The difficulties in reviewing ergovaline

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Abstract

The endophytic alkaloid, ergovaline, is a secondary metabolite of a number of endophytes associated with perennial ryegrass. Ergovaline is known to protect ryegrass against attack by a range of insect pests, but can also negatively affect domestic ruminants. We have recently reviewed the physiological responses and metabolism of ergovaline and the effect of its consumption on animal production. The main focus of this paper is on the difficulties encountered in preparing these reviews. These include the lack of a cost-effective source of ergovaline to allow for robust dose-dependent trials resulting in ergovaline treatments based on seed sources presented in a range of processed forms, or as ryegrass pasture in which ergovaline is often associated with other alkaloids. Intake of ergovaline is often difficult to predict because ergovaline concentration of the diet on offer may not represent the concentration in the diet consumed. Furthermore, the mean live weight and level of production of the experimental animals is not always available to allow a prediction of ergovaline intake. Any change in gut-fill in response to ergovaline consumption needs to be accounted for when assessing any impact on body weight. Experimental objectives need to be clear, as the effects of short-term ergovaline exposure can be masked in a production system by subsequent compensatory growth/production. By identifying these difficulties, we hope that future work on ergovaline may be more consistent and of greater value to further reviews.

Introduction

Endophytic fungi are associated with many plant species. Of agricultural significance in New Zealand is *Neotyphodium lolii,* found in natural selections of perennial ryegrass (*Lolium perenne*). The main endophytic alkaloids produced as secondary metabolites by *N. lolii* are lolitrem B, ergovaline and peramine (di Menna et al. 2012). Although these confer protection against animal and insect herbivory and enhance the survival of ryegrass tillers (Popay & Hume, 2011), lolitrem B and ergovaline pose a potential risk to animal health, welfare and production. Lolitrem B is present in much higher concentrations than ergovaline in the native, 'wild-type', 'standard' strain of *N. lolii* and the 'ryegrass staggers' associated with intake of lolitrem B is well documented (Fletcher & Harvey 1981). However, the commercial availability of 'novel' strains of *N. lolii* with different alkaloid profiles compared with standard endophyte, has increased interest in the impact of ergovaline *per se* to New Zealand animal production.

In the process of reviewing the physiological responses and metabolism of ergovaline and the effect of its consumption on animal production, we encountered problems which limited the completeness of the review and the robustness of the conclusions. The aim of this paper is to document these difficulties in the hope that future work on ergovaline may avoid some of the problems and address the unknowns and thus better contribute to our understanding of its importance to New Zealand farmers.

Basic requirements

There are three basic requirements to consider when assessing the impacts of any substance consumed by animals. These are daily intake, physiological action and animal response. We encountered difficulties and large amounts of variation in assembling published information in all of these areas. Ergovaline intake is the product of daily dry matter (DM) intake and the ergovaline concentration of the dry matter consumed. The action of ergovaline depends on its bioavailability and physiological effects of the molecule and any animal response needs to be assessed in terms of animal welfare and production.

Intake of ergovaline: a function of DM intake and ergovaline concentration

DM intake

DM intake can be accurately recorded in pen-feeding trials and can be predicted using digesta markers in grazing experiments (Bluett et al., 2001), although such estimates are subject to large prediction errors (±15-20%) and are costly. Most frequently we found daily DM intake had to be estimated from published energy requirements based on mean live weight and level of production (milk production and/or live weight gain) and an assumption of the metabolisable energy (ME) concentration of the pasture consumed (Nicol & Brookes 2007). Some field trials failed to record the data needed for such estimation (e.g., Clark et al. 1996). This approach to estimating DM intake is satisfactory for short-term relatively 'steady-state' trials but is unrealistic for longer-term studies in which animal parameters vary widely (e.g., Eerens et al. 1994).

Ergovaline concentration

The second essential figure needed to estimate ergovaline intake is the ergovaline concentration of the diet consumed. The ergovaline concentration in ryegrass varies widely (from <0.2 to >1.5 mg/kg DM) within year (Figure 1), between years and most importantly within a plant as leaves contain a much lower concentration of ergovaline than pseudostem and stems (Figure 2).

