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An observational study of naturally occurring gastrointestinal nematode infections in a seasonal grazing cow-calf herd in southern Ontario

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Little work has been done to determine the prevalence of gastrointestinal nematodes (GIN) in Ontario cow-calf herds. A prospective single cohort study was conducted during the grazing seasons of 2014 and 2015. Twenty-four crossbred cows were randomly assigned to one of six rotationally grazed fields each year, blocked by calving date and parity ($n=48$). Feces were collected and weight and body condition score (cows) recorded at approximately 28 d intervals. Fecal egg counts (FEC) were performed using the Wisconsin method. No clinical signs of GIN parasitism were observed. Cow FEC stayed relatively low throughout both pasture seasons (mean 8 ± 20.02 eggs per gram of feces (epg), $n=301$). Most calf FEC were also low throughout both seasons (mean 42 ± 86.20 epg, $n=268$). The sample time (a proxy for days on pasture (DOP)) had a significant effect on cow epg ($P < 0.001$) and calf epg ($P < 0.001$). Cow and calf FEC peaked at the fourth sample, after 55 to 72 DOP; 4 epg (95% CI 2.57, 6.32) and 24 epg (95% CI 15.82, 37.19) for cows and calves, respectively. Mean calf FEC did not have a significant effect on calf weaning weight ($P = 0.9$).

Key words: Epidemiology; gastrointestinal nematode; cow-calf; grazing; weaning weight

Abbreviations: GIN, gastrointestinal nematode; FEC, fecal egg count; epg, eggs per gram; DOP, days on pasture; WW, weaning weight

Gastrointestinal nematodes (GIN) are one of the most important classes of internal parasites to the beef industry in terms of negative biological and economical effects (Ward et al. 1991; Corwin 1997; Stromberg and Gasbarre 2006). By controlling GIN infections, a weaning weight advantage of 7 to 22 kg has been seen as well as improved average daily gains (Ciordia et al., 1987; Stromberg and Vathauer, 1997; Guichon et al., 2000). Many studies have been conducted looking at the epidemiology of nematodes with the aim to improve GIN parasite control in cattle, including pasture management techniques, timing of turnout, and timing of anthelmintic use (Nansen and Jørgensen 1987; Wohlgemuth et al. 1990). However, it is known that the epidemiology of GIN varies with geographic location (Craig, 1988). Understanding the

epidemiology of GIN in a specific region is critical in developing an efficient and effective control program (Waller, 2006). There is little known about the epidemiology of GIN in naturally infected cow-calf herds in temperate continental climates, such as southern Ontario. The objective of this study was to describe the change in GIN fecal egg count (FEC) over the grazing season and the time after placement on pasture when the maximum FEC is observed.

MATERIALS AND METHODS

Study Design

This study was part of a larger trial (Mackie, 2016) being conducted on anthelmintic use in cow-calf production. That trial ran from 21 May to 29 October in 2014 and 13 May to 13 October in 2015. Cow-calf pairs were randomly allocated, by calving date and parity, to one of two anthelmintic treatments (fenbendazole or ivermectin) or no treatment (negative control). This study included cow-calf pairs not treated with an anthelmintic (i.e. negative control group). Each of the two years, 24 cow-calf pairs were enrolled for a total of 48 cow-calf pairs. Each year, four cow-calf pairs were randomly assigned to one of six paddocks resulting in six replications per year. In 2014, of the 24 cows enrolled five were primiparous and 19 multiparous. Cows from 2014 were re-enrolled into the same treatment group, but randomized to pastures, the following year, provided they still met the enrollment criteria. Primiparous cows were used to replace cows that were culled or did not calve within the calving window in the second year of the trial. Therefore, in 2015 of the 24 cows enrolled 11 were primiparous and 13 multiparous. Cows that gave birth to a single, healthy calf, did not have any difficulty calving and did not experience disease (e.g. metritis, mastitis, pneumonia) around the time of calving, were eligible for inclusion in the trial. Cows that had twins or were the recipient of a cross-fostered calf were not eligible

for the trial. Cows with past behavioural issues were also excluded as a safety precaution due to the frequent handling required.

