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ARTICLE

Assessment of ergot (*Claviceps purpurea*) exposure in pregnant and postpartum beef cows

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Abstract: Cows were fed ration for 9 wk containing 5, 48, 201, and 822 μg kg^{-1} ergot alkaloids. The objective was to evaluate the impact of ergot consumption in beef cow–calf operations. Ergot alkaloids up to 822 μg kg^{-1} did not alter the weight of peripartum and postpartum beef cows ($P = 0.93$) or nursing calves ($P = 0.08$), rectal temperature ($P = 0.16$), or plasma prolactin concentrations ($P = 0.30$) at moderate ambient temperatures. Ergot did not influence the time (>1 ng mL^{-1}; $P = 0.79$) or the progesterone concentration ($P = 0.38$) at the time of first postpartum rise or the size of the first (14 ± 0.6 mm; $P = 0.40$) and second (13 ± 0.5 mm; $P = 0.41$) follicles to ovulate. The maximum size of the first postpartum corpus luteum (CL) was 4 mm larger in the 822 μg kg^{-1} ergot group compared with the control ($P = 0.03$) for the first ovulation post partum, but not for the second ($P = 0.11$). There was no effect of ergot exposure on the number of days until the appearance of the first (43 ± 4 d; $P = 0.95$) or second (52 ± 4 d; $P = 0.98$) CL post partum. Ergot alkaloid concentrations up to 822 μg kg^{-1} did not affect pregnancy rates ($X^2 = 0.36$). In conclusion, ergot alkaloid exposure for 9 wk to concentrations as high as 822 μg kg^{-1} did not alter performance in pregnant and postpartum beef cattle at moderate ambient temperatures.

Key words: Claviceps purpurea, ergot alkaloids, beef cows, productivity, ovarian function.

Résumé : Les vaches ont reçu des rations contenant 5, 48, 201 et 822 μg kg^{-1} d'alcaloïdes de l'ergot pendant 9 semaines. L'objectif était d'évaluer l'impact de la consommation de l'ergot dans les opérations vaches–veaux de boucherie. Les alcaloïdes de l'ergot jusqu'à 822 μg kg^{-1} n'ont pas modifié le poids des vaches de boucherie périnatales et post-partum ($P = 0{,}93$) ni celui des veaux allaitants ($P = 0{,}08$), la température rectale ($P = 0{,}16$) ou les concentrations plasmatiques de prolactine ($P = 0{,}30$) à des températures ambiantes modérées. L'ergot n'a pas eu d'influence sur le temps (>1 ng mL^{-1}; $P = 0{,}79$) ou la concentration de progestérone ($P = 0{,}38$) au moment de la première levée post-partum ou la taille des premières (14 ± 0,6 mm; $P = 0{,}40$) et deuxièmes (13 ± 0,5 mm; $P = 0{,}41$) follicules à ovuler. La taille maximale du premier corpus luteum (CL) post-partum était 4 mm plus grande dans le groupe ayant reçu 822 μg kg^{-1} d'ergot par rapport au groupe témoin ($P = 0{,}03$) pour la première ovulation post-partum, mais pas pour le deuxième ($P = 0{,}11$). Il n'y a pas eu d'effet d'exposition à l'ergot sur le nombre de jours avant l'apparition des premiers (43 ± 4 j; $P = 0{,}95$) ou deuxièmes (52 ± 4 j; $P = 0{,}98$) CL post-partum. Les concentrations d'alcaloïdes de l'ergot jusqu'à 822 μg kg^{-1} n'ont pas eu d'effet sur les taux de gestation ($X^2 = 0{,}36$). En conclusion, l'exposition aux alcaloïdes de l'ergot pendant 9 semaines à des concentrations aussi hautes que 822 μg kg^{-1} n'a pas modifié la performance chez les vaches de boucherie gestantes et post-partum à des températures ambiantes modérées. [Traduit par la Rédaction]

Mots-clés : Claviceps purpurea, alcaloïde de l'ergot, vaches de boucherie, productivité, fonction des ovaires.

Introduction

Animal productivity and performance are important for livestock producers to maximize economic return. Animal consumption of ergot alkaloids may cause a range of effects including but not limited to, convulsions, gangrene, hyperthermia, agalactia, and reduced weight gain and feed intake (Carson 1977; McMullen and Stoltenow 2002; Burrows and Tyrl 2012; Klotz 2015).

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Animals grazing endophyte-infected tall fescue (*Lolium arundinaceum*) or consuming grain contaminated with *Claviceps* spp. will likely encounter ergot alkaloids, potentially causing adverse effects.

The vasoconstrictive effects of the ergot alkaloids, and reduced blood flow may affect hormonal control involving reproduction, digestion, and the central nervous system, as well as nutrient delivery and metabolism (Strickland et al. 2012). Decreased circulating prolactin with increasing ergot alkaloid concentrations suggest a subclinical effect (Stamm et al. 1994). For this reason, decreased prolactin is considered a sensitive indicator of exposure and is commonly used for this purpose (Klotz 2015).

The alkaloids produced in fescue, which are commonly found in the United States, differ from those produced in grain infected by *Claviceps purpurea* (Canty et al. 2014). While the clinical manifestations and effects of ergotism and fescue toxicosis are similar (Yates et al. 1985), most studies have focused on fescue rather than grain infected by *C. purpurea*.

The Canadian Food Inspection Agency (CFIA 2015) has set 2–3 mg kg^{-1} as the recommended tolerance concentration of ergot alkaloids in cattle feed. The basis for this recommendation is unclear. However, we have speculated it to be based primarily on the clinical effects such as gangrene, which can be viewed excessive, if subclinical disease such as decreased animal productivity and performance are considered. Clinical effects of ergot alkaloids have been documented at concentrations as low as 0.473 mg kg^{-1}, which is below the Canadian guidelines (Craig et al. 2015).

The main objective of this study was to evaluate the effects of low-concentration ergot consumption (*C. purpurea*) in cow–calf operations and the recovery from exposure. The endpoints examined included calf and cow weights, rectal temperature, prolactin and progesterone concentrations, and ovarian function. Ergot exposure in pre- and post-partum beef cows was hypothesized to decrease both cow and calf weights, decrease cow prolactin concentrations, increase cow rectal temperatures, and increase the time for the cows to return to normal cyclicity.

Materials and Methods

Grain collection and feed preparation

Contaminated ergot wheat screenings were collected from a seed cleaning plant in Weyburn, Saskatchewan using a sampling spear to ensure representative sample collection.

Treatment pellets were created from the ergot-contaminated wheat screenings by the University of Saskatchewan's Canadian Feed Resource Centre in North Battleford, Saskatchewan. Three types of ergot-contaminated pellets at concentrations including 221, 731, and 2981 μg kg^{-1} were formulated for the study. Control pellets containing normal background ergot concentrations (18 μg kg^{-1}) were purchased from CO-OP Feeds in Saskatoon, Saskatchewan. All pellets were comprised barley, oat hull, canola, and wheat screenings, which were formulated to meet the nutritional requirements of the beef cows when fed in combination with the remainder of the total mixed ration.

