# Effect of sample handling and storage on ergovaline concentration in fresh tall fescue samples

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## Introduction

Ergovaline is an ergot alkaloid produced by an endophyte (*Neotyphodium coenophialum* Morgan-Jones and Gams), found in tall fescue (*Schedonorus arundinacea=Festuca arundinacea* Schreb.) and causes a range of disorders across livestock species. The concentration of ergovaline within a plant can vary by variety, management, time of year, location and weather conditions (Smith *et al.* 2009). Due to the significant economic impact of fescue toxicity in livestock, many samples are tested every year in research and diagnostic laboratories. Ergovaline is known to be unstable and affected by many variables; therefore sample handling is critical to obtaining accurate and consistent results. Currently, a number of laboratories in the United States can perform ergovaline analysis, but there is little information in the literature on how these results are affected by sample handling from the pasture to the laboratory or during storage of samples at the laboratory.

## Methods

A horse farm in central Kentucky (USA) provided a pasture for our sampling. This pasture had been sampled for over 4 years and had shown higher than average ergovaline concentrations. On day 0, approximately 0.9 kg of tall fescue was collected, placed on ice and transported to the UK Veterinary Diagnostic Laboratory (VDL). The material was cut to 5 cm lengths, frozen in liquid nitrogen and milled using a Stein Mill (Steinlite Corporation, Atchinson, KS, USA). The sample was mixed thoroughly before being divided into 42 individual whirl-pak bags and randomly assigned to 1 of 14 treatments (Table 1) with 3 replicates assigned to each treatment. The treatments were analysed for ergovaline at the assigned date and adjusted for moisture content. Each sample was extracted for 1 hour using a 50% aqueous 2-propanol solution that is 1% lactic acid and analysed using reverse phase HPLC with flurescence detection. Fresh standards were prepared daily and ergotomine was used as an internal standard. Ergovaline values are reported as the summed peak areas for ergovaline and ergovalinine on dry matter basis. Ergovaline and ergovalinine are stereoisomers (Fig. 1) that are often present in extract solutions. Degree of epimerization from ergovaline to ergovalinine depends on many variables including type of extraction solvent, amount of light and heat and time in solution (Smith *et al.* 2009). This extraction method was developed from similar published works (Spiering *et al.* 2002).

**Table 1. Storage method, time and temperature for each treatment.**

| Treatment # | Storage Method | Time in Storage | Temperature |
|---|---|---|---|
| 1 | Control | 2 hours | -20°C |
| 2 | Ice | 2 hours | 1°C |
| 3 | Light + Heat | 2 hours | 38.5± 1°C |
| 4 | Ambient temp. | 1 day | 21.8± 0.4°C |
| 5 | Ambient temp. | 3 days | 21.8± 0.4°C |
| 6 | Ambient temp. | 5 days | 21.8± 0.4°C |
| 7 | Refrigerator | 1 day | 5°C |
| 8 | Refrigerator | 3 days | 5°C |
| 9 | Refrigerator | 6 days | 5°C |
| 10 | Freezer | 1 day | -20°C |
| 11 | Freezer | 7 days | -20°C |
| 12 | Freezer | 14 days | -20°C |
| 13 | Freezer | 21 days | -20°C |
| 14 | Freezer | 28 days | -20°C |

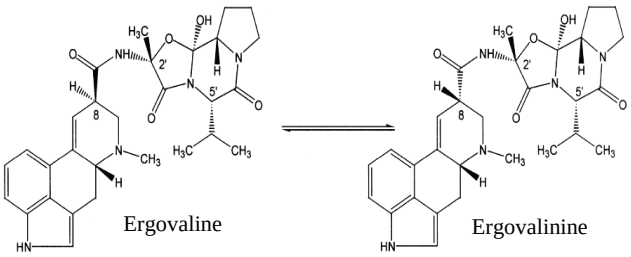


**Figure 1. Ergovaline and Ergovalinine are stereoisomers.**

## Results

A date by treatment interaction was determined therefore results were separated by date. For both May and August, the ice treatment was not different from the control and considered the ideal method of transport (data not shown). When the plant samples were stored at ambient temperature, there was a significant reduction in ergovaline content after one day regardless of harvest date (Fig. 2a,b).