Cows and calves were weighed (scale accurate to 2 kg) and the body condition scores (BCS on scale of one to five) of cows were recorded at turnout and throughout the pasture seasons. Technicians recorded BCS and were blinded to treatments. Cattle were observed for signs of clinical parasitism; this included weight loss, edema of the lower jaw and/or clinical signs of diarrhea. In 2014, all cows and calves were sampled at turnout (day 0), day 41 to 44 and every 28 d thereafter. The large gap between the first and second sampling times was due to unanticipated complications with the weigh scale. During the second year of the study, cows and calves were sampled at day 0, day 14, day 28, and every 28 d afterwards. Before cattle were removed from pasture in October, final samples were collected.

Study Site and Cow-Calf Pairs

This study was conducted at the Elora Beef Research Centre in Central Wellington, Ontario. At this facility, cross-bred cows were overwintered in straw bedded group housing. Cows had outdoor access on a concrete pad, water *ad libitum* and a total mixed ration formulated to meet nutrient requirements for beef cows (NRC 2000). Previous anthelmintic use on this herd consisted of a single application of a generic ivermectin product (Noromectin® administered at 0.5 mg kg⁻¹), for calves at weaning that coincides with housing in the fall. Cows were not historically treated. The 48 cow-calf pairs enrolled over the two years did not receive an anthelmintic in either of the years. Over the two grazing seasons, a total of 48 cow-calf pairs were rotationally grazed. The cattle grazed for an average of 155 and 140 days in 2014 and 2015, respectively, starting in May. There was a slight variation in grazing time in study animals due to

labor constraints. All animals housed on the same field were placed on and removed from pasture on the same day. However, animals on different fields were placed on or removed from pasture over a one week period. While on pasture, cows and calves had free access to water and a mineral supplement, including salt. Breeding was done using artificial insemination with teaser bulls used to detect estrous on pasture. All cattle enrolled in this trial were cared for meeting the guidelines laid out by the Canadian Council on Animal Care (CCAC, 2009) and the trial was approved by the University of Guelph Animal Care Committee (AUP #2678).

Pasture History

Cow-calf pairs in this study were housed on one of six fields that were subdivided from one large field. The fields have been dedicated to pasture and cattle grazing since 1985, with occasional reseeded of common grass species and some legumes. Each field was approximately 1.62 ha (four acres) and was subdivided into eight paddocks using electric fencing. The dominate forage species included: orchard grass, fescue, Kentucky bluegrass, brome grass, white clover, vetch. A stocking density of four cow-calf pairs per field was used (0.41 ha per cow-calf pair). Cattle were moved to a new paddock every two to eleven days, depending on visual assessment of pasture conditions and forage availability. Pastures were left vacant for an average of 27 days before they were re-grazed by cattle. Contact between cow-calf pairs on different fields was limited due to the pattern of rotation of cattle from paddock-to-paddock and the presence of alleyways between fields.

Sample Collection and Laboratory Analysis

A separate plastic sleeve was used per rectum to collect a fecal sample from each cow and each calf, between 1000 and 1300 h on collection day. Each fecal sample was placed directly

into a separate 120 mm sterile specimen container. The containers were placed directly in a cooler with ice packs after collection. The samples were transferred to a refrigerator (4°C) within three hours of collection and processed within seven days of collection. The Modified Wisconsin Flotations Technique was used to perform fecal egg counts (FEC), following the Animal Health Laboratory, University of Guelph protocol (M. Lake, personal communication). Five grams of feces, on a wet basis, were measured out and mixed with 10 ml of water. The mixture was strained through a filter and pressed until dry. The strained feces-water mixture was placed in a test tube for centrifugation at 264 x g for 5 minutes. The supernatant was poured off and a sucrose solution was added and mixed. Specific gravity of the sucrose solution used was between 1.30 to 1.33 units. The tube was filled again with the sucrose solution carefully to the top and a cover slip was placed on the tube. The tube was placed in the centrifuge for an additional 5 minutes (264 x g). The cover slip was lifted off the tube and placed on a slide. A correction factor of 1.6 was used to compensate for egg loss during the process; this is because a recovery rate of 62.5% is expected for this technique (Egwang and Slocombe, 1982). Slides were examined under 10x magnification and all nematode (family Trichostrongylidae) eggs were counted. The minimum detection limit for the Wisconsin method is one to two eggs per gram of feces (Animal Health Laboratory, University of Guelph).