Ergot alkaloid extraction and measurement

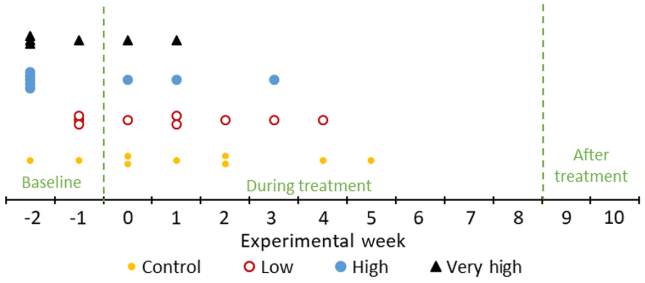
Feed samples were evaluated for ergot alkaloid concentration using an extraction procedure followed by high-performance liquid chromatography-tandem mass spectrometry analysis (HPLC-MS/MS) on an Agilent 1100 HPLC system with a Micromass Quattro Ultima Pt mass spectrometer operated in positive mode. An Agilent Zorbax Eclipse XDB-C18 narrow bore 2.1 mm × 150 mm, 5 μm p/n 993700-902 column was used. Ergot extraction and analysis were carried out as described previously in Chapter 2 section 2.3.1 (Grusie et al. 2017). Five gram samples of ground feed were extracted for 10 min using a 25 mL volume of 85/15 solvent (85% acetonitrile: 15% 10 mmol L^{-1} ammonium acetate, *v*/*v*). To clean the matrix, 50 mg Agilent Bondesil-PSA 40 μm was mixed with 1 mL of the filtered extraction. The solution (400 μL) was transferred to an Agilent auto-sampler vial with insert and placed into the HPLC auto-sampling tray. The total ergot alkaloid concentration was determined by summing the six ergot alkaloids: ergosine, ergocornine, ergocristine, ergocryptine, ergotamine, and ergometrine.

Experimental design and animal husbandry

This study was approved by the University Committee on Animal Care and Supply before experimentation. Animals in this experiment were cared for in accordance to the guidelines of the Canadian Council on Animal care (Olfert et al. 1993) under the University of Saskatchewan Animal Care Protocol 20140044. Animals were monitored using a humane intervention scoring system developed for the study. The scoring system monitored food and water intake, appearance and behaviour (pain and distress), vital signs, and vasoactive and neurological signs.

Thirty-six pregnant Hereford cross beef cows (576 ± 109 kg) were selected based on projected calving date at the University of Saskatchewan Research Farm. Cows were randomly assigned to treatment groups including, control ($n = 10$), low ($n = 10$), high ($n = 10$), and very high ($n = 6$) ergot alkaloid concentrations. Each of the groups was housed in an outdoor pen for a minimum of 2 wk before the start of the study. During this period, the animals were acclimated to the new surroundings and introduced to the control pellet

Fig. 1. Timeline of calving, relative to the initiation of ergot feeding, based on the experimental weeks for each animal. Control ($n = 9$) received 5 μg kg^{-1}, low ($n = 9$) received 48 μg kg^{-1}, high ($n = 8$) received 201 μg kg^{-1}, and very high ($n = 6$) received 822 μg kg^{-1} ergot during ergot feeding. Colour online.



ration. Following birth, the calves remained in the same pen as their mothers.

Exposure to the contaminated feed began in April 2015 for a 9 wk period and the study concluded at the end of August 2015. The experiment was designed to include 2 wk of clean ergot-free (control) pellet consumption (weeks −2 and −1) to collect baseline measurements on the cows. During the following 9 wk (weeks 0–8), the animals were fed their designated ergot-contaminated pellets. For weeks 9 and 10, the animals were returned to the control pellets. During the final 7 wk (weeks 11–17), the animals were housed on a grass mix pasture. Pellets were not consumed during the final 7 wk period. In retrospect analysis of the calving dates, the calving took place between weeks −2 and 5 of the study (n = w−2 = 9, w−1 = 5, w0 = 5, w1 = 5, w2 = 3, w3 = 2, w4 = 4, w5 = 1) (Fig. 1).

Due to the large number of animals and practical considerations, the study was divided into two data collection days. Blood samples and other assessment endpoints from control and low groups were collected on Mondays, the high and very high groups were collected on Thursdays. On the collection day, the calves were separated from the cows.

Diets and feeding procedure

Animals were targeted to consume 2% of their body weight (dry matter basis) during the study. The diets were based on the average weight of the animals in each of the groups. The diets consisted of 8.5 kg of dry chopped hay (grass per alfalfa mix), 2 kg of barley for energy, and 3.5 kg of experimental pellets. The total average daily intake as fed was 14 kg per animal representing a total daily intake on a dry matter basis of 12.7 kg. Feeding of the pellets was done under observation and all animals consumed approximately the same quantity of pellets. Physical facilities did not allow for individual feeding.

The targeted total daily intake of ergot alkaloids for each of the four groups based on the total mixed ration was 0 (control), 50 (low), 200 (high), and 800 μg kg^{-1} (very high) on a dry matter basis. To obtain these intake amounts in the animals, the control animals received 3.5 kg of the clean pellets, the low exposure animals received 2.7 kg of the 221 μg kg^{-1} total ergot alkaloid pellets and 0.8 kg of the clean pellets, the high exposure animals received 3.5 kg of the 731 μg kg^{-1} total ergot alkaloid pellets, and the very high exposure animals received 3.5 kg of the 2981 μg kg^{-1} total ergot alkaloid pellets.

To minimize animal handling, the animals were group fed. The pellets for all the cows in each group were hand mixed and spread along a feed trough to reduce any feed competition between the cows in the morning. In addition, 70 g of 1:1 (calcium to phosphorous) mineral per animal was sprinkled on top of the distributed pellets. The chopped hay was spread along the trough using a tractor with a weigh scale and the barley was spread on top of the hay for each group in the afternoon to prevent selective consumption of feed type by the cattle.

Animals had ad libitum access to water and a CO-OP 2:1 Beef Cattle Range Mineral Block (Saskatoon, SK, Canada). The animals were administered 3 mL of Vétoquinol Vitamins AD-500, a mix of vitamins A (500 000 IU mL^{-1}), D (75 000 IU mL^{-1}), and E (5 IU mL^{-1}), during the acclimation period before the start of the study.

Animal weights

Animals were weighed weekly approximately 1 h after receiving their designated pellets. Any calves older than 4 d of age were run through the chute system and weighed. If a calf was younger than 4 d their weight was obtained on the subsequent week on the appropriate collection day.

