>78.2</td>
<td>73.0</td>
<td>81.9</td>
<td>0.61</td>
<td>&lt; 0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Ergosinine</td>
<td>ND</td>
<td>85.5</td>
<td>80.9</td>
<td>88.2</td>
<td>0.44</td>
<td>&lt; 0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Ergotamine</td>
<td>55.5</td>
<td>71.8</td>
<td>71.8</td>
<td>81.3</td>
<td>0.95</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
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<tr>
<td>Ergotaminine</td>
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<td>74.4</td>
<td>79.2</td>
<td>1.01</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

ND: No Data

N/A: Not Available

<sup>1</sup>no added alkaloids
<table>
<thead>
<tr>
<th>Alkaloid concentration (ppb)</th>
<th>P- value</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEM</td>
<td>Dose</td>
</tr>
<tr>
<td>Initial LW$^1$ (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34 ppb</td>
<td>1.51</td>
<td>0.72</td>
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<tr>
<td>930 ppb</td>
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<tr>
<td>1402 ppb</td>
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</tr>
<tr>
<td>2447 ppb</td>
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<tr>
<td>Final LW (kg)</td>
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</tr>
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<td>28.7</td>
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<tr>
<td>31.3</td>
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</tr>
<tr>
<td>DMI (g d$^{-1}$)</td>
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<tr>
<td>1469.4</td>
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<td>ADG (g d$^{-1}$)</td>
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<td>40.1b</td>
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<tr>
<td>Ln Prolactin Conc ($\mu$g/L)$^\chi$</td>
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<td>Hot carcass weight (kg)</td>
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<td>47.1a</td>
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<td>Grade rule (mm)</td>
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<td>17.1</td>
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<td>16.7</td>
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<td></td>
</tr>
<tr>
<td>15.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a,b Means within rows with different letters differ ($P < 0.05$).
LW: live weight; DMI: dry matter intake; ADG: average daily gain.

N.S.= not significant.

\(^1\)no added alkaloids

\(^\gamma\)Rectal temperature recorded on d 72.

\(^\times\)Mean prolactin (\(\mu g/L\)) in performance lambs (\(n = 36\)) from serum samples collected at three stages during the study: 1) at the commencement of the study, 2) LW 35 kg, 3) At market weight (\(\geq 45\) kg).
Chapter 6 – General Discussion

Ergot alkaloids have been the cause of copious human epidemics since early fifth century AD, with increased awareness and advances in technology dramatically reducing outbreaks, with the risk to human health now low. Although many of the same ergot alkaloids have been acknowledged and researched for centuries, a shift in use of contaminated grain to livestock consumption has seen a reduction in documented and evaluated data for clinical disease in livestock. With conflicting feed regulations recommending the maximum allowable levels of ergot alkaloids in ruminant feed to be 2-3 ppm, other literature suggests that the general guideline for all livestock should be approximately 5-10 µg ergot alkaloids/kg body weight (Belser-Ehrlich et al., 2012). Moreover, doses as low as 0.6-1 µg of ergot alkaloids/kg body weight have been suggested to avoid vasoconstrictive effects (EFSA, 2012). Consequently, with such a vast array of allowable concentrations circulating around harmful levels of ergot alkaloids in feed, misunderstandings persist amongst the plant and animal industries today.

Unfortunately, the majority of documented data surrounding ergot alkaloids are based on fescue sources, even though cereal ergot is likely to have somewhat different effects on livestock due to the types of alkaloids produced and the differences in alkaloid profiles (Shelby, 1999; Miller and Richardson, 2013). As such, Chapter 2 explored the common clinical symptoms of ergot poisoning in livestock, demonstrating that indicators such as feed refusal can be associated with vomitoxins, however can also be observed with ergot toxicosis (Scott et al., 1985; Trenholm et al., 1989). Further to the topic on fescue toxicosis, common ergot alkaloids present in forages such as ergovaline is currently unidentified in grain ergot, demonstrating how the knowledge from forage to grain ergot cannot be directly transferred. However economical facts, such as an estimated loss of more than $860 million per year in the USA is impacted by fescue toxicosis in all agricultural
industries (Perumbaklam et al., 2007; Duckett et al., 2014), establishes the concern for detrimental impacts to advance from cereal ergot worldwide.