Meteorological Data

Most climatic data was obtained from the Agricultural and Forest Meteorology Group - Elora Research Station/Guelph Turfgrass Institute, School of Environmental Sciences, Ontario Agricultural College, University of Guelph. Due to absence of data from the Agricultural and Forest Meteorology Group from 1 July to 31 December 2015, data were sourced from Environment Canada. Total precipitation in millimeters, the mean average relative humidity (%)

and the mean average temperature (°C) were calculated for each month. These data were compared to the 30-year average (1981 to 2010) for Waterloo-Wellington, Ontario, from Canadian Climate Normals station data. Data was also included in the statistical models for cow and calf FEC as the outcome of interest.

Data Analysis

The statistical software SAS (Version 9.4, SAS Institute Inc., Cary, NC, USA) was used to perform all statistical analyses. A significance level of $P \leq 0.05$ was set. Summary and descriptive statistics were performed to provide an initial overview of the data.

The number of days the cow-calf pairs were on pasture equalled the difference between the date of the observation and the date the cattle were placed on pasture. Cow age was dichotomized into multiparous and primiparous. Calf sex was initially categorized into three categories: bull, steer, and heifer. However, there was no difference in model estimates, significant covariates, or outcomes between bulls and steers, so calf sex was dichotomized into male and female to produce a similar model. The natural logarithm of cow and calf FEC was applied with a bias correction term (bct) of 0.25 ($\ln(\text{FEC} + 0.25)$) due to FEC of zero in the data set. This logarithm transformation was performed to improve the normality of the FEC data bringing the distribution closer to normal. Mean and standard deviation of calf and cow FEC were calculated. Dates sampled were categorized into seven sampling times, as cows and calves were repeatedly sampled six and seven times throughout the 2014 and 2015 pasture seasons (Table 1). The categorical variable 'sample time' was created to group repeated measures together between the two years. Due to differences in cattle turnout times between years, repeated samples were not collected on the same days of the year in 2014 and 2015 (i.e. DOP were not equivalent in the calendar). Precipitation, relative humidity and temperature were

averaged for all days between sampling times and were included in the FEC statistical models. A dummy variable for early and late turnout was also created to test for difference in turnout time in 2015. Half of the cow-calf pairs in 2015 were turned out between 13 to 15 May and were categorized as early turnout. The second half was turned out two weeks later (i.e. late turnout). All predictor variables underwent univariable screening to determine which were offered to the models.

PROC MIXED was used to construct three mixed models manually, using backwards stepwise elimination. Pearson pairwise correlation coefficients were assessed for age of calf at sampling, DOP, and sample time (a proxy for DOP). Collinearity, between predictor variables that questioned the assumption of independence (Pearson correlation coefficient ≥ 0.8), was assessed by testing pair-wise correlations, using PROC CORR.

The outcomes of interest were the natural logarithm of calf FEC (LCAFEC), natural logarithm of cow FEC (LCOFEC), and unadjusted calf weaning weight (WW: the last calf weight collected in the fall). Unadjusted weaning weight was chosen over adjusted weaning weight so the effects of all explanatory variables (cow age, sex of calf, age, etc.) could be examined individually. Causal diagrams were constructed and assessed for all models, looking for possible confounders and intervening variables. For the first model examining LCAFEC, the following variables were included in the initial multivariable model: year, sample time, sex, dam parity, calf average daily gain, turnout and repeated measures. The repeated measures were LCOFEC, calf weight, cow weight and weather variables. The LCOFEC model included year, sample time, parity, turnout and the repeated measure cow weight and weather variables.

The third model examined unadjusted calf WW, a single measurement, as the outcome of interest. Since the outcome was measured once explanatory variables could not be repeated measures. Therefore, the mean and standard deviation of the natural logarithm of FEC for each cow and calf were calculated and included as explanatory variables. The initial model included year, sex, parity, birth weight, age of calf, mean LCAFEC and standard deviation (SD), mean LCOFEC and SD, and cow weight at weaning.

Included in the models were all explanatory variables, possible two-way interactions and quadratic terms for all continuous variables. First a liberal p-value ($P \geq 0.2$) was used to determine which terms were removed from the model. Different error structures were also tested to look for possible improvement in the model (lowest AIC). Variables with $P > 0.05$ were then removed. All main effects and interactions of interest that were not significant (i.e. $P > 0.05$) at the beginning were placed back in the model at the end to check for a change in significance.