Cows were weighed after the calves were moved. Prepartum cow weights were adjusted for fetal and conceptus weight according to the Nutrient Requirements for Beef Cattle (NRC 2000).

A baseline weight was calculated to compare the weight change between the animals. This calculation was done by averaging the weights of the first 2 wk before the study (weeks −1 and −2). This weight was considered as the baseline value (100%). A gain or loss of weight will result in a value >100% or <100%, respectively.

Rectal temperatures

Rectal temperatures were recorded as the cows were weighed using a digital rectal thermometer. The temperature was taken twice to ensure a correct reading. In the case that the two readings were different, a third temperature was taken to determine the average reading. To compare rectal temperature between animals, a baseline value was calculated for each animal in the same manner as the baseline weight values.

Blood collection

Blood was collected from the jugular vein of the cows at the same time they were weighed and 1 h after receiving the experimental pellets. Approximately, 20 mL of blood was collected. The side from which the blood was taken was alternated weekly to minimize vascular damage. Collection was done using 18-gauge needles and green-grey collection tubes with heparin separators (BD Vacutainer). The blood collection took approximately 2 h in total. Following collection, blood samples were centrifuged for 15 min at 9000*g* at room temperature. Plasma was collected in 5 mL storage vials creating two aliquots per animal. The plasma aliquots were stored at −20 °C until further analysis.

Prolactin measurement

The prolactin concentration was determined using enzyme-linked immunosorbent assay (ELISA) at the University of Saskatchewan Endocrine Lab in the Western College of Veterinary Medicine following the manufacturer's procedure. The ELISA kits used were prolactin bovine 96-well plates (Catalog No. CEA846BO) purchased from Cedarlane Labs (Burlington, ON, Canada). The detection range for this kit was 2.47–200 ng mL^{-1} and the sensitivity was <0.98 ng mL^{-1}. The kits intra- and inter-assay coefficient of variations (CVs) were 11% and 26%, respectively. To compare prolactin concentrations between animals a baseline value was calculated (in the same manner as baseline weights) for each animal.

Progesterone measurement

Progesterone concentrations were determined via radioimmunoassay (RIA) at the University of Saskatchewan Endocrine Lab at the Western Collage of Veterinary Medicine using ImmuChem Coated Tube Progesterone ^{125}I RIA Kits (Catalog No. 07-270102; ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) following previously used techniques (Pfeifer et al. 2009). The detection range for the assay was 0.15–20 ng mL^{-1} with a sensitivity of 0.02 ng mL^{-1}. The intra- and inter-assay CVs were 9% and 12%, respectively.

Ovarian parameters

All animals in the very high group ($n = 6$) along with the first six animals to calve in the control group were examined twice weekly (Monday and Thursday) starting approximately 2 wk post calving using Color Doppler and B-mode ultrasonography. Color-mode, which detects blood flow, was used to confirm the presence of a corpus luteum (CL). A linear 7.5 MHz transrectal ultrasound probe was used with the MyLabFive ultrasound system (Indianapolis, IN, USA). Ultrasound examinations took place after blood collection was completed for the day. Animals to be examined were gathered and ran through the locking chute system where video segments of both the left and right ovaries were recorded for further analysis. Immediately after all the examinations were conducted for that day, the recorded video segments were analyzed using the MyLabFive system. For each ovary, all follicles >4 mm and the CL (if present) were drawn onto a recording sheet. The sizes in millimetre of each of the follicles and the CL were measured using the MyLabFive system program and recorded with the drawing. Ultrasound examinations continued twice weekly for all selected cows until two consecutive ovulations (i.e., a follicle was replaced by a CL) were detected. Once all ultrasound examinations were completed, the drawings were used to backtrack from the appearance of the CL to determine which specific follicle ovulated.

Pregnancy rates

Bulls were placed with the cows on week 9 of the experiment and removed on week 15. All cows were checked for the presence of a fetus 17 wk after the bulls were introduced to the cows (week 26 of the experiment). Physical palpation and ultrasound were used to confirm pregnancy.

Statistical analysis

Animal variables were compared by calculating their change from baseline (weeks −2 and −1) as described above. The change from baseline data during and following treatment was analyzed using IBM SPSS statistics version 23 (Armonk, NY, USA). A P value of <0.05 was considered a statistical difference. One-way analysis of variances were used to determine statistical differences between the treatment groups for cow weights, calf weights, rectal temperatures, prolactin and progesterone concentrations, and time until first progesterone rise. t tests were used to analyze ovarian follicle size, CL size, and days to CL appearance. A chi-squared analysis was used to determine pregnancy rate differences. Weekly data for cow weights, calf weights, rectal temperatures, and prolactin concentrations were analyzed using the proc mixed model repeated measures procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The model included analysis of the ergot treatment effect (Tx), the difference between during treatment and after treatment effect (D vs. A), and the interaction between the treatment and during vs. after effects (Tx × D vs. A). Calving month was integrated into the analysis as a covariate. P values of <0.05 were considered to be significant.

Results

For analysis, four animals were excluded from the study. One cow was removed from the control group as she was found to be nonpregnant during the study. One cow in the low ergot group died in week 1. The death was caused by uterine perforation by the fetal feet, which was unrelated to ergot treatment. The remaining two cows were removed from the high group. One cow from the high ergot group was removed due to nerve injury during parturition and the death of the calf.

Table 1. Ration components and ergot concentration in each treatment diet.

Total mixed ration (As feed)	Ergot concentration in the pellet (μg kg^{-1})	Amount fed per animal daily (kg)			
		Control	Low	High	Very high
Chopped grass hay	0	8.5	8.5	8.5	8.5
Barley	0	2.0	2.0	2.0	2.0
Control pellets	18	3.5	0.8	0	0
Low pellets	221	0	2.7	0	0
High pellets	731	0	0	3.5	0
Very high pellets	2981	0	0	0	3.5
Total daily intake as fed	0	14.0	14.0	14.0	14.0
Total daily intake dry matter	0	12.7	12.7	12.7	12.7
Ergot alkaloid concentration in ration (μg kg^{-1} of dry matter intake)		5.0	48	201	822

Note: Animals were group fed daily.

Table 2. Baseline [weeks −1 and −2 average; mean ± standard deviation (SD)] weights, rectal temperatures, and prolactin concentrations of the animals prior to ergot treatment.

Treatment	Baseline measurements (weeks −1 and −2 average)			
	Cow weight (kg ± SD)	Calf weight[a] (kg ± SD)	Rectal temperature (°C ± SD)	Prolactin concentration (ng mL^{-1} ± SD)
Control (n = 9)	554 ± 123	48 ± 10	39.0 ± 0.34	50.5 ± 15.0
Low (n = 9)	595 ± 99	48 ± 10	39.2 ± 0.46	55.9 ± 24.6
High (n = 8)	574 ± 91	48 ± 8	39.1 ± 0.29	65.6 ± 32.9
Very high (n = 6)	578 ± 131	49 ± 8	38.9 ± 0.17	54.9 ± 18.4

[a]Calculated using the first weight after birth.