Figure 1 Seasonal pattern of ergovaline concentration in total herbage samples of ryegrass containing two endophyte strains (from Fletcher et al. 2000)

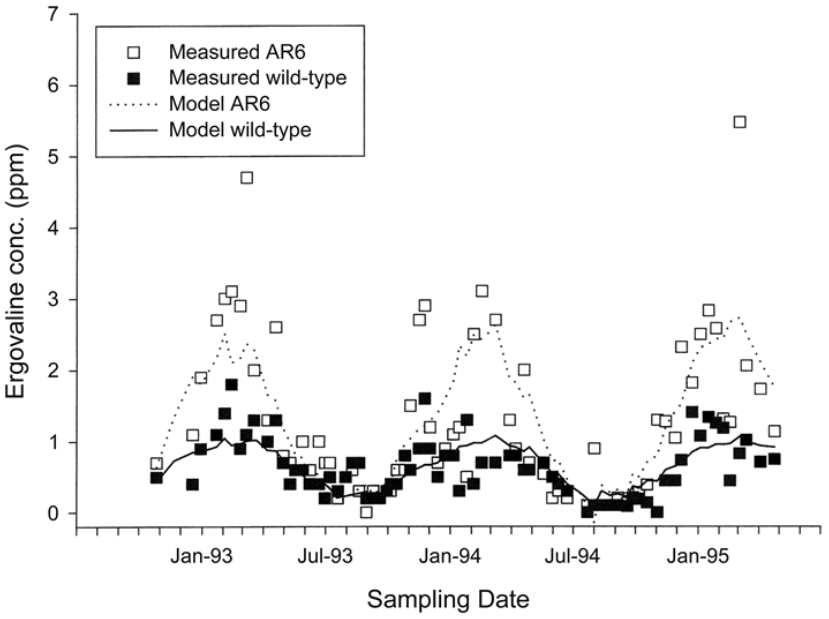
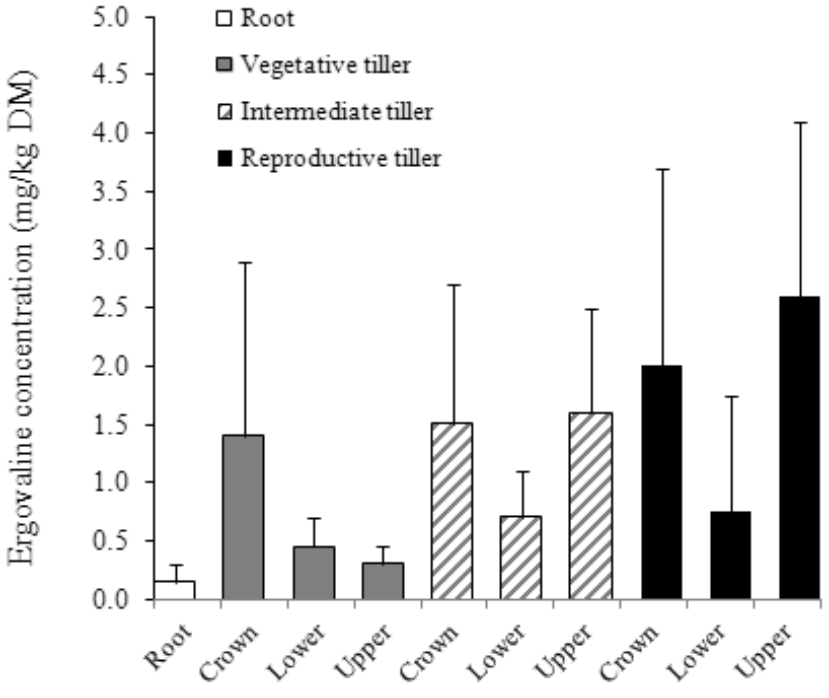


Figure 2 Ergovaline concentration of parts of individual ryegrass plants (from Lane et al. 1997)



Individual tillers within the same plant can also vary widely in ergovaline concentration (Mace et al. 2014). The implications of these variations across-year and within-plant are that a mean ergovaline concentration may represent a wide range within an experimental period (e.g., Bluett et al. 2005). Of greater concern is that the ergovaline concentration of pasture is typically assessed by sampling to ground level, which is likely to over-estimate ergovaline concentration in the pasture consumed by the animal. The ergovaline concentration and pasture mass (kg DM/ha) of both pre- and post-grazing pasture is required to estimate the ergovaline concentration of the diet consumed.

