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Estimation of ergovaline intake of cows from grazed perennial ryegrass containing NEA2 or standard endophyte

C.C. EADY¹, J.R. CORKRAN¹, K.M. BAILEY¹, G.A. KERR¹ and A.M. NICOL²
¹*Agriseeds Ltd, 2547 Old West Coast Road RD1, Christchurch, 7671, New Zealand*
²*AMN Consulting, 1248 Old West Coast Road, RD 1, Christchurch 7671, New Zealand*
 ceady@agriseeds.co.nz

Abstract

Ergovaline concentration was measured monthly from December 2015 to April 2016 in herbage of perennial ryegrass pastures containing NEA2 or standard endophyte (SE) in the Waikato, Manawatu and Canterbury. Ergovaline concentrations were then combined with estimated pasture intake, pasture botanical composition and cow liveweight data from experimental dairy farm systems in the Waikato and Canterbury to estimate dairy cow ergovaline intake (mg/kg LW^{0.75}/day). For the diploid ryegrass 'Trojan' with NEA2 estimated ergovaline intake ranged from 0.01 to 0.029 mg/kg LW^{0.75}/day, levels at which no significant animal production effects have been reported. Ergovaline intake of tetraploid ryegrass 'Bealey NEA2' was at least an order of magnitude lower than this. For the diploid ryegrass, 'Bronsyn' with SE, ergovaline intake was 0.016 to 0.056 mg/kg LW^{0.75}/day, which at the highest level carried a ~20% risk of causing animal production effects for 15% of the samples. AR1 and AR37 pastures, used as controls, contained nil or trace amounts of ergovaline.

Keywords: alkaloid, *Epichloë festucae* var. *lolii*, *Lolium*

Introduction

The perennial ryegrass endophyte *Epichloë festucae* var. *lolii* provides the plant protection against insect attack by producing alkaloids, mainly lolitrem B, peramine, ergovaline and epoxy-janthitrems (Popay & Hume 2011). These compounds, apart from peramine, can be associated with detrimental effects on animals at particular levels of intake. Lolitrem B is a neuro-toxin associated with the neuromuscular disorder 'ryegrass staggers' in grazing animals (di Menna *et al.* 2012). Some strains of *Epichloë festucae* var. *lolii* endophyte including AR1 (Fletcher 1999a), AR37 (Hunt & Newman 2005) and NEA2 (Tian *et al.* 2013) produce little or no lolitrem B and are commercially marketed in perennial ryegrass as offering insect protection with reduced likelihood of animal health and production issues.

NEA2 and AR37 produce the alkaloids ergovaline and epoxy-janthitrems, respectively, that can have

detrimental animal affects above certain intake levels. Epoxy-janthitrems are tremorgenic compounds (Finch *et al.* 2012) and AR37 is marketed with the caveat that it can cause ryegrass staggers (<https://www.agricom.co.nz/rd/endophyte-information/ar37>).

Ergovaline plays a role in producing physiological effects in animals similar to those of fescue toxicosis, with a review by Klotz & Nicol (2016) concluding the effects of ergovaline are dose related. Reduced serum prolactin concentration and increased respiration rate have been reported at low ergovaline intake (0.02-0.04 mg/kg LW^{0.75}/day), increased core temperature at moderate intake (0.04-0.06 mg/kg LW^{0.75}/day), and economically important symptoms of reduced DM intake, milk production or liveweight gain usually associated with ergovaline intake above 0.07 mg/kg LW^{0.75}/day. The review concluded that "quantitative data on the ergovaline profiles of novel endophytes in both the grazed and residual portions of the pasture are needed".

This study aimed to measure ergovaline concentration in the grazed component of several perennial ryegrass cultivars containing novel or SE, including the diploid commercially sold as 'Trojan NEA2' and the tetraploid cultivar sold as 'Bealey NEA2'. Ryegrass stubble, below a simulated grazing height (4-5 cm), could not be tested in this study as the National Forage Variety Trial (NFVT) protocol stipulates that grass plots cannot be cut to ground level.