Table 3. The mean cow weight expressed as a percent of baseline during and after ergot treatment.

Treatment[a]	During treatment (weeks 0–8)			After treatment (weeks 9–17)		
	Percent weight of baseline[b]	SD	P value[c]	Percent weight of baseline[b]	SD	P value[c]
Control (n = 9)	103.7	1.93	0.93	105.4	3.17	0.47
Low (n = 9)	103.0	2.67		102.9	3.99	
High (n = 8)	102.9	3.85		105.1	4.03	
Very high (n = 6)	102.9	4.17		105.9	5.72	

Note: SD, standard deviation.
[a]Control = 5; low = 48; high = 201; very high = 822 μg kg^{-1} total daily ergot alkaloid consumption.
[b]Baseline = the average of w−1 and w−2, represented as 100%.
[c]One-way analysis of variance, P = probability of no treatment effect (IBM SPSS statistics version 23; Armonk, NY, USA).

The second cow–calf pair was removed from the high group due to calving almost a full month later than the cohorts. With these changes, the number of animals in the control group was reduced to nine, the low group was reduced to nine, and the high group was reduced to eight. The number remained unchanged in the very high group at six cows.

Feed analysis, animal data, and ambient temperatures

The feed components along with the actual ergot concentrations are shown in Table 1.

The mean raw data for cow weight, calf weight, rectal temperature, and prolactin are shown in Table 2. Data compared with baseline values can be found in Tables 3–5 and Figs. 2*b*, 3*b*, 4*b*, and 5*b*. Weekly data can be found in Figs. 2*a*, 3*a*, 4*a*, and 5*a*.

The ambient temperature during the ergot feeding period (weeks 0–8) was moderate ranging from 5 to 29 °C with an average temperature of 21 °C. The temperature after the ergot feeding period (weeks 9–17) was also moderate ranging from 15 to 30 °C with an average temperature of 23 °C.

Cow weights

Cow weights were not affected by ergot treatment during (P = 0.93) or after (P = 0.47) the exposure

Table 4. The mean calf weight expressed as a percent of baseline during and after ergot treatment of the cows.

	During treatment (weeks 0–8)			After treatment (weeks 9–17)		
Treatment[a]	Percent weight of baseline[b]	SD	*P* value[c]	Percent weight of baseline[b]	SD	*P* value[c]
Control ($n = 9$)	136.5	23.9	0.08	219.6	46.9	0.15
Low ($n = 9$)	147.5	20.4		239.1	41.6	
High ($n = 8$)	162.6	19.1		261.2	26.1	
Very high ($n = 6$)	158.4	20.1		251.0	30.9	

Note: SD, standard deviation.
[a]Control = 5; low = 48; high = 201; very high = 822 μg kg^{-1} total daily ergot alkaloid consumption.
[b]Baseline = the average of w–1 and w–2, represented as 100%.
[c]One-way analysis of variance, *P* = probability of no treatment effect (IBM SPSS statistics version 23; Armonk, NY, USA).

Table 5. The mean cow plasma prolactin concentration expressed as a percent of baseline during and after ergot treatment.

	During treatment (weeks 0–8)			After treatment (weeks 9–12)		
Treatment[a]	Percent prolactin concentration of baseline[b]	SD	*P* value[c]	Percent prolactin concentration of baseline[b]	SD	*P* value[c]
Control ($n = 9$)	92.0	14.2	0.30	73.8	26.6	0.87
Low ($n = 9$)	98.0	25.9		73.0	32.1	
High ($n = 8$)	83.6	14.8		66.5	21.4	
Very high ($n = 6$)	81.6	16.5		63.7	30.0	

Note: SD, standard deviation.
[a]Control = 5; low = 48; high = 201; very high = 822 μg kg^{-1} total daily ergot alkaloid consumption.
[b]Baseline = the average of w–1 and w–2, represented as 100%.
[c]One-way analysis of variance, *P* = probability of no treatment effect (IBM SPSS statistics version 23; Armonk, NY, USA).

Fig. 2. Cow weights during (9 wk) and after (9 wk) ergot treatment feeding. Control ($n = 9$) received 5 μg kg^{-1}, low ($n = 9$) received 48 μg kg^{-1}, high ($n = 8$) received 201 μg kg^{-1}, and very high ($n = 6$) received 822 μg kg^{-1} ergot during ergot feeding. All cows received 2 wk of control diet and 7 wk of pasture for the duration of the after treatment feeding. Weekly mean (± standard error) cow weight (*a*) and percent cow weight change from baseline (*b*) (mixed model repeated measures, SAS version 9.3; SAS Institute Inc., Cary, NC, USA). Colour online.

