



Recently Identified Coumestans in Bovine Plasma

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Introduction

Coumestans are plant derived, non-steroidal compounds occurring predominantly in legumes. Coumestans may negatively affect the reproductive functions of cattle at concentrations greater than 18 mg/kg DM in pasture¹. Acting as oestrogen antagonists, they interact directly with oestrogen receptors, resulting in suppression of progesterone production and adversely affect conception and embryo development². The project was designed to quantify key coumestans and other metabolites and evaluate their influence on follicular development, conception and early embryonic development at 7 days post ovulation.

Methodology

Samples were collected from an established lucerne (*Medicago sativa* var. Genesis) pasture, over eight timepoints for eight weeks. Blood samples were collected from 20 head of cattle, over eight timepoints. Cattle were separated into 3 treatment groups (4 weeks on lucerne, 7 weeks on lucerne, 7 weeks on ryegrass before artificial insemination) and a control (n=5). Plasma and pasture samples were stored and extracted using a previously published method³. Targeted and non-targeted analysis were conducted using high pressure liquid chromatography (HPLC) coupled with triple quadrupole mass spectrometry (Agilent 6470 Triple Quadrupole UHPLC/MS) and quadrupole time of flight mass spectrometry (Agilent 6530 LC/MS QTOF), respectively in negative ion mode. Metabolites were identified by comparing accurate mass, retention time and mass spectra with analytical standards and available libraries.



Figure 1: Cattle on established lucerne pasture (var. Genesis).

References:

¹Reed; *Agriculture* **2016**, 6(3), 35.

²Ferreira-Dias, *et al.*; *Theriogenology* **2013**, 80, 684-692.

³Wyse, *et al.*; *Metabolites* **2021**, 11(8), 550.

Results

Four abundant coumestans were identified in the pasture, coumestrol, 4'methoxycoumestrol, 3'methoxycoumestrol and coumestrin (coumestrol-3-O-glucoside). Other coumestans including lucernol, sativol and 11,12-dimethoxy-7-hydroxycoumestan were also annotated. The metabolite 3'methoxy-coumestrol had the greatest relative abundance of all coumestans identified. Three additional coumestans were found in bovine plasma, with the novel metabolite coumestrin also annotated. Coumestrol concentrations in the plasma ranged from 0.16 - 2.6 ng/mL. Embryo recovery ranged from 40 - 100%.

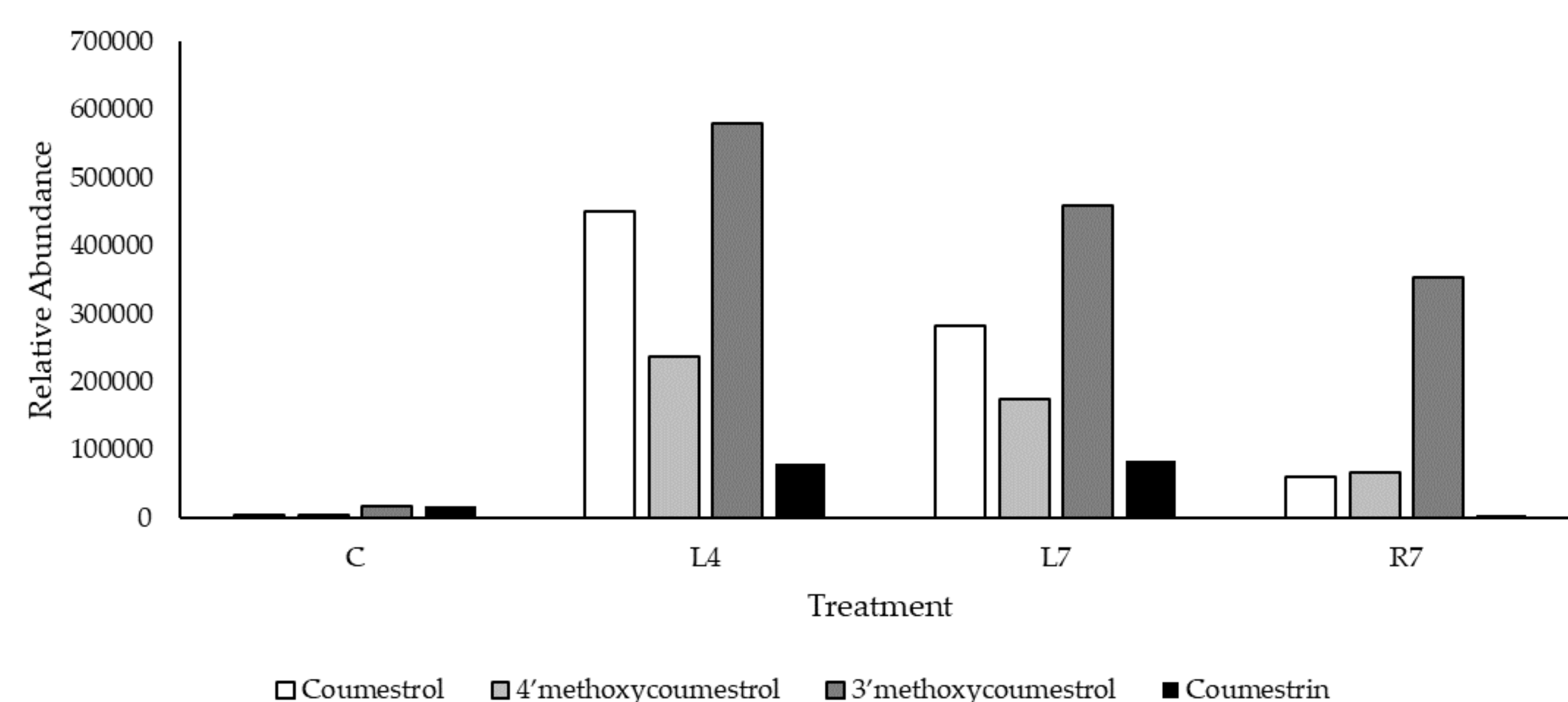


Figure 2: Relative abundance of coumestans found in pasture samples, coumestrol, 4'methoxycoumestrol, 3'methoxycoumestrol and coumestrin. Treatments were as follows: control (C) - ryegrass, L4 - 4 weeks on lucerne, L7 - 7 weeks on lucerne, R7 - 7 weeks on clover:ryegrass mix.

Conclusion

The present study observed the effect of coumestans, and their effects on embryo stage, and quality. The results suggest that high concentrations of coumestrol in pasture and plasma affected embryo stage, reduced progesterone production and potentially contributed to early embryonic loss. Over time, we have identified both precursors and metabolites of coumestans in lucerne and various other legume pastures and are now tracking key metabolites abundance in bovine plasma in replicated feeding trials. Comparison to known standards will assist us in quantification of all annotated coumestans in both pasture legume and plasma samples.

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