When NEA2 was released it was believed to be a single endophyte strain, but subsequent genotyping showed it is a mix of several endophytes. 'Trojan NEA2', as sold and used in this study, contains two biochemically distinct endophytes (NEA2 and NEA6, both protected by Plant Variety Rights) and each representing approximately 50% of the endophyte content (breeding lines are tested before multiplication). Recently, through the use of additional molecular markers, NEA6 in 'Trojan' has been shown to be two separate endophyte strains (Fletcher *et al.* 2017), however, alkaloid analysis has shown that their profiles are similar (unpublished data). 'Bealey NEA2', as sold, contains two endophytes, approximately 85% of strain NEA2 and 15% NEA6.

Three locations within the two New Zealand mega-

environments for pasture production (Chapman *et al.* 2017a), one in the upper (Waikato) and two in the lower (Manawatu and Canterbury) were chosen. Pasture samples were taken to measure ergovaline concentration over 5 months (November to April) when it is at its highest (Easton 1999). These data were then combined with pasture intake data for lactating dairy cows on experimental dairy farms in Canterbury and the Waikato to give estimates of ergovaline intake. The ergovaline intakes were then compared to those of Nicol & Klotz (2016) to assess the risk of ergovaline induced effects on animal health and production.

Methods

Pasture samples were taken from four National Forage Variety Trial sites; two non-irrigated sites in the Waikato (two adjacent sites at DairyNZ, Scott Farm, Newstead; 37°77'S 175°36'E) sown in 2014, a non-irrigated Manawatu site (Massey University, Number 4 Dairy Farm; 40°24'S 175°36'E), and an irrigated site in Canterbury (Rolleston Dairy Farm; 43°57'S 172°31'E), both sown in 2015. At Scott Farm, diploid and tetraploid ryegrass cultivars were established in separate but adjacent sites. 'Base AR37' provided a non-ergovaline control for the tetraploid site. All sites had four replicates in a row by column design, and were managed to broadly simulate rotational grazing under high nutrient supply as described by the NZPBRA (<http://www.nzpbra.org/forage-trials/>). Sites were maintained as ryegrass monocultures with herbicides applied to control grass and broadleaf weeds. The cultivar x endophyte combinations sampled, and endophyte percentage of seed sown at the sites are presented in Table 1.

Samples were taken from three replicate plots for each cultivar x endophyte combination at each site in December 2015, January, February, March, and April 2016. Pre-graze plots (2500-3000 kg DM/ha) were cut to a height of 4-5 cm with a mower; six subsamples from the catcher were removed and after mixing, a

~200g fresh weight (FW) of this herbage was placed in a plastic bag, sealed, and placed on dry ice. Samples were stored frozen (<-18 °C) before freeze-drying. Freeze-dried samples were chopped, mixed and ground (<1 mm sieve) on a Cyclone Sample Mill (Thomas Scientific, 1654 High Hill Rd, Swedesboro, NJ 08085, USA) and stored in sealed vials at 2°C and 20% relative humidity.

Ergovaline analysis was undertaken by the AgriBio Science Centre, 5 Ring Rd, Bundoora VIC 3083, Australia (unpublished method). For cross-checking, a subset of 30 replicate samples was analysed for ergovaline concentration by AgResearch Ltd, Palmerston North, New Zealand (Moore *et al.* 2015).

Estimated cow dry matter intake of pasture and supplement was obtained from Waikato (Newstead) and Canterbury (Lincoln) DairyNZ P21 experimental farms (<https://www.dairynz.co.nz/about-us/research/pastoral-21/>) from pre- and post-grazing pasture mass assessment by calibrated plate meter (www.dairynz.co.nz). Both sites ran two farm systems, a 'low input high efficiency' system (LSE) and a 'high input high efficiency' system (HSE) in Canterbury, and a 'current' and 'future' system in Waikato as described in Chapman *et al.* (2017b). Pasture composition was assessed monthly by sampling herbage before dissecting a subsample into component species, drying and weighing. Cow liveweight and milksolids production were also recorded and converted to monthly mean values. The average estimated ryegrass dry matter intake for each month was combined with cow liveweight and the herbage ergovaline concentration (mg/kgDM) to calculate ergovaline intake of cows (mg/kg LW^{0.75}/day).