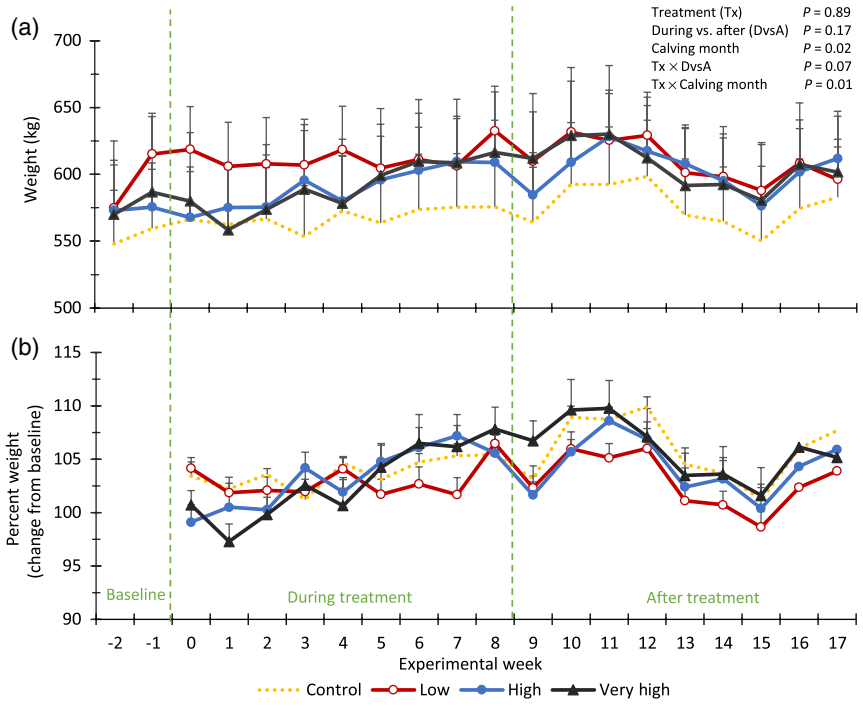
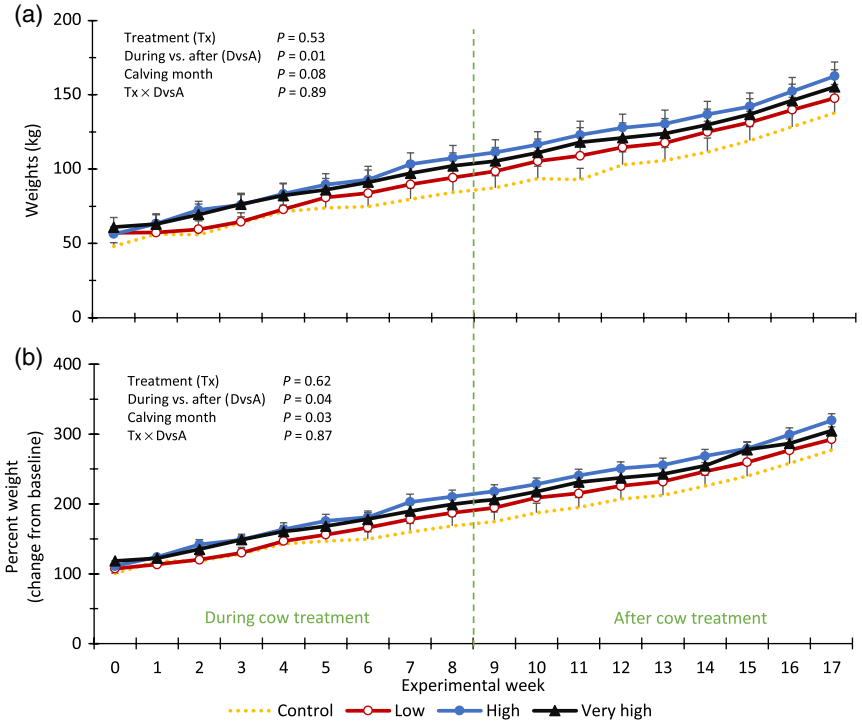


Fig. 3. Calf weights during (9 wk) and after (9 wk) ergot treatment feeding to the cows. Control cows received 5 μg kg^{-1}, low cows received 48 μg kg^{-1}, high cows received 201 μg kg^{-1}, and very high cows received 822 μg kg^{-1} ergot during ergot feeding. All cows received 2 wk of control diet and 7 wk of pasture for the duration of the after treatment feeding. Control ($n = 9$), low ($n = 9$), high ($n = 8$), and very high ($n = 6$) once all calves were born. Baseline was calculated using the calves weight the first week after calving. Weekly mean (± standard error) calf weights (*a*) and percent calf weight change from baseline (*b*) (mixed model repeated measures, SAS version 9.3; SAS Institute Inc., Cary, NC, USA). Colour online.



period (Table 3). Furthermore, weekly cow weights showed no treatment ($P = 0.89$) or time (during vs. after ergot treatment; $P = 0.17$) effect (Fig. 2*a*) after accounting for calving month ($P = 0.02$). Percent weekly cow weights (percent of baseline measurements) showed a treatment effect ($P = 0.002$) and an effect during vs. after ($P = 0.05$) ergot treatment periods (Fig. 2*b*). Based on the Tukey's adjusted post hoc comparisons, the very high ergot group (104.38% ± 0.57%) had greater percent body weight change from baseline (averaged over during and after treatment period) compared with control (104.01% ± 0.34%) and low ergot (102.94% ± 0.33%) groups ($P < 0.05$). The high (103.95% ± 0.43%) ergot group had a greater percent body weight than the low ergot group, but had a lower value than the control group ($P < 0.05$). The high and very high groups did not differ; similarly, control and low ergot did not differ from each other.

Calf weights

Calf weights were not affected by ergot treatment during ($P = 0.08$) or after ($P = 0.61$) the exposure period (Table 4). Weekly and percent calf weights were not affected by ergot treatment effect ($P = 0.53$ and 0.62, respectively), but did produce an effect for time (during vs. after ergot treatment periods; $P = 0.01$ and 0.04; Fig. 3). Overall, calves were growing throughout the study (caving month $P = 0.08$ and 0.03) but there was no differential effect of treatment (treatment × during vs. after interaction $P = 0.89$ and 0.87).

Rectal temperatures

Cow rectal temperatures were found to be similar for all treatment groups both during ($P = 0.16$) and after ($P = 0.07$) the ergot treatment period. Weekly rectal temperature data exhibited an interaction between treatment and time (during vs. after treatment periods; $P < 0.001$; Fig. 4*a*). Weekly rectal temperatures compared with baseline measurements displayed no interaction between treatment and during vs. after ($P = 0.11$) nor a treatment ($P = 0.37$) or during vs. after effect ($P = 0.52$; Fig. 4*b*).

Prolactin concentrations

Cow plasma prolactin concentrations were not affected by ergot treatment during ($P = 0.23$) or after ($P = 0.87$) the exposure period (Table 5). Weekly prolactin concentrations showed no treatment ($P = 0.38$) nor time effect (during vs. after $P = 0.71$; Fig. 5*a*). Weekly prolactin concentrations compared with baseline measurements also presented no treatment ($P = 0.43$) nor time effect (during vs. after $P = 0.63$; Fig. 5*b*).

Fig. 4. Cow rectal temperatures during (9 wk) and after (9 wk) ergot treatment feeding. Control ($n = 9$) received 5 μg kg^{-1}, low ($n = 9$) received 48 μg kg^{-1}, high ($n = 8$) received 201 μg kg^{-1}, and very high ($n = 6$) received 822 μg kg^{-1} ergot during ergot feeding. Weekly mean (± standard error) cow rectal temperatures (*a*) and percent cow rectal temperature change from baseline (*b*) (mixed model repeated measures, SAS version 9.3; SAS Institute Inc., Cary, NC, USA). Colour online.

