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## Effects of pelleting diets containing cereal ergot alkaloids on nutrient digestibility, growth performance and carcass traits of lambs



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## 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  $\sim$  3 ppb; Low (L),  $\sim$  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 feces 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 feces from lambs fed H as compared to C diets. In the growth experiment, ram and ewe lambs (live weight 24.6  $\pm$  1.08 kg) were randomly assigned to diets, weighed weekly and fed to a slaughter weight of  $\geq$  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.

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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; PL, pelleted diet with ~169 ppb added alkaloids; MH, mash diet with ~433 ppb added alkaloids; PL, pelleted diet with ~169 ppb added alkaloids; PH, pellete

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#### 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 analyzed 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 feces were determined using the procedures of Krska et al. (2008) in combination with a Romer Labs<sup>®</sup> 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  $16 \times 100$  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  $\times$  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<sup>®</sup> 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  $\mu$ L distilled water. Each sample was vortexed to suspend particles, then centrifuged at 9391  $\times$  g for 2 min. The sample material was then carefully re-suspended in distilled water, placed on a microscope slide, covered with an 18  $\times$  18 coverslip and sealed with acetone.

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