Ergovaline concentrations were statistically analysed with a linear mixed model (fitted by REML) using Genstat 18 (VSN International 2015) which accounts for the unbalanced occurrence of cultivars in sites and the pooled samples (for the Canterbury December sampling, the 3 initial plots for each treatment were accidentally pooled before being analysed). The data were log-transformed, and separate variances for each site were fitted along with strata for replicates, plots and harvest dates within plots. The fixed model was (site x cultivar x time) (all site by cultivar by time combinations) and the random model was site/replicates/plot/time (time periods nested in plots, nested in replicates, nested in trials). Samples with no detectable ergovaline had half of the minimum detectable level substituted for their value to enable the data to be logged. Where all observations of a cultivar or site had no detectable alkaloid, that entry was removed from the analysis so that the standard errors were not biased downwards. Ergovaline concentration data from different laboratories were assessed by regression analysis.

Table 1 Regions, ryegrass x endophyte combinations sampled, and the endophyte percentage of the seed sown. 'Base AR37' was the non-ergovaline control for the tetraploid site at Scott Farm, Newstead.

Cultivar/endophyte	Waikato	Manawatu	Canterbury
'Trojan NEA2'	88	88	88
'Bealey NEA2*'	90*	86	86
'Bronsyn SE'	98	98	98
'Trojan AR1'	99	98	98
'Base AR37*'	not tested		

* tetraploid site at Newstead, Waikato.

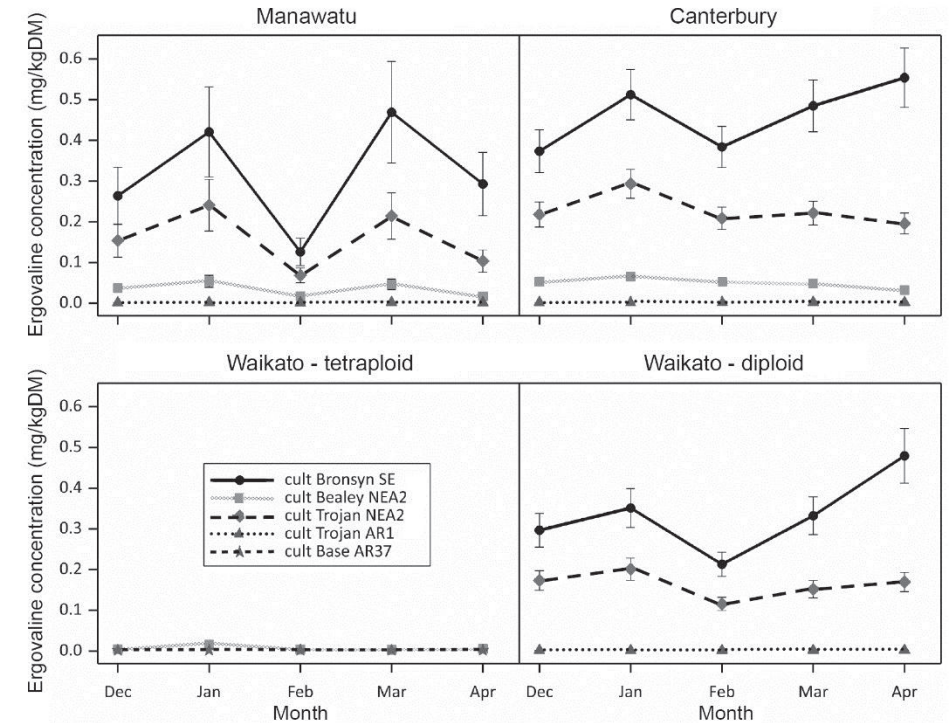


Figure 1 Mean ergovaline concentration (mg/kg DM) of the cultivar x endophyte combinations in the grazed zone (above 4-5 cm) from December 2015 to April 2016 at the four sites. LSD_{5%} given as bars.

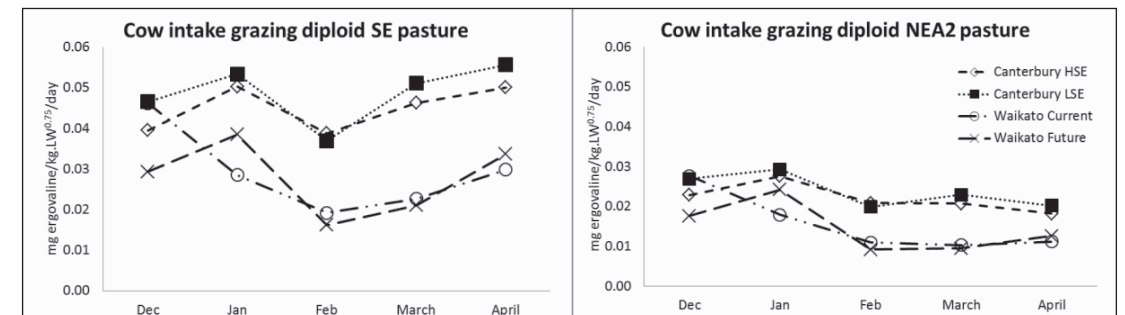


Figure 2 Estimated ergovaline intake of dairy cows (mg/kg LW^{0.75}/day) for the diploid SE ryegrass (left) versus diploid NEA2 ryegrass (right), based on data from four dairy farm systems.