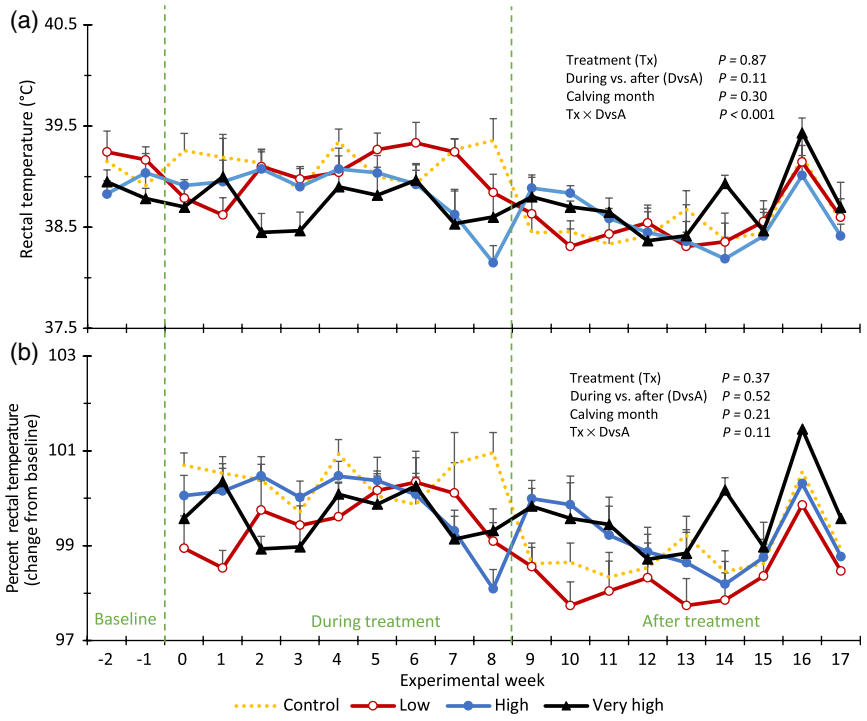


Fig. 5. Cow plasma prolactin concentrations during (9 wk) and after (4 wk) ergot treatment feeding. Control ($n = 9$) received 5 μg kg^{-1}, low ($n = 9$) received 48 μg kg^{-1}, high ($n = 8$) received 201 μg kg^{-1}, and very high ($n = 6$) received 822 μg kg^{-1} ergot during ergot feeding. Weekly mean (± standard error) plasma prolactin concentrations (*a*) and percent plasma prolactin change from baseline (*b*) (mixed model repeated measures, SAS version 9.3; SAS Institute Inc., Cary, NC, USA). Colour online.

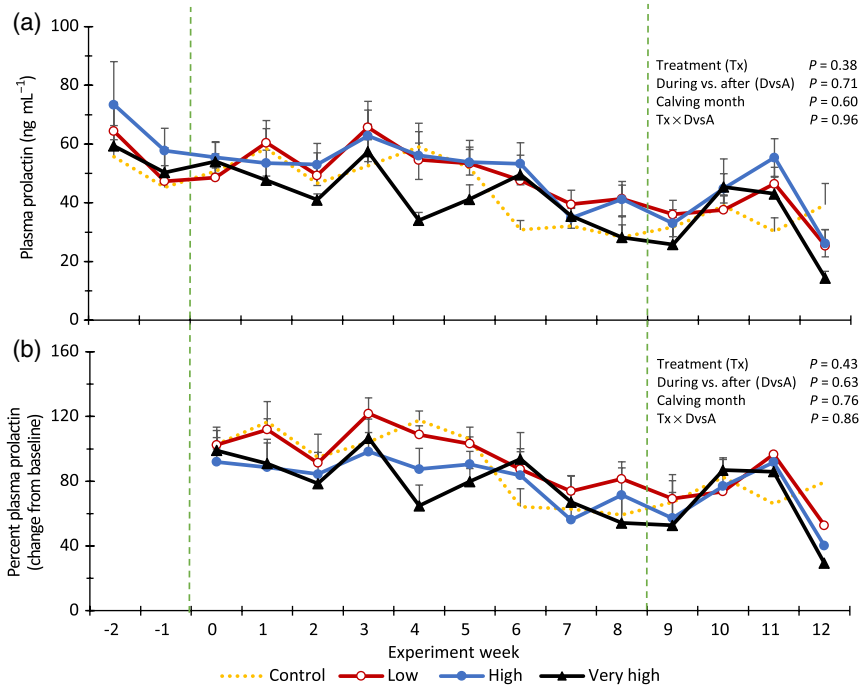


Fig. 6. Weeks [± standard deviation (SD)] until first rise (>1 ng mL^{-1}) of progesterone post partum (*a*) and progesterone concentration (±SD) at first rise post partum (*b*) of cows receiving 9 wk of ergot treatment feeding (one-way analysis of variance, IBM SPSS statistics version 23; Armonk, NY, USA). Control ($n = 9$) received 5 μg kg^{-1}, low ($n = 9$) received 48 μg kg^{-1}, high ($n = 8$) received 201 μg kg^{-1}, and very high ($n = 6$) received 822 μg kg^{-1} ergot during the exposure period. Colour online.

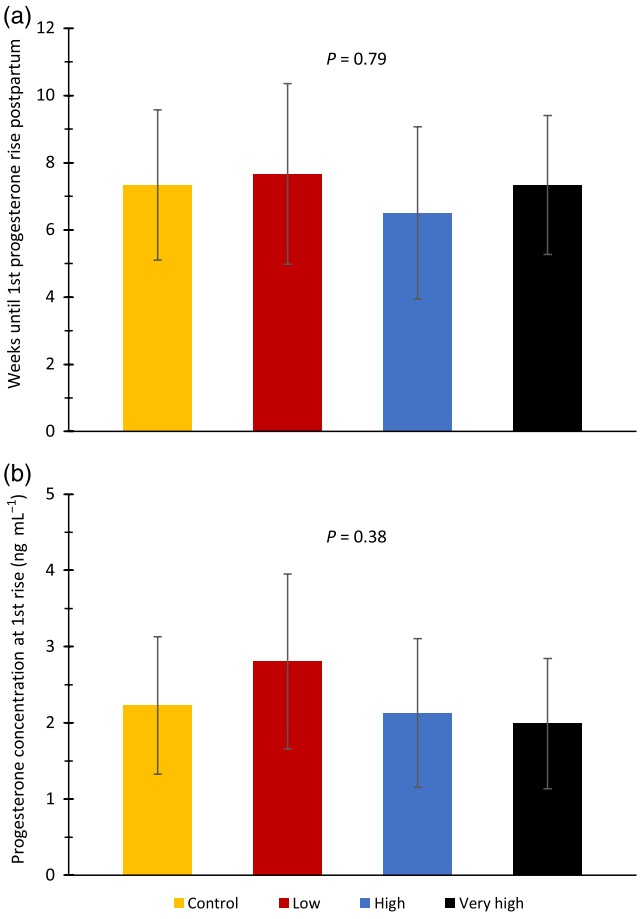
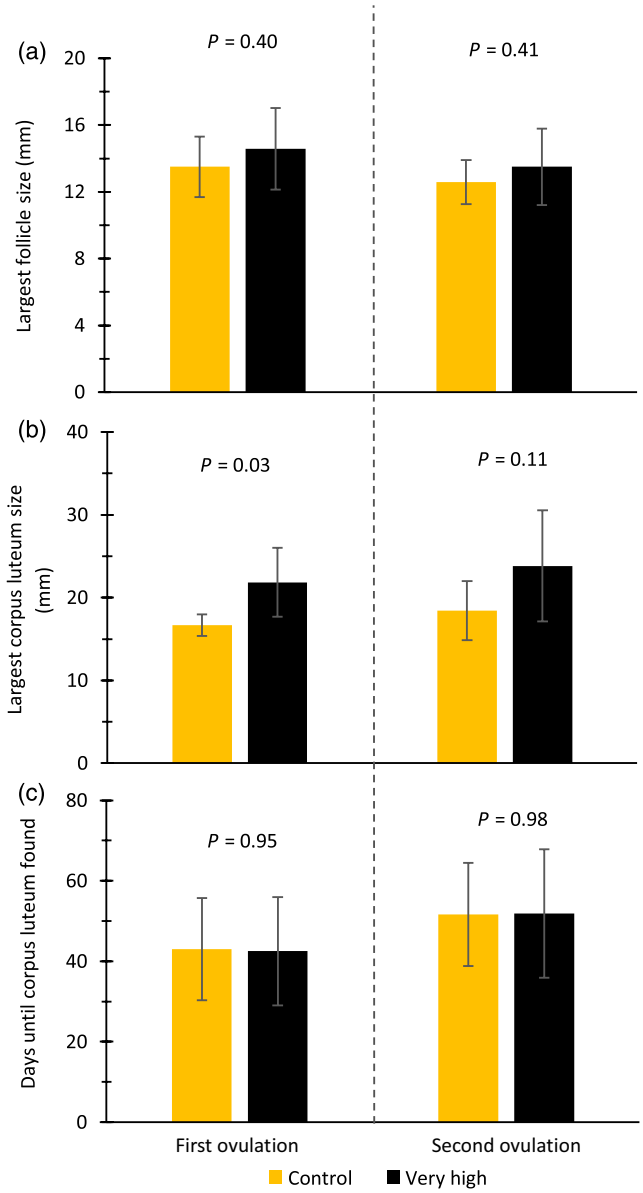