In view of the vast majority of research focusing on either fescue alkaloids, or non-ruminant consumption of ergot alkaloids, there is a great need for research on cereal ergot, especially with increasing use of cereal grains in livestock production systems. Although noticeable differences in tolerance levels between species have been documented and briefly summarised in Chapter 2, uncertainty remains around ruminants and their ability to detoxify alkaloids more readily than non-ruminant species (EFSA, 2012).

Another concern is the issue of accurately detecting alkaloids, which has been briefly explored within this thesis, demonstrating that particle size can influence alkaloid recovery results in combination with the method of analysis used to determine ergot alkaloid concentration. It is thus evident that a major issue keeping ergot studies from progressing and moreover preventing anyone from determining what the allowable limits of cereal ergot should be is the variability between laboratories from the method of detection used. Further to the lack of knowledge surrounding the major impacts of cereal ergot ingestion on ruminants, the concern as to whether feed processing and grain storage can further increase ergot alkaloid concentrations negatively (particularly the proportions of $R$ and $S$ epimers) is of concern in the agricultural world, which has been explored within this thesis.

6.1 Method of detection

High performance liquid chromatography mass spectrometry (HPLC-MS) was applied to both studies to detect low concentrations of ergot in feed samples, with the second study (Chapter 5) incorporating electrospray ionisation (ESI). Chapter 4 also explored how particle size can
change the recovery rate of alkaloids, with a reduced particle size increasing alkaloid recovery by 24%. This chapter also noted that only $R$ epimers were identified in this particular lab (PDS), producing concentrations which did not incorporate $S$ epimers, which appeared to be an issue when comparing two different results in Chapter 5.

Chapter 5 used a different lab (Biomin) which could identify both $R$ and $S$ epimers in combination with ESI to increase the accuracy in detecting lower concentrations of alkaloids in grains. Upon sending the same samples to PDS, used in Chapter 4, results were surprising as they revealed that at least 78% more alkaloids were detected using Biomin as compared to PDS, demonstrating how the method of detection used can be greatly variable ($R$ epimers; PDS diet 4: 1007 ppb; Biomin diet 4: 1790 ppb). The range between samples from the same feed source between both laboratories was great, with lower concentrations containing the most variability between samples analysed by PDS (e.g. no added alkaloids ranged between 68 to 138 ppb). Reductions to the repeatability between samples have been previously recognised by Grusie et al. (2016) establishing the differences in covariance between samples with lower alkaloid concentration (284% CV) compared with samples of higher concentration (17% CV).

The importance of standardising sampling and sample preparation when analysing ergot contaminated grain may improve great variabilities in estimating concentrations in bulk samples (Songsermsakul and Razzazi-Fazeli, 2008; Grusie et al., 2016). Additionally, investigating the recovery of alkaloids in faeces is novel, requiring studies where added known amounts of different alkaloids to faeces has been established. Thus, this demonstrates an area of future research which can develop a better understanding of alkaloid absorption, toxicity impact and help explain the rumen’s detoxifying capabilities.
It is therefore evident that whilst labs have the ability to detect ergot alkaloids in feed sources, a consistent method of detection is crucial in accurately determining the total alkaloid concentration. With studies demonstrating the concern for livestock consuming concentrations of alkaloids below the current recommendations, without consistent lab analysis within investigations, ergot studies cannot assist in determining what the allowable limits of ergot should be. Ongoing studies need to establish a standardised method of detection to improve future ergot studies and decrease the variability which can be produced from using different laboratories.