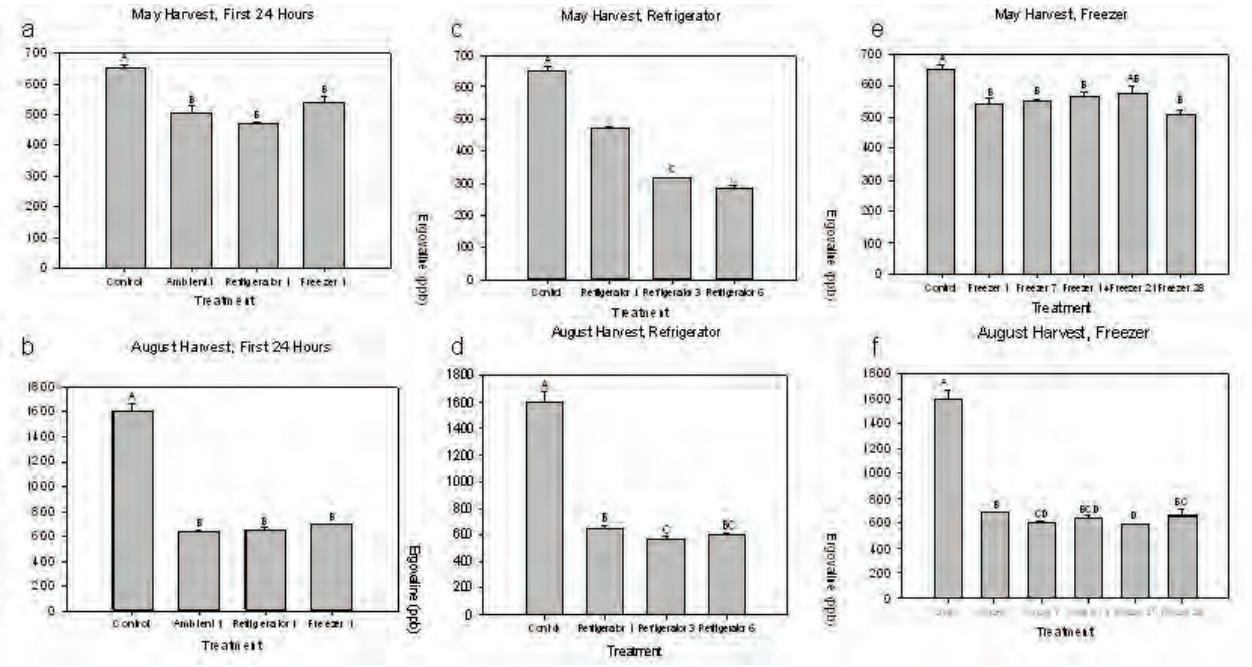


**Figure 2. a-f, Effect of treatments on ergovaline concentration (superscripts are differences at *P*<0.05).**

A similar loss of ergovaline was observed after a day of refrigerated (5°C) storage. In May, significant ergovaline was lost in the refrigerator compared to the control on days 1 and 3. Day 3 and 6 were not different from each other (Fig. 2b). In August, Day 6 was not different from days 1 and 3, but was different from the control (Fig. 2d). Finally, samples in May stored in the freezer were different from the control at all dates except day 21, which was not different from any other freezer treatment or the control (Fig. 2e). In August the control was different from all freezer treatments, but there was not a progressive reduction in ergovaline by storage time as expected (Fig. 2f). These results suggest that a significant fraction of ergovaline is lost in the first 24 hours after harvest regardless of storage method (Fig. 2a,b).

## Conclusion

Based on this research, optimal results for ergovaline concentration in fresh tall fescue samples will be obtained by analysing samples the same day as harvest. When this is not possible, samples should be immediately stored in a freezer until analysis. Further testing is planned to confirm these results.

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