Fig. 7. Three ovarian parameters were compared between cows postpartum in the control ($n = 6$; 5 μg kg^{-1}) and the very high ($n = 6$; 822 μg kg^{-1}) ergot treatment groups. The parameters were observed for both the first and second ovulation. Largest diameter [± standard deviation (SD)] measured of the ovulating follicle (*a*). Largest diameter (±SD) measured of the corpus luteum (*b*) and number of days (±SD) until the corpus luteum was observed (*c*) (*t* test, IBM SPSS statistics version 23; Armonk, NY, USA). Colour online.



Progesterone measurements

The number of weeks until first progesterone rise post partum (Fig. 6*a*) and the progesterone concentration at that first rise (Fig. 6*b*) were monitored. A rise in progesterone was considered a concentration greater than 1 ng mL^{-1}. Both the number of weeks until first progesterone rise post partum ($P = 0.79$) and the concentration at that first rise ($P = 0.38$) were not effected by the ergot treatment.

Ovarian measurements

The largest follicle (Fig. 7*a*), largest CL (Fig. 7*b*), and days until CL was appearance (Fig. 7*c*), were recorded by ultrasonography for the first and second ovulations post partum. No differences were found for the largest follicle observed for the first ($P = 0.40$) or second ($P = 0.41$) ovulation post partum between the control and very high ergot treatment groups. The size of the CL was found to be larger in the very high treatment group compared with the control group for the first ovulation ($P = 0.03$), however, this difference was not apparent for the second ovulation ($P = 0.11$). No differences were observed in the number of days until the appearance of the CL for the first ($P = 0.95$) or second ($P = 0.98$) ovulation comparing the control and very high treatment groups.

Pregnancy rates

Cows were checked for pregnancy 17 wk (week 26 of experiment) after bull exposure (removed on week 15). There were no differences in pregnancy rates ($X^2 = 0.36$) between the ergot treatment groups; control (7/9), low (8/9), high (8/8), and very high (6/6).

Discussion

This study examined the effects of ergot alkaloid consumption at concentrations up to 822 μg kg^{-1} (total mixed ration) in pregnant and postpartum beef cattle to assess performance and reproductive endpoints during the exposure and recovery period.

The study determined that low-concentration ergot exposure to pregnant and postpartum beef cows did not alter weight gain of the cows or the calves. Ergot concentrations up to 822 μg kg^{-1} of total dry matter intake did not alter cow prolactin concentrations, rectal temperature, or the return to postpartum cyclicity.

Calving month was incorporated into the weekly statistical analysis as a covariate in treatment effect; therefore, any statistical differences associated with calving month were not considered to be relevant in the discussion.

The findings indicate that feeding up to 822 μg kg^{-1} of total dry matter intake had no effect on cow weight gain during the early postpartum period. The interaction between treatment groups and calving month observed in the weekly weight data (Fig. 2*a*) was most likely associated to the weight variation between the cows as this interaction disappeared when comparing the cows' weights to their baseline values (Fig. 2*b*). It should be noted that control group remained amongst the middle of the treatment groups indicating there was no dose-response relationship or trend related to ergot alkaloid consumption up to 822 μg kg^{-1} on weight gain. If ergot exposure had reduced weight gains, one would expect at minimum the very high ergot exposed treatment group to exhibit a reduced weight gain as the ergot exposure increased.

This finding is in contrast with Burfening. (1994) who found that average daily gain deceased linearly with ergot consumption from 0% to 1.6% of ergot in the diet. While it is difficult to determine the actual ergot alkaloid concentration in the cited study, it was likely much higher than the concentration used in the current study. Depending upon the feed type and growth conditions, the 1.6% ergot content represents approximately 10 000 μg kg^{-1} alkaloid content. In the present study, if exposure concentrations had been increased by 10-fold, a linear decline may have been observed.

Most studies establishing reduced weight gain and intake as a consequence of ergot alkaloid consumption have been done using endophyte-infected tall fescue (Mahmood et al. 1994; Paterson et al. 1995; Foote et al. 2013; Koontz et al. 2015). Estimated ergot concentrations in these studies range from approximately 5500 μg kg^{-1} to unknown concentrations of up to 75% infectivity of endophyte in pasture. Alkaloids produced by *C. purpurea* are expected to act in a similar fashion by interacting with the serotonergic receptors involved in the regulation of gut motility, thereby, negatively affecting the motility and passage rate through the gut (Klotz 2015). The lack of effect found in the present study may be due to the different alkaloid composition in the endophyte-infected fescue compared with those found in *C. purpurea* or more likely related to the substantially lower ergot alkaloid concentration in the present study.

