Characterisation and Quantification of Coumestans in Lucerne Grazed Cattle

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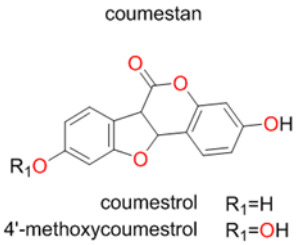
Coumestans are secondary plant metabolites, occurring predominantly in legume species, and have been recorded to negatively impact both cattle and sheep fertility[1]. Coumestans act as anti-oestrogens as they interact directly with oestrogen receptors[2]. In comparison to isoflavones, which have a limited impact on oestrus, coumestans have greater potential to suppress oestrus[3].

The most important coumestan synthesised in legumes is coumestrol. It was first isolated in lucerne and has later been identified in various other pasture species[4,2]. Concentrations of coumestrol *in vitro* are typically high in oestrogenic potency, similar to the potency of oestradiol[5]. Coumestrol acts similarly to oestradiol when administered exogenously, subsequently resulting in ovulation failure as a result of disruption to follicular growth[5]. Lucerne is an integral part of mixed farming systems in much of the southeast New South Wales. Current project was therefore, designed to characterise and quantify key coumestans in various cultivars of lucerne and consequently *in vivo* plasma concentrations in cattle grazed on lucerne.

Lucerne samples were collected from cultivated field experiments in Wagga Wagga (35.11° S, 147. 36° E), Australia at various growth stages of the pasture. Shoot samples were extracted in methanol using high pressure speed extractor. A three week field trial was conducted on cattle grazing lucerne (after synchronisation of their oestrous cycle). Blood samples were collected every four days for harvesting plasma to measure coumenstans using previously published method[6]. Targeted analysis was conducted using high pressure liquid chromatography coupled with quadrupole – time of flight mass spectrometry (UPLC-MS QToF). Compounds were identified by comparing accurate mass, retention time and mass spectra with analytical standards and available libraries. Concentrations of coumestands varied significantly with variety of lucerne. Further analysis to quantify coumestan levels at various stages on plant growth and blood plasma is currently under investigation.



[1]Ha *et al.*; Scientific Reports. **2019**, *(9), 1934.*

[2]Nehybova *et al.*; Anti-Cancer Agent ME. **2014**, (14), 1351-1362.

[3]Kelly *et al.*; Aust. J. Agric. Res. **1976**, (27), 253-259.

[4]Sirtori *et al.*; Ann. Med. **2005**, (37), 423-438.

[5]Ferreira-Dias *et al.*; Theriogenology. **2013**, (80), 684-692.

[6]Ludwif *et al.*; Free Radical Bio. Med. **2015**, (89), 758-769.