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# **METABOLISM OF RUMEN INFUSED SKATOLE AND EFFECTS ON HEPATIC GENE EXPRESSION IN SHEEP**

A thesis presented in partial fulfilment  
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## ABSTRACT

Skatole (3-methylindole) contributes to the unique flavour palate known as pastoral flavour (characterised by 'sheepy' or 'grassy' odours) that differentiates meat products of pasture-fed ruminants from those finished under grain-based production systems. Pastoral flavour is undesirable to some consumer groups who are sensitive or unaccustomed to meat from temperate pastoral production systems yet is largely appreciated by traditional consumers. The biosynthesis of skatole in the rumen requires bacterial deamination of the amino acid L-tryptophan, hence its rate of production can be manipulated by reducing the rumen formation of free amino acids or decreasing the activity of skatole forming microbes. Ruminants grazing New Zealand improved pasture species, in particular white clover, ingest sufficient rumen degradable protein to allow post-prandial skatole formation at a rate greater than the first-pass detoxification capacity of the liver. Skatole accumulation in body fat occurs when its absorption from the reticulorumen exceeds the pre and post-hepatic detoxification capacity of the body.

It was hypothesised that under conditions of minimal rumen skatole production a 72 hour administration of exogenous skatole would result in an increase in rumen skatole to a plateau concentration and induce differential expression of detoxification genes in the ovine liver. These hypotheses were tested using a continuous intraruminal infusion of skatole (140 mg/h), measurement of skatole concentration in rumen fluid, peripheral plasma and inter-muscular fat and transcriptional analysis of hepatic tissue using DNA microarrays.

Twelve, ten month old castrate male sheep (*Ovis aries*) from a single sire were exposed to a contrasting level of skatole for a 72 hour period. Rumen skatole production was minimised by feeding a diet with low rumen degradable protein content. Sheep received a constant rumen infusion of propylene glycol carrier with or without skatole (28 mg/mL). Samples of rumen fluid and peripheral blood were collected at 0, 2, 4, 6, 8, 12, 16, 24, 48 and 72 hours for determination of skatole concentration. Sheep were euthanased 72 hours after commencement of infusion and samples of liver and inter-

muscular fat were collected. Total hepatic RNA was isolated, purified and used for microarray hybridisation. cDNA was synthesised and cyanine dye incorporated in preparation for hybridisation to expressed sequence tag bovine microarrays with greater than 97% homology to ovine protein coding sequence. The experiment consisted of an 18 array augmented loop design balanced for dye bias.

Rapid appearance of rumen-infused skatole in the peripheral blood and inter-muscular fat confirmed the high rate of skatole absorption and deposition in sheep. A two compartment model fitted to the rumen and blood concentration of treated sheep enabled estimation of the rate of transfer from rumen to peripheral plasma ( $k = 0.23$ ) and the rate of elimination from peripheral circulation ( $k = 2.10$ ). A negative correlation ( $P < 0.05$ ) between the rate of elimination and level of skatole deposition in inter-muscular fat was also found.

Expressed sequence tags with significant ( $FDR < 0.01$ ) differential expression in either direction represented about 14% of those assessed. Genes encoding enzymes with xenobiotic detoxification activity were induced in ovine hepatic tissue in response to skatole exposure. Of these, only five had a fold change greater than 2.0; three encoded cytosolic phase I oxidoreductase enzymes involved in detoxification; aldehyde dehydrogenase 1 family member A1, NAD(P)H dehydrogenase quinone 1 and leukotriene B4 12-hydroxydehydrogenase. The metabolic oxidoreductase enzyme stearoyl-CoA desaturase was also induced along with phase II detoxification enzyme glutathione S-transferase. Induction of these genes, specifically those with known catalytic activity towards toxic xenobiotics, indicates that the ovine liver is a site of detoxification for skatole or its intermediary metabolites. Further investigation is required to determine the role of these genes in the regulation of skatole detoxification in the ovine liver and the possibility to reduce pastoral flavour in forage grazing ruminants through modulation of the activity of these genes or enzymes.

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|                |   |
|----------------|---|
| pH             | hydrogen potential                      |
| BW             | bodyweight                              |
| CA             | California                              |
| cDNA           | complementary DNA                       |
| cm             | centimetre(s)                           |
| CP             | crude protein                           |
| d <sub>2</sub> | deuterium                               |
| DM             | dry matter                              |
| DNA            | deoxyribonucleic acid                   |
| °C             | degree Celsius                          |
| EB             | equilibration buffer                    |
| EST            | expressed sequence tag                  |
| FDR            | false discovery rate                    |
| FID            | flame ionization detection              |
| GC-MS          | gas chromatography-mass spectrometry    |
| g              | gram(s)                                 |
| <i>g</i>       | gravities of centrifugal force          |
| >              | greater than                            |
| h              | hour                                    |
| HPLC           | high performance liquid chromatography  |
| HCl            | hydrochloric acid                       |
| IL             | Illinois                                |
| i.m.           | intramuscular                           |
| i.v.           | intravenous                             |
| kg             | kilogram                                |
| <              | less than                               |
| L              | litre                                   |
| <i>m/z</i>     | mass-to-charge ratio                    |
| MJ             | mega joule                              |
| ME             | metabolisable energy                    |
| m              | metre(s)                                |
| MeOH           | methanol                                |
| 3MI            | 3-methylindole                          |
| 2MI            | 2-methylindole                          |
| µg             | microgram(s)                            |
| µL             | microlitre(s)                           |
| mg             | milligram(s)                            |
| mL             | millilitre(s)                           |
| mm             | millimetre                              |
| min            | minute(s)                               |
| MO             | Missouri                                |
| M              | molarity, moles per litre               |
| ng             | nanogram(s)                             |
| NJ             | New Jersey                              |
| NY             | New York                                |
| NZ             | New Zealand                             |
| NADPH          | nicotine adenine dinucleotide phosphate |
| n              | number of observations                  |
| ON             | Ontario                                 |

|                  |                           |
|------------------|---------------------------|
| OD               | optical density           |
| o.d.             | outer diameter            |
| %                | percent                   |
| PMT              | photomultiplier tube      |
| PCR              | polymerase chain reaction |
| PD               | propane-1,2-diol          |
| ®                | registered trademark      |
| rpm              | revolutions per minute    |
| RDP              | rumen degradable protein  |
| RNA              | ribonucleic acid          |
| SPE              | solid phase extraction    |
| TX               | Texas                     |
| TM               | trade mark                |
| UV               | ultraviolet light         |
| USA              | United States of America  |
| UK               | United Kingdom            |
| VFA              | volatile fatty acid       |
| VFI              | voluntary feed intake     |
| v                | volume                    |
| H <sub>2</sub> O | water                     |
| w                | weight                    |
| WI               | Wisconsin                 |