An effect of time (i.e., during vs. after treatment) and calving month was found in both the weekly and weekly change from baseline calf weight data (Fig. 3). All of the treatment groups exposed to ergot demonstrated increased weight gains in the calves and numerically the control calves had the least body weight at the end of the study. It was anticipated that ergot exposure in the cows would have resulted in reduced milk production (prolactin inhibition) and consequently reduced nutrition (milk) available for the calves; however, this parameter was not quantified in the current study. We acknowledge that the inability to undertake individual feeding is a limitation of the present study. Although we were not able to absolutely ensure that each cow consumed equal amounts, in our assessment, the supervised feeding of treatment pellets was closer to individual feeding than pen feeding, and therefore, we treated the cows as experimental units. We would like to stress that the information provided in this manuscript provides some critical ground work for future studies and potential regulation changes despite the aforesaid limitation.

Prolactin has been functionally linked, together with other mechanisms, to the initiation and maintenance of milk secretion and mammogenesis (Fell et al. 1974; Houdebine et al. 1985). Decreased prolactin production in the lactating cow has the potential to negatively affect calf weight gain post partum. Multiple studies have observed a decline in prolactin production in dairy cattle as a result of the consumption of ergot alkaloids (Carson 1977; Strahan et al. 1987; Paterson et al. 1995; Munkvold et al. 1997; Ilha et al. 2003). However, this effect was not observed in the current study. The difference related to the current study and past research may be attributed to the type of cow (i.e., dairy vs. beef), the source of ergot alkaloids (i.e., endophyte vs. *C. purpurea*), and (or) the ergot alkaloid concentration. If the exposure to ergot by the cows had included treatment groups approaching 10 000 μg kg^{-1}, a negative impact on calf weight gain may have been observed. Milk production related to prolactin synthesis may be a more sensitive bioindicator of ergot exposure in high producing dairy breeds. Milk production was not evaluated in the present study.

Ergot alkaloids have the ability to cause arterial vasoconstriction, thereby, diminishing blood circulation (Seaman 1980; Shelby 1999; Strickland et al. 2009). Animals exposed to ergot alkaloids have been found to

have a reduced ability to remove body heat particularly in hot climates or retain body heat in cold climates (Carson 1977; Rhodes et al. 1991; Strickland et al. 2009; Spiers et al. 2012). Although, an interaction between treatment and time (during vs. after) was found in the weekly cow rectal temperature data (Fig. 4*a*), the values were within the normal body temperature range of 36.7–39.1 °C for cows (Erickson et al. 2004). Furthermore, this interaction was not evident in the weekly change from baseline rectal temperature data (Fig. 4*b*). Therefore, with interpretation based on both recorded rectal temperature and percent of baseline values, the anticipated dose-response hyperthermia with increasing ergot concentrations was not evident under the current ambient temperature conditions at the ergot alkaloid concentrations consumed in this study.

It is noteworthy that the ambient temperature was approximately 21 °C and no extreme environmental temperature conditions were encountered. Thermoregulation was unlikely to be altered under the moderate climatic conditions encountered in this study. This conclusion may not be valid under extreme cold conditions encountered in Canadian prairies during the winter or during the extreme hot weather in the summer in Southern United States.

The return to normal ovarian cyclicity in postpartum cows in a timely manner is important for livestock farmers to maximize economic returns. This study evaluated the time of first postpartum progesterone rise and the concentration, timing of first postpartum ovulation, and size of the ovulatory follicle at that time to assess the impact of ergot exposure on the return to normal cyclicity in cows.

A progesterone concentration above 1 ng mL^{-1} is an accepted indication of the progression of the estrus cycle and the onset of ovarian activity (Díaz et al. 1986; Patterson et al. 1989).

Some researchers have demonstrated decreased progesterone concentrations in cattle with ergot alkaloid consumption (Mahmood et al. 1994; Jones et al. 2003; Poole et al. 2016), whereas other studies found no effect of ergot alkaloids on progesterone (Burke et al. 2001; Schuenemann et al. 2005). The present study supported the latter conclusion, there was no observed effect on either the time of first progesterone rise above 1 ng mL^{-1} (all treatment groups) or the time of first ovulation, ovulatory follicle size, and the first or second CL (control vs. 822 μg kg^{-1} ergot alkaloid group). The present results relating to no effect on the follicle size or diameter of the CL are consistent with other studies (Ahmed et al. 1990; Jones et al. 2003; Seals et al. 2005).

Mahmood et al. (1994) suggested that animal age can alter the effect of ergot alkaloids on progesterone. Grazing endophyte-infected tall fescue reduced progesterone in weaned heifers, however, yearling heifers were not as sensitive to the ergot alkaloids. It is plausible that the source of the ergot alkaloids, dose, duration, and time of ergot exposure may all contribute to the varied observations reported in literature related to plasma progesterone concentrations. It is interesting to note that the preliminary results indicate pregnancy rates were not altered in the current study subsequent to ergot exposure. It should be noted that the limited number of animals used in this study makes it difficult to detect minor differences in pregnancy rates. A future study warranted with larger group sizes would be required to confirm or refute these preliminary findings. However, considering all of the measurements together (timing of first progesterone rise, ovulatory follicle and CL size, timing of first ovulation, pregnancy rates) it appears that the consumption of ergot alkaloids at concentrations up to 822 μg kg^{-1} for 8 wk in peri-parturient and early postpartum period in beef cows does not impact reproduction and return to cyclicity. This information is important for cattle producers as normal reproductive performance is necessary to keep cow–calf operations profitable. Delays in conception related to ergot alkaloids can be a major production loss. Ergot alkaloid concentrations up to 822 μg kg^{-1} appear to be acceptable in beef cattle feed without adverse reproductive effects.

Since no clinically relevant alterations were observed during the treatment period, the assessment of recovery from ergot exposure in cattle from a reproductive perspective could not be evaluated. The lack of alterations in the "after" treatment period for 9 wk suggests there are also no delayed effects associated with the consumption of ergot alkaloid concentrations up to 822 μg kg^{-1}.

At the present time, there is considerable controversy related to current tolerance or feed guidelines related to the consumption of ergot-contaminated feed by cattle. Since no effects were observed at concentrations approaching 822 μg kg^{-1}, tolerance guidelines based on reproductive performance, prolactin concentration, or weight gain could be established near the 822 μg kg^{-1} value. This recommendation may vary under extreme climactic conditions or perhaps with dairy cattle, with more sensitive metabolic requirements.

Conclusion

This study was conducted to assess the potential loss of productivity and cow–calf production due to consumption of ergot alkaloids produced by *C. purpurea*. Three concentrations of ergot alkaloids were evaluated at or below 822 μg kg^{-1} of total dry matter intake. Endpoints measured were unaffected by ergot exposure included cow weight, calf weight, rectal temperature, prolactin concentration, progesterone concentration, and postpartum ovarian function. There was no impact on the overall performance of cow–calf production at moderate ambient temperatures.

Further studies should explore the effects of ergot alkaloids produced by *C. purpurea* above 822 μg kg^{-1} but less than the current Canadian guidelines under varying

climatic conditions and duration of exposure. Modifications of the guidelines may be influenced based on this updated species-specific dose-response information.

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