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

A thesis presented in partial fulfilment
of the requirement for the degree of

Master of Science
in
Animal Science

at Massey University, Palmerston North,
New Zealand.

Matthew Hervey Deighton

2008

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.

ACKNOWLEDGMENTS

I wish to extend my sincere thanks to my supervisors, Dr Warren McNabb, Dr Nicole Roy and Dr David Pacheco, for their support and encouragement in completing this thesis and for their guidance throughout this research project. Thanks to Warren for allowing me to undertake this course of study part-time