

## The effect of coumestans in lucerne cultivars on ruminant reproduction

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### Introduction

Research conducted at Charles Sturt University is currently evaluating key legume-produced phyto-oestrogens that play an important role in both plant defence and disruption of the reproductive system in grazing livestock. Phyto-oestrogenic secondary metabolites are of increasing interest to livestock producers, veterinarians, reproductive practitioners, and farm managers across Australia, as determination of threshold concentrations in both fresh and dried legume fodder are required for optimal reproductive management of livestock. Our research is currently addressing the issue of the role of phyto-oestrogens on fertility in cattle grazing lucerne (*Medicago sativa*) by quantifying levels of key metabolites both *in planta* and *in vivo* by metabolic profiling using highly sensitive UPLC/MS QToF instrumentation.

Phyto-oestrogens typically belong to two distinct chemical classes; flavonoids (isoflavones) and non-flavonoids (lignans and coumestans). They play an integral role in plant defence against predation and herbivory, and bioaccumulate *in planta* during periods of stress and microbial infection. When present at threshold levels, phyto-oestrogens also cause disruptions in mammalian reproductive systems upon consumption due to their structural similarities with mammalian oestrogens and their affinity for oestrogen receptors<sup>1</sup>. Phyto-oestrogens have been reported to suppress follicular development, reduce oestrogen and amplify androgen levels, disrupt normal endocrine function, and stimulate the production of multi-oocytes in cattle<sup>2</sup>. In heifers they reduce the release of luteinising hormone (LH) due to its effect on release of gonadotrophin-releasing hormone (GnRH)<sup>3</sup>. Effects on reproductive functions are typically dependent on the dosage or bioavailable total concentrations and the reproductive status of the cow<sup>4</sup>. Infertility can result without any evident signs and is detected by assessment of concentrations within the diet or plasma, and clinical signs on the reproductive tract and organs<sup>5</sup>. However, threshold levels of key metabolites have not been well ascertained in cattle grazing lucerne.

### Methods

Metabolomic profiling of plant secondary metabolites was performed on fresh and dried lucerne extracts and plasma samples collected from heifers grazing replicated lucerne or ryegrass paddocks. Analysis was performed by UHPLC-MS- QToF in an attempt to characterise key coumestans and isoflavones and quantify known phyto-oestrogens present, to contrast with previously reported threshold levels for cattle consumption. Coumestan and flavonoid standards were obtained for quantification purposes. Extraction of lucerne foliage was performed via a pressurised solvent extraction system (E-916 Büchi, Switzerland). Extraction of cattle plasma samples in a replicated lucerne feeding trial was performed following weekly blood serum collection as per Ludwig et al. (2015).

### Results

Numerous coumestans and isoflavonoids were successfully annotated and identified in glasshouse and field-grown lucerne with concentrations generally higher in field-grown lucerne (cv. Genesis). The most abundant metabolites present included coumestrol, gentistein and daidzein, with numerous other phyto-oestrogenic metabolites detected at lesser or trace concentrations. While drying of lucerne increased the concentration of many metabolites, concentrations of phyto-oestrogens were generally unaffected or reduced by drying in contrast to fresh tissue. The abundance of key phyto-oestrogens increased linearly in plasma samples of grazing heifers over time, with coumestrol concentrations exceeding reported threshold levels after 21 days of grazing in some cases.

## Conclusions

Our findings suggest a need for 1) determination of total phyto-oestrogen accumulation and impacts on reproductive health in cattle grazing non-irrigated lucerne genotypes and 2) further evaluation of threshold levels of total phyto-oestrogens observed in freshly grazed lucerne genotypes versus lucerne hay or silage.

## References

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