Initial calf egg counts were removed from the LCAFEC model as they were all zero and thus the analysis of variance assumption could not be met. A zero-inflated model was investigated, but as all measurements at the initial time point were zero and there were no zeros at other time points, this model was inappropriate and could not be fit. Since multiple observations were collected from the same cows and calves over the pasture seasons REPEATED statements were investigated in the first two FEC models. This was to account for within cow/calf and field auto-correlation. All error structures were investigated (AR, ARH, TOEP, TOEP (2-6), TOPEH, TOPEH (2-6), UN, and UN (2-6) (SAS/STAT(R) 9.2 User's Guide, Second Edition)). There was less within calf correlation than expected in the LCAFEC model (i.e. correlations between the repeated measures of an individual calf) so an error structure was not included. A REPEATED statement was included to allow for variation in time among

sample times. In the LCOFEC model, a REPEATED statement was included with the autoregressive (AR) error structure. A RANDOM statement was included in all three models to account for clustering within fields.

All models were checked for confounders as variables were removed looking for changes in the coefficients of $\geq 20\%$ and changes to p-values. Residual analysis was completed. Residuals were plotted against predicted outcomes and explanatory variability, to look for non-linearity, homoscedasticity and outliers. To assess normality, histograms of residuals and normal probability plots, as well as four tests offered by SAS 9.4 (Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises and Anderson-Darling) were used. Influential points or outliers were compared to the original data collection records and in the case of entry errors corrections were made. The models were repeated with the influential observations removed and differences in coefficients were noted. Major changes in the coefficients would suggest additional analysis to give reason for removal of the observation.

RESULTS

The cattle were on pasture for a mean average of 154.5 (153 to 156) and 140.0 (138 to 142) days for the 2014 and 2015 pasture seasons, respectively. No influential points, outliers or confounders were removed from any of the models.

Meteorological Data

Though there were slight differences between weather data observed in 2014/2015 and the 30-year averages, none of the meteorological data was statistically significant in the LCAFEC or LCOFES models ($P > 0.05$).

Fecal Egg Counts

At turnout both years, cows and calves had an average baseline FEC of 8.05 ± 13.07 epg and 0 ± 0 epg, respectively. Over the two pasture seasons, there were a total of 268 FEC observations for calves, 44 out of 312 total calf observations (14%) were missing FEC. For cows there were a total of 301 FEC observations, 11 out of 312 total cow observations (3.5%) were missed. Most of these missing FEC were a result of feces collection (i.e. no fecal matter present in the rectum at time of sampling).

Fecal samples in cows had a mean of 8 ± 20.02 epg, ranging from 0 to 191 over both years. Egg counts in cows stayed relatively low and steady throughout both pasture seasons (Table 2). In the final LCOFEC model (Fig. 1) time and cow weight had a significant effect ($P < 0.05$). As cow weight increase one kilogram cow FEC dropped by 0.0074 epg ($P = 0.001$). Cow egg counts peaked slightly after 55 to 72 DOP, at sampling time 4, to 4.03 epg (95% CI 2.57, 6.32). At this timepoint a few individual cows had a high FEC, thus inflating the mean and increasing the standard deviation.

Fecal samples in calves ranged from 0 to 817 epg with an arithmetic mean of 42 ± 86.20 epg (Table 3). There was a large amount of variation in egg counts between individual calves and cows in the herd. A total of 24% (65/268) of fecal samples collected from calves had zero or undetectable levels of GIN eggs. For cows 15% (44/301) has zero or undetectable levels of GIN eggs.

The final model for the LCAFEC (Fig. 2) only included the variable 'sample time' ($P < 0.001$). None of the other explanatory variables, interactions, or quadratics, which were initially included in the multivariable model were significant.

Covariance parameter estimates for LCAFEC showed little variation at the second sampling conducted between 27 May and 11 June (13 to 15 DOP). The majority of the variation was seen in samples taken between 10 June and 9 July (27 to 44 DOP). Calf egg counts peaked at the fourth sample collection, between 8 July and 6 August (55 to 72 DOP). The LCAFEC model showed that the estimates during the fourth sample time were not statistically different from the fifth, sixth, or final sample collection ($P > 0.05$). The estimated FEC for calves at the peak of egg production between 8 July and 6 August was 24 ep_g (95% CI 15.82, 37.2). Time of turnout did not have a significant effect on the outcomes of interest (e.g. LCAFEC, LCOFEC, $P > 0.05$).