Another issue in determining the intake ergovaline is whether the reported ergovaline concentration is ergovaline alone, includes other ergovaline derivatives, or ignores the other derivatives and attributes all animal responses to ergovaline. Some simpler laboratory analyses only detect common structural components (e.g., the tetracyclic ergoline ring) and only provide a measure of total ergot alkaloids (e.g., Realini et al. 2005). Conversely, Rowan & Shaw (1987) reported that ergovaline made up 40% of the ergot alkaloids detected in perennial ryegrass with ergotamine, ergosine, ergoptine, ergocryptine, and ergocornine making up the majority of the remainder. These percentages will likely vary in the make-up of the total alkaloid content present depending on cultivar, environment and time-of-year interactions. As ergovaline is the only ergot alkaloid that has been consistently evaluated and reported in New Zealand literature, not assessing the concentrations of the other ergot alkaloids present in perennial ryegrass will overestimate the animal response associated with intake of ergovaline.

Physiological Action of Ergovaline

Bioavailability

Even when the actual animal intake of ergovaline is known, there are sources of variation in ergovaline bioavailability which can limit its physiological action. There are currently no data describing the bioavailability of ergovaline from ryegrass leaf or seedhead tissues. In tall fescue, Goff et al. (2012) demonstrated that the maturity of level of the plant tissue affects ergovaline bioavailability. In addition to plant maturity, routes of administration (e.g., feeding compared to intravenous infusion), and form of administration (e.g., ground compared to whole seed) will also affect ergovaline bioavailability. As researchers attempt to control the variation in ergovaline intake mentioned above and maximise bioavailability, the route of ergovaline administration ranges from a grazing

treatment, a top-dressed bunk-fed treatment, a direct dose to the gastrointestinal tract (administered through a ruminal cannula), to intravenous or intraperitoneal administration of ergovaline. Even within studies that used direct ruminal dosing, variable ergovaline bioavailability was likely as studies used either whole seed (Siegel et al. 1984), ground seed (Koontz et al. 2013), or a seed extract (Foote et al. 2013). As the level of experimental control increases, so does the departure from a realistic application of ergovaline in terms of a grazing animal.

Biotransformation of ergovaline by rumen or gut microbes could create a difference between the intake of ergovaline and the amount actually available for absorption by the animal. There are surprisingly few data available on this aspect, but it could be an additional source of variation among animals, and especially experiments where different diets and treatments create different ruminal environments (e.g., pH levels) and consequently alter the microbial populations such that the ability to metabolise or conjugate ergovaline may change.

The exact mechanisms by which a molecule of ergovaline arrives intact into the body and cause a response are not fully elucidated. There are uncertainties about the percent of available ergovaline that is actually absorbed, the percent of absorbed ergovaline that is detoxified by liver, the percent of absorbed ergovaline that is excreted via the hepatic biliary system, and the remaining percentage of ergovaline that is available to cause a physiological effect and ultimately an animal response. This is further complicated by the possibility that ergovaline could bioaccumulate in tissues (Klotz et al. 2009) and far exceed the plasma half-life (e.g., - Jaussaud et al. 1998). This would result in variable recovery of ergovaline molecules, as those residing in tissue depots are gradually turned over or released (Aiken et al. 2013).

Physiological effects

The physiological effects of ergovaline are increasingly well defined, but not necessarily well understood. Ergot alkaloids like ergovaline have the ability to bind various biogenic amine receptors and the ability to assume roles as full agonists, partial agonists, and antagonists (Eckert et al. 1978). This results in a diverse array of physiological effects such as vasoconstriction and disruptions, in gastrointestinal activity, and endocrine action that may all impact livestock production (Strickland et al. 2011). The diversity of receptor interactions, functional roles, and consequential effects of ergovaline create the potential for diversity in measured animal responses. The level of animal-to-animal variation reported also suggests that there is still an unknown influence (e.g. – a U.S. review of tall fescue studies (Paterson et al., 1995) reported a range of 30 to 100% reduction of live weight gain in steers). This could be a consequence of genetic effects increasing an animal's tolerance or sensitivity to ergovaline (indicating a potential for selection of 'tolerant' animals?). Previous damage to hepatic tissue by conditions such as facial eczema or liver flukes could decrease an individual's ability to metabolise and excrete ergovaline. This could all culminate with an interaction of the effects of ergovaline with adverse environmental conditions. Because of the potential for this interaction, the environmental conditions reported in literature (if reported) are another aspect that makes interpreting the effects of ergovaline challenging.