6.2 Feed preparation and heat application

The question of how heating and therefore pelleting feed may increase the bioavailability of ergot alkaloids has been of recent interest in the grain industry. Merkel et al. (2012) demonstrated that after baking rye flour at 190°C for 9 to 17 minutes, a substantial reduction in concentrations of ergotamine and ergosine were observed. In Chapter 4, it was evident that whilst pelleted feed had the same total concentrations of alkaloids, some individual alkaloids, mainly ergotamine and ergosine, reduced in pelleted diets, similar to the results from Merkel et al. (2012). Moreover, the perception that heating increased the bioavailability of alkaloids proved to be invalid, as lambs receiving pelleted diets performed better than those fed mashed diets. It is speculated that this may be from the epimerisation of alkaloids from the toxic (R) configuration to the biologically inactive (S) configuration, as sample preparation and particle size were consistent between feed sources, thus not supporting the anecdotal evidence that pelleting increases toxicity of ergot alkaloids, but more likely that this perception arises from the difficulty in visually detecting ergot bodies in pelleted feed. In future studies using different sources of grain and recording the temperature throughout the pelleting process would be valuable in determining the
optimal temperature responsible for lowering the concentration of more toxic alkaloids in each grain type.

6.3 Maximum alkaloid concentrations in sheep diets

Current regulations recommend that the toxic concentration of ergot alkaloids for ruminants is a maximum dosage of 2-3 ppm. However in Chapter 4 it was evident that concentrations of 433 ppb (pellet) impacted growth, NDF and ADF digestibility, as well as prolactin concentrations, although reduction in ADG and serum prolactin were most marked in lambs fed mashed feed. In contrast, Chapter 5 revealed that feeding 2447 ppb decreased ADG and serum prolactin, with doses similar to the first investigation having no negative impact on sheep performance or nutrient metabolism. Although both studies had similar alkaloid profiles when observing the concentration of $R$ epimers, it is evident that concentrations of alkaloids present in diets appear to be poor predictors of animal performance.

It is also important to understand how alkaloid profiles can cause more severe impacts than other feed sources with a similar total alkaloid concentration. The proportions of $R$ and $S$ epimers can determine whether the animal consumes a toxic or safe concentration of alkaloids. Furthermore to this topic, understanding how sex, age and production phases can influence how alkaloids are metabolised and thus determine the impact this can have on the animal’s performance and nutrient digestibility, is an essential component to determining what the recommended dosage should be.

With this in mind, based on study 1, if a similar alkaloid profile was consumed in similar conditions by lambs, it would be recommended to give a maximum of approximately 200-400 ppb without impacting performance and nutrient digestibility. On the other hand, study 2 exposed that a much greater concentration of alkaloids could be fed to lambs based on this alkaloid profile and
similar environmental conditions, determining that up to 2000 ppb would be the recommended dosage in this scenario. Based on McLeannan et al. (2016) findings, when a decrease in environmental temperature decreased the tolerance of steers to digest sorghum ergot, suggesting that if there was a change in environmental temperature but the same ergot alkaloid concentration present, negative physiological and growth effects may be seen in livestock, demonstrating the complexity concerning the interaction of ergot alkaloids and environmental conditions. Hannah et al. (1990) identified that extreme high or low temperatures are required to enhance the toxicity of the ergot when the animal is out of the thermoneutral zone, establishing that the conditions experienced by animals in these two studies may have not allowed the potential toxicity of the ergot alkaloids to be expressed completely. Thus, recommendations can only be made based on these 2 studies, as a very different outcome could be established in higher environmental temperatures and/or consuming a different alkaloid profile even with the same total alkaloid concentration.