Final Weaning Weight Model

The initial calf weights at the beginning of the pasture seasons were 86 ± 16 kg and 72 ± 18 kg for 2014 and 2015, respectively ($n=48$). Turnout weights were statistically different between pasture years ($P = 0.01$). Birth weights were not statistically different between pasture years (40 ± 2 kg in 2014 and 38 ± 2 kg in 2015; $P = 0.15$). At the end of the grazing season, the calves averaged an unadjusted WW of 272 ± 40 kg for 2014 and 244 ± 40 kg for 2015, which were significantly different ($P = 0.021$). Mean LCAFEC did not have a significant effect on WW of calves ($P = 0.90$). Cow parity and calf sex were not significant in the model ($P > 0.05$). Significant fixed effects in the model included birth weight ($P = 0.005$) and cow weight ($P < 0.001$). As birth weight of a calf increased by one kg the expected WW increased 3.14 kg (95% CI 1.14, 5.15). In addition, an increased dam weight by one kg resulted in a slight increase in WW of 0.23 kg (95% CI 0.15, 0.32).

DISCUSSION

Egg counts were zero for all calves at turnout; this was likely a result of absence of prior exposure to the parasite. As mentioned, there was a large amount of variation in the GIN FEC of calves and cows in the study herd. This over-dispersion is typical of GIN in a grazing herd (Stromberg and Gasbarre, 2006). In northern temperate regions, such as Ontario, there are four well defined seasons. The temporal pattern seen is also relatively typical of GIN infections in cows and calves. In the spring, there is resumption of development of larvae observed amongst the cows (Ranjan et al. 1992). With this resumption, calves become infected and start expelling GIN eggs in the feces after only 13 to 15 DOP. There is a rise in egg counts in the spring and into the summer. The significant decrease in cow FEC from sample 1 to 2 was unexpected. This decline may have been due to a change in fecal consistency and volume with diet change (i.e. stored feed to pasture). Thus, the FEC in egg may have declined or increased eggs were excreted with a higher volume of feces. In the months of September and October, the typical start of autumn, a decrease in FEC was seen in cows in this study. However, the calf FEC were not statistically lower during these months. Some work in Quebec on spring born calves has shown egg counts peak later in the fall and then drop off (Ranjan et al. 1992). Similar work shows a constant increase in calf FEC in the autumn in North and South America following weaning (Entrocasso 1988; Slocombe and Curtis 1989; Snyder 1993). During the winter period, there is a break in the cycle as there is no transmission of GIN infections occurring (Gibbs 1988). For this reason, it is typical to see egg counts drop later in the fall as larvae enter a state of hypobiosis due to environmental conditions. If the study was to be continued into the winter months, one would expect to see calf FEC decrease as the cow FEC drops.

Another limitation of this study was that the composition of GIN genera that made up the FEC was unknown. This information is important as prepatent periods and pathogenicity vary

between genera and species. Prepatent periods can range anywhere from two to six weeks (Sutherland and Scott 2010). There is limited genera or species specific research conducted but *O. ostertagi* peak egg production is seen on average 15 d (11 to 19 d) after the adults start producing eggs (Stromberg and Gasbarre 2006). Peak egg production was seen in calves between 55 to 72 DOP in this study. Therefore, the majority of adult GIN would have started producing eggs after 40 to 57 DOP. Based on these data and the fact that anthelmintics target L4 and adult GIN, the best time to apply an anthelmintic to kill as many adults as possible should be between 40 to 57 DOP (late June, early July).

Little work has been done on strategic use of anthelmintics on cow-calf herds in Canada. Studies conducted in the southern and mid-west U.S. show decreased FEC and improved calf performance with strategic use of anthelmintics later in the grazing season treating 90 d before weaning (Hersom et al. 2011) and treating mid-summer (Myers and Hoechst-Roussel 1988; Stromberg and Vathauer 1997). Applying an anthelmintic after cattle have spent 40 to 57 DOP presents a challenge for many cattle producers. Few cow-calf producers in southern Ontario have the labour or infrastructure to complete such a recommendation during the grazing period. Most producers refrain from handling cattle post spring turnout, as many operations are unable to manage the logistics of bringing cattle in from pasture and handling cattle as individuals.