Results

Regression analysis of the ergovaline concentrations from the 30 paired samples analysed by AgriBio Science Centre and AgResearch showed a high level of agreement with the constant not significantly different from 0, the slope not significantly different from 1, and an overall 95.5% correlation (data not shown).

Over all sites and months the mean ergovaline concentration in diploid 'Bronsyn SE' was 0.21 mg/kg DM, higher than diploid 'Trojan NEA2' (0.11 mg/kg DM), which in turn was higher than in the tetraploid 'Bealey NEA2' (0.02 mg/kg DM) (P<0.05 in all cases). Ergovaline concentration varied over time, and was

lower in February than in January or March (P<0.05). There were a small but significant (P<0.01) cultivar x site and cultivar x time interaction but these were only 5% of the variation due to the main effects, and did not change the rankings between the cultivars, although there were small changes in the relative differences (Figure 1).

The estimated ergovaline intake of cows (mg/kg LW^{0.75}/day) for the four associated farm systems were calculated (Appendix), with intake of diploid SE and diploid NEA2 cultivars presented in Figure 2. For SE the estimated ergovaline intake for cows ranged from 0.016 - 0.046 mg/kg LW^{0.75}/day for the Waikato,

and 0.037 - 0.056 for Canterbury. The diploid NEA2 ergovaline intake ranged from 0.009 - 0.029 mg/kg LW^{0.75}/day. For tetraploid NEA2 and control cultivars the intakes were negligible (below 5 x 10⁻³).

Discussion

Ergovaline concentration in herbage

The ergovaline concentrations in the pasture above the 4-5 cm cutting height, representing the grazed zone of ryegrass (Figure 1) are consistent with those of Lane *et al.* (1997), who reported ~0.25 mg/kg DM for leaf, and Spiering *et al.* (2002, 2005) who reported 0-0.15 mg/kg DM in leaves.

Ergovaline concentration did not follow the expected seasonal pattern of rising through summer before falling in late autumn (Fletcher *et al.* 2000; Thom *et al.* 2013) for any of the sites, although levels at the Manawatu site did drop in April (Figure 1). Ergovaline concentration in Canterbury and Waikato pastures did not drop in March or April, possibly in response to February being the second warmest on record in New Zealand (NIWA 2017). All sites recorded a dip in ergovaline concentration in February. It was assumed that the warmer North Island sites would have higher ergovaline concentrations (Fletcher *et al.* 1999b), however, the Canterbury site showed the highest values.

Most commonly, ergovaline concentration has been reported from harvesting pasture to ground level, as this provides a consistent cutting height for sampling. However, as ergovaline concentration increases towards the base of SE containing ryegrass plants (Spiering *et al.* 2005), with up to a 10-fold greater concentration in the plant crown (Lane *et al.* 1997), harvesting to ground level is likely to result in an overestimate of ergovaline intake (mg/kg LW^{0.75}/day) by stock. In this work, pastures were sampled to an estimated 'grazed horizon', using recommended pre- and post-graze levels (e.g. DairyNZ 2017a; DairyNZ 2017b), to provide more appropriate levels of ergovaline concentration (mg/kg DM) in the herbage ingested by grazing animals.

The high level of agreement in the analysis of paired samples between two laboratories adds confidence in the ergovaline concentration data reported by other authors in New Zealand.

Ergovaline intake by cows

This study provides a framework for estimating ergovaline intake by grazing animals, and the likelihood of any adverse effects on them.

The estimated ergovaline intakes of cows in all the NEA2-based systems (Figure 2) were less than 0.03 mg/kg LW^{0.75}/day, a level below which no significant effects on animal production or liveweight gain have been reported (Nicol & Klotz 2016). This study suggests that the ergovaline intake of dairy cows from

well managed NEA2-based ryegrass pasture is unlikely to affect animal health or production.

