The potential of a plant extract to provide fluorescent chlorophyll derivatives to act as markers of faecal contamination on carcasses

MRF Lee[1], MB Scott[1], RI Richardson[2], EJ Kim[1], F Lundby[3], A Veberg Dahl[3], ND Scollan[1]
[1]Aberystwyth University, Aberystwyth, Ceredigion, United Kingdom, [2]University of Bristol, Langford, Somerset, United Kingdom, [3]Nofima Mat, As, Osloevein, Norway *Email: michael.lee@aber.ac.uk*

Introduction Cleanliness in the abattoir is of the utmost importance and strategies are carried out on farm to ensure that the animals arrive at the abattoir with limited faecal matter clinging to the hide. Currently carcasses are checked by 'eye' and trimmed to remove contaminated areas. Small areas of faecal contamination may not be visible to the eye and may harbour millions of pathogenic bacteria. Spectroscopic imaging is a rapidly evolving research area, with the potential to provide real-time solutions for the detection of faecal contamination on carcasses (Ashby *et al*. 2007). Chlorophyll is ubiquitous in green plants and thus grazing diets. During digestion in the gut, chlorophyll is only partially degraded to coloured and fluorescent intermediates: the phaeophytin, chlorophyllide, phaeophorbide and pyrophaeophorbide derivatives of chlorophylls a and b (Lee *et al*. 2009). This study assessed the potential of a chlorophyll containing feed (PX - an extract from lucerne; *Medicago sativa*) to provide fluorescent markers in the faeces which could then be used for on-line detection in the abattoir.

Materials and methods Forty-four Belgium-Blue steers were maintained on grass silage until the end of April 2008 and then at pasture until housing in October 2008. Animals were then allocated to 1 of 5 treatments: grass silage (G); grass silage + 75g/kg silage DM intake of PX (lucerne extract, Desialis, France; GLPX); grass silage + 150 g/kg silage DM intake PX (GHPX); straw + standard concentrate (C); straw + PX-concentrate (22% PX) (CPX). Straw and grass silage were offered *ad libitum* and concentrate was fed at a rate of 8 kg DM/d. Animals were kept on treatment for 16 weeks with feed samples taken daily and bulked per week. Before slaughter faecal samples were collected. Chlorophyll catabolites were determined by HPLC in the feed and faeces and analysed using a general ANOVA. Fluorescence emission spectra were measured directly on the faeces. The fluorescence emission spectra were measured with excitation at 382 and 430 nm, using an optical bench system. The spectra were collected by an imaging spectrograph (Acton SP-150, Acton Research Corporation, Acton, MA) connected to a sensitive charge coupled device (CCD-camera) (Roper Scientific NTE/CCD-1340/400-EMB, Roper Scientific, Trenton, NJ). Cut-off filters at 400 nm (for the 382 nm excitation) (Melles Griot 03FCG049) and 475 nm (for the 430 nm excitation) (Melles Griot 03FCG068) were positioned in front of the spectrograph slit to suppress excitation light reflected from the samples. Exposure time was 10 and 5 sec for excitation at 382 and 430 nm, respectively. The temperature of the samples was 4 °C. All the samples were measured twice and an average was used in the analysis. The resulting spectra were analyzed using PCA (Principal Component Analysis) and catabolite concentration using general ANOVA.

Results Intake of chlorophyll and its catabolites was different ($P<0.001$) across the 5 treatments: 12.9, 15.3, 0.56, 24.1 and 10.4 g/d for GLPX, GHPX, C, CPX and G, respectively. This corresponds with the levels of chlorophyll and its catabolites in faeces (Figure 1), where CPX was higher than C and G ($P<0.05$) but there was no difference between silage based treatments. These results are confirmed in the PCA cluster analysis for the fluorescent spectra emitted from the faeces which showed no difference between GHPX, GLPX and G but with clear separation from CPX (highest intensity) and C (lowest intensity).

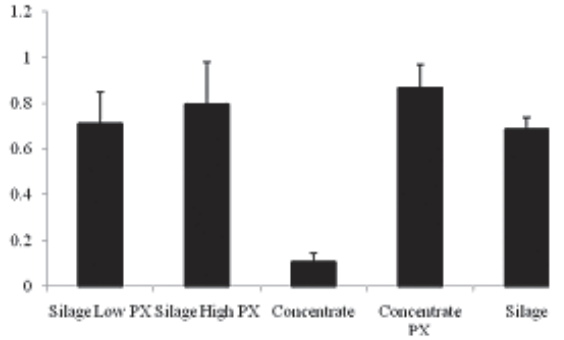

Figure 1 Concentration (mg/g DM) of chlorophyll catabolites

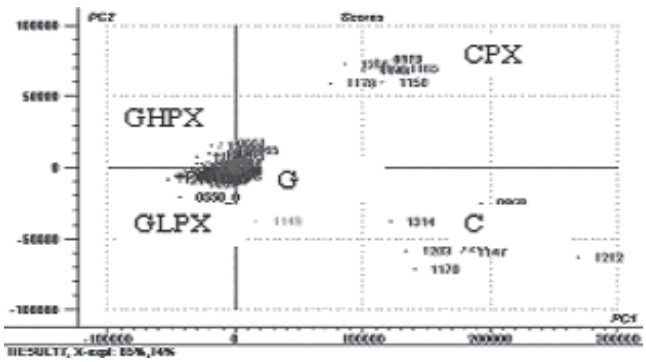

Figure 2 PCA cluster analysis of faecal fluorescent spectra in faeces

Conclusions Inclusion of PX within a concentrate significantly increased the level of chlorophyll catabolites in faeces but had little effect increasing the levels when animals were offered silage. This suggests potential for its use to increase fluorescent intensity in concentrate finishing systems making it easier to detect small traces of faecal contamination on carcasses in the abattoir and thereby improve product safety.

Acknowledgements The authors gratefully acknowledge funding from an EU sixth framework programme ProSafeBeef.

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https://doi.org/10.1017/S204047001000275X Published online by Cambridge University Press