In addition to the possibility of environmental interactions, there is the potential for synergy or antagonism of ergovaline effects with effects of other alkaloids (Bluett et al. 2001). A major challenge in reviewing ergovaline and its given effects is its association and similar seasonal profile with lolitrem B in most New Zealand studies. These studies do not eliminate the possibility of an interaction with the predominant lolitrem B with ergovaline. A further limitation in clearly identifying ergovaline effects *per se* was the poor documentation of the alkaloid profiles of 'novel' endophytes such as that marketed as 'NEA2'.

Animal responses

A review of the animal responses to ergovaline consumption identifies a number of potential effects which include; decreased circulatory prolactin concentration, increased respiration rate, and increased rectal temperature. These changes are exaggerated at higher environmental temperature (Hannah et al. 1990) and are dose- dependent (Leyton et al. 2004). These physiological responses are not inevitably associated with a reduction in animal production (live weight gain, milk production and reproductive rate) which are only observed at greater ergovaline intake.

There were difficulties in assessing the economic significance of some animal production responses. For example, a reduction in gut fill has been suggested to be responsible for most of the lower 'live weight gain' associated with ergovaline consumption (Table 1, from Emile et al. 2000), but on the other hand rumen contents have been shown to increase in ergovaline-treated groups (Koontz et al. 2013).

Table 1 The liveweight gain of heifers during (days 1-98) and after (days 98-147) ergovaline-containing (+E) or ergovaline-free (-E) hay (from Emile et al. 2000)

Main diet		Tall fescue hay	
	Day	+E	-E
Liveweight (kg)	1	294	289
LWG (kg/day)	1-98	0.94	0.99
Live weight (kg)	98	380	388
Main diet		Maize silage	
LWG (kg/day)	98-147	1.06	0.88
Live weight (kg)	147	432	431

(9-month-old Friesian heifers , n=10 per group)

The length of the trial period and the timing of ergovaline exposure can have an important impact on the experimental results. There is considerable evidence for compensatory growth and/or milk production in animals following their reduction due to exposure to ergovaline

(Emile et al. 2000; Thom et al. 2013). Thus trials which conclude at the end of ergovaline exposure will identify greater effects than those of a more 'system' type which include a period of ergovaline exposure in a longer term experiment. There is also a suggestion that the animal response to ergovaline consumption may be less with a higher quality diet (ME concentration) compared to lower quality diets (Layton et al. 2004), but this requires confirmation.

The bulk of the work reviewed compares positive and negative ergovaline treatments and while results from these may or may not demonstrate differences, they do little to help establish the 'threshold' ergovaline intake responsible for its various effects. More dose-response experiments are required to better define critical intake levels of ergovaline.

Recommendations for future work elucidating the importance of ergovaline to New Zealand farmers

In view of the difficulties we encountered in reviewing the impact of ergovaline we make the following suggestions for future work in the area.

Where DM intake cannot be measured directly, mean live weight and level of animal production need to be recorded so DM intake can be estimated. Feed-sampling protocols must allow for an estimate of the ergovaline concentration of the diet *consumed.*

The definition of 'ergovaline concentration' used in an experiment must be clear. An assessment of other ergot alkaloids present in ryegrass is warranted given ergovaline does not make up 100% of the bioactive ergopeptine alkaloids. There is a need to calibrate the physiological dose of ergovaline supplied by various routes.

More dose-response trials are required to better establish 'threshold' intakes for physiological and animal production responses. This particularly applies to heat stress responses, changes in upper critical temperature etc. An opportunity exists to select animals for tolerance to ergovaline consumption (standard dose).

Future work should aim to avoid any confounding of ergovaline consumption with that of other secondary plant metabolites. More research on the involvement of the gut microbiome on ergovaline bioavailability and biotransformation is warranted. Scientists should recognise that experiments that use increasingly refined and controlled ergovaline treatments do not mirror actual production settings and ergovaline delivery. The resultant conclusions should acknowledge this, as physiological effects and animal responses to ergovaline are defined.

Researchers should consider re-weighing ergovaline exposed animals along with control groups 3-5 days after the end of ergovaline exposure to account for any ergovaline-induced changes in gut fill. Furthermore, the selection of an appropriate experimental design to test for short-term effects or longer-term system impact should be considered.

The absence of the data and knowledge identified here prevents our ability to make robust recommendations on threshold ergovaline intake or critical dietary ergovaline concentrations. Although we encountered the difficulties and lack of knowledge identified above and devised this check-list when reviewing ergovaline, many are applicable to nutritional and physiological studies on other dietary components.

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