6.4 Future Directions

The uncertainty and complexities around alkaloid detection methodologies are a major impediment to making headway in the area of cereal ergot as differential analysis of epimers and the difficulties in estimating recoveries are issues which need to be further addressed prior to carrying out any further animal experiments. Following standardisation of analytical detection methods and the work carried out in Chapter 5 of this thesis, additional animal studies investigating different physiological stages for example reproducing stock (pregnant ewes and reproducing males) would be greatly informative, as the current regulations do not take into account different physiological stages or sex. It is clear that these types of investigations are needed for future studies on accurate alkaloid regulations, with further establishment on different ruminant intakes.
necessary such as cattle vs. sheep tolerance. However, it is first essential to establish a standard method of alkaloid detection, to minimise the variability between sample analysis such as that received from low alkaloid concentrations analysed by PDS in Chapter 5.

Further to standardising the methodology used for ergot alkaloid detection, increasing the understanding of alkaloid ingestion in ruminants will benefit in determining what the maximum allowable limit should be. Studies involving the collection of ruminal samples to determine whether ruminal metabolism of alkaloids is possible and whether or not the rumen does in fact increase the concentration of ergot alkaloids during the fermentation process (as seen by Ayers et al., 2009) may help in the understanding of whether tissue residue is possible and whether the issue of food safety needs to be of concern in the future. However, in order to minimise any metabolism of ergot alkaloids, investigations in the use of binders to prevent ingestion of toxic alkaloids may be the most effective treatment available in the near future, reducing any form of wastage in the industry.

6.5 Concluding Remarks

This thesis reveals that the comparability of experiments on ergot is quite complex, due to the interaction between different alkaloid profiles involved, along with the varying sources of alkaloids. Moreover, the selected method of alkaloid detection further complicates these comparisons, as variability between analysed samples is often great.

Although this thesis provides greater knowledge on cereal ergot ingestion in sheep, it cannot be concluded that these levels are deemed safe to sheep as every contaminated crop produces different alkaloid profiles and furthermore different alkaloid concentrations, complicating the issue. Alkaloid chemical analysis of the feed needs to be conducted prior to
feeding livestock contaminated feed and therefore caution needs to be taken on a case by case basis, with highly contaminated feed voided for consumption to reproducing stock and young. Furthermore, impacts on different life stages such as pregnancy and lactation need to be examined to understand whether these animals have increased sensitivity to alkaloid toxicity and how these situations can be minimised

Thus, commercial cereal grain and screenings produced on farm are potential sources of contaminated grain, with initial exposure commonly going unnoticed. The question concerning how we can best gain a clearer understanding of ergot alkaloids and their influence on livestock firstly revolves around standardising an accurate method of detecting alkaloids, as this is one of the main disputes keeping research from proceeding. In addition, the research outcomes presented reinforces the need to examine the biological effects of individual alkaloids and how they can impact ruminants. Therefore, this thesis has provided insight into the complex interactions of ergot alkaloids, along with a preliminary introduction on alkaloids and their epimers on performance and digestion in young ruminants.
6.7 References


Appendix 1.1

To whom it may concern,

This is a statement describing our contribution to the published journal article presented in Chapter 2 of this thesis:


The authors’ contributions were as follows:

- S.C-M. collected literature and wrote the manuscript;
- T.M., K.S., and Y.W. perceived the concept and co-wrote the manuscript
- B.B., J.M., and A.V.C. performed the critical review and co-wrote the manuscript

Yours sincerely,

Stephanie Coufal-Majewski
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Damaris Critchlow (Frontiers Zendesk)

Apr 20, 11:00

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Journal Operations Specialist: Dimitri Christodoulou
EPFL - Innovation Park, building I
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To whom it may concern,

This is a statement describing our contribution to the published journal article presented in Chapter 4 of this thesis:


The authors’ contributions were as follows:

- S.C-M., K. S., T.M. and A.V.C. designed the experiment
- S.C-M. conducted the experimental procedures and laboratory analysis
- S.C-M., K.S., and A.V.C. analysed and interpreted the data.
- S.C.-M. drafted the manuscript
- K.S., T.M., Y.W., B.B., J.M. and A.V.C. critically revised the manuscript

Yours sincerely,

Stephanie Coufal-Majewski