For SE pastures the intake range was 0.037 - 0.056 mg/kg LW^{0.75}/day in Canterbury, higher than the 0.016-0.046 mg/kg LW^{0.75}/day in Waikato. The highest ergovaline intakes correspond to a 20% chance of animal production effects in Canterbury in January, March and April, and a 15% chance of ergovaline effects in the Waikato in January according to the criteria used by Nicol & Klotz (2016).

In practice, the ergovaline intake from ryegrass by dairy cows is often diluted by the mixed botanical composition of pasture, feeding of summer crops such as chicory or turnips, and the supply of supplements such as grain or silage. A major difference in the DairyNZ P21 farms was their pasture botanical composition, with an average ryegrass content of 79.8% in Canterbury and only 54.8% in the Waikato, throughout the sampling period. The lower levels in the Waikato were mainly due to the presence of C4 summer grasses known to be well adapted to the Waikato climate (Field & Forde 1990) and not hosts of Epichloë-type endophytes (Clay 1990).

While it has been suggested that ergovaline effects might be more likely in northern New Zealand due to climate induced elevated ergovaline concentrations (Fletcher *et al.* 1999b), this study indicates that this is not necessarily the case. Overall ryegrass ergovaline concentrations were lower in the Waikato than Canterbury, and the lower ryegrass content in Waikato pastures and more common feeding of summer crops and supplements may naturally mitigate ergovaline intake, by reducing the level of ryegrass intake.

Whether ergovaline intake (mg/kg LW^{0.75}/day) would change if animals grazed below the 4-5 cm sampling height used in this study cannot be determined. While ergovaline concentration are higher in the base of a pasture than the grazed region (Spiering *et al.* 2005, Lane *et al.* 1997), this is usually counter-balanced by a reduced DM intake as animals graze closer to the ground as a result of decreased bite size and intake rate (Cosgrove & Edwards 2007).

The ergovaline intake from NEA2 pastures was below the 0.475 mg/kg DM (or 0.045 mg/kg LW^{0.75}/day) threshold level for fescue induced ergovaline effects suggested by Stamm *et al.* (1994) and Bohnert & Merrill (2006), respectively. Also, Lane *et al.* (1997) suggested that fescue and ryegrass ergot profiles are different with symptoms caused by ergovaline in fescue seemingly much stronger.

In the Nicol & Klotz (2016) review only four papers reported negative effects of ergovaline intake on production (liveweight gain or milk yield) when intake was below 0.07 mg/kg LW^{0.75}/day, all of which involved SE, and thus could not be separated from the

possible effect of the associated lolitrem B. The lolitrem B concentration of NEA2 containing ryegrass in this simulated grazed zone was below the threshold for detection (~0.05 ppm), so NEA2, will likely avoid any possible additive effects of these alkaloids as suggested by Bluett *et al.* (2001).

Conclusion

To date there is no perfect endophyte commercially available, and farmers and plant breeders must balance the advantages of insect control and plant persistence of different endophyte strains, with potential disadvantages of animal welfare and production issues.

This study provided a framework for estimating ergovaline intake by stock, and suggests that the ergovaline intake of dairy cows from well managed NEA2-based ryegrass pastures is unlikely to affect animal health or production.

ACKNOWLEDGEMENTS

Chris Roach and Anna Clement of DairyNZ for providing the P21 farm systems data. David Baird for statistical analysis. DairyNZ for assistance in funding this work.

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Appendix Calculation of estimated ergovaline intake of dairy cows on four farmlets

Canterbury (top) and Waikato (bottom) experimental farm systems. Data for: cow weight, milksolids production, pasture intake, pasture composition, with calculated (shaded) ergovaline levels and ergovaline intake (mg/kg LW^{0.75}/day) for diploid SE and diploid NEA2 ryegrass x endophyte combinations.