The natural logarithm of calf FEC did not have a significant effect on unadjusted WW in this study. The majority of the literature suggests that increases in egg counts result in a drop in calf performance seen as decreased average daily gain, weight gain, and/or WW (Stromberg and Gasbarre 2006; Sutherland and Scott 2010; Walker et al. 2013). It is possible that this study lacked a sufficiently large sample size to detect a correlation between LCAFEC and growth performance. It is also possible that the level of GIN parasitism found in the calves was not high

enough to hinder the performance of the calves. It has been suggested that grazing calves might not require anthelmintic treatment while they are nursing (Gasbarre 2014).

CONCLUSION

The epidemiological pattern of calf FEC suggests that the later part of June and early July might be the most appropriate time to treat calves in southern Ontario for subclinical GIN infections (between 40 to 57 DOP). The natural logarithm of FEC of the calves did not have a significant affect on unadjusted WW.

No cows or calves in the study had clinical signs of parasitism (e.g. diarrhea, weight loss, pitting edema of lower jaw). To the authors knowledge, there is no published work correlating GIN epg and severity of infection in cows or calves. In addition, reliable thresholds have not been established to determine at what FEC cows or calves should be treated for internal parasitism. This is a clear gap in the literature. Additional work is required to establish an acceptable range for FEC in cows and calves in cow-calf herds pastured in Ontario.

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Table 1. Sample intervals, dates and days on pasture for cow-calf pairs in both 2014 and 2015 pasture seasons

Sample time	Range of days on pasture	Date range		Median days on pasture	Difference (d)
1	1	13 May	29 May	1	
2 ^a	13 to 15	27 May	11 June	14	14
3	27 to 44	10 June	9 July	35.5	21.5
4	55 to 72	8 July	6 August	63.5	28
5	83 to 100	5 August	3 September	91.5	28
6	110 to 128	1 September	1 October	119	27.5
7	140 to 156	1 October	29 October	148	29

^a Observations for sample time 2 were collected only in 2015.

Table 2. Raw arithmetic means and standard deviations of cow fecal GIN egg counts (FEC) expressed as eggs per gram, in naturally infected cows seasonally grazed in southern Ontario; 139 and 162 fecal observations total in 2014 and 2015, respectively

Sample Time	2014	2015
1	10.02 $\pm$ 10.02	6.08 $\pm$ 12.26
2	N/A	2.01 $\pm$ 2.66
3	4.55 $\pm$ 4.61	3.75 $\pm$ 4.96
4	8.79 $\pm$ 18.99	34.67 $\pm$ 51.02
5	5.61 $\pm$ 11.02	17.56 $\pm$ 28.69
6	2.25 $\pm$ 4.80	5.64 $\pm$ 7.74
7	1.20 $\pm$ 1.03	3.39 $\pm$ 6.13

Table 3. Raw arithmetic means and standard deviations of calf fecal GIN egg counts (FEC) expressed as eggs per gram, in naturally infected calves seasonally grazed in southern Ontario; 126 and 142 fecal observations total in 2014 and 2015, respectively

Sample Time	2014	2015
1	0 ±0	0 ±0
2	N/A	0.048 ±0.16
3	89.80 ±114.05	0.72 ±1.01
4	52.87 ±59.33	39.69 ±57.09
5	49.84 ±41.92	58.37 ±66.28
6	36.91 ±50.67	36.34 ±61.99
7	109.26 ±205.86	41.54 ±67.42

Fig. 1. The natural logarithm of cow fecal egg counts (LCOFEC) estimates ($\pm$ SE), with bias correction term of 0.25, over the grazing season in southern Ontario - the LCOFEC estimates with letters that differ are statistically different from each other, $P \leq 0.05$

Fig. 2. The natural logarithm of calf fecal egg counts (LCAFEC) estimates ($\pm$ SE), with bias correction term of 0.25, over the grazing season in southern Ontario - the LCAFEC estimates with letters that differ are statistically different from each other, $P \leq 0.05$