Canterbury	High SE						Low SE					
	Dec	Jan	Feb	Mar.	April	Av.	Dec	Jan	Feb	Mar.	April	Av.
Av. Cow weight (kg)	502	506	511	519	524	512	509	519	518	529	542	523
Mean daily milksolids (kg)	2.0	1.8	1.7	1.7	1.5	1.7	2.0	1.9	1.8	1.7	1.5	1.8
Total intake (kg DM/day)	17.0	16.0	18.3	18.9	17.0	17.4	16.7	15.7	17.1	17.5	16.4	16.7
Silage (kg DM/day)	1.1	0.5	2.2	3.4	2.4	1.9	0.0	0.0	1.3	1.0	2.3	0.9
Grain (kg DM/day)	2.7	2.6	3.4	3.7	3.5	3.2	0.5	0.1	0.2	0.3	0.5	0.3
Pasture intake (kg DM/day)	13.2	13.0	12.8	11.8	11.2	12.4	16.2	15.6	15.6	16.2	13.7	15.5
Clover (%)	12.5	15.0	12.5	9.0	7.0	11.2	15.0	23.0	30.0	25.0	13.0	21.2
Other (%)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Ryegrass (%)	83.5	81.0	83.5	87.0	89.0	84.8	81.0	73.0	66.0	71.0	83.0	74.8
NEA2 ergovaline (mg/kg DM)	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2
NEA2 ergovaline (mg/cow/day)	2.4	2.9	2.2	2.3	2.0	2.4	2.9	3.2	2.2	2.5	2.3	2.6
SE ergovaline (mg/kg DM)	0.4	0.5	0.4	0.5	0.6	0.5	0.4	0.5	0.4	0.5	0.6	0.5
SE ergovaline (mg/cow/day)	4.2	5.4	4.2	5.0	5.5	4.8	5.0	5.8	4.0	5.6	6.3	5.3
Cow LW ^{0.75}	106.1	106.7	107.5	108.7	109.5	107.7	107.2	108.7	108.6	110.3	112.3	109.4
NEA2 Ergovaline intake (mg/cow/kg LW ^{0.75} /day)	0.023	0.028	0.021	0.021	0.018	0.022	0.027	0.029	0.020	0.023	0.020	0.024
SE Ergovaline intake (mg/cow/kg LW ^{0.75} /day)	0.039	0.050	0.039	0.046	0.050	0.045	0.047	0.053	0.037	0.051	0.056	0.049
Waikato	Current						Future					
Av. Cow weight (kg)	475	481	483	496	508	489	501	492	480	483	486	488
Mean daily milksolids (kg)	1.6	1.3	1.1	0.9	0.7	1.1	1.8	1.5	1.1	0.9	0.8	1.2
Total intake (kg DM/day)	16.9	15.9	22.0	20.1	15.8	18.1	16.5	17.8	22.7	18.9	15.6	18.3
Silage (kg DM/day)	0.0	0.0	2.0	2.9	3.1	1.6	0.0	0.0	1.9	2.9	2.4	1.4
Grain (kg DM/day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pasture intake (kg DM/day)	16.9	15.9	20.0	17.2	12.7	16.5	16.5	17.8	20.8	16.0	13.2	16.9
Clover (%)	5.1	13.5	9.1	3.7	7.5	7.8	23.9	13.5	4.3	4.7	9.9	11.3
Other (%)	2.2	33.9	43.7	54.3	40.1	34.8	13.3	21.9	57.7	54.3	35.1	36.5
Ryegrass (%)	92.7	52.6	47.2	42.0	52.4	57.4	62.8	64.6	38.0	41.0	55.0	52.3
NEA2 ergovaline (mg/kg DM)	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2
NEA2 ergovaline (mg/cow/day)	2.8	1.8	1.1	1.1	1.2	1.6	1.9	2.5	0.9	1.0	1.3	1.5
SE ergovaline (mg/kg DM)	0.3	0.4	0.2	0.3	0.5	0.3	0.3	0.4	0.2	0.3	0.5	0.3
SE ergovaline (mg/cow/day)	4.7	2.9	2.0	2.4	3.2	3.0	3.1	4.0	1.7	2.2	3.5	2.9
Cow LW ^{0.75}	101.7	102.7	103.0	105.1	107.0	103.9	105.9	104.5	102.5	103.0	103.5	103.9
NEA2 Ergovaline intake (mg/cow/kg LW ^{0.75} /day)	0.028	0.018	0.011	0.010	0.011	0.016	0.018	0.024	0.009	0.010	0.013	0.015
SE Ergovaline intake (mg/cow/kg LW ^{0.75} /day)	0.046	0.028	0.019	0.023	0.030	0.029	0.029	0.039	0.016	0.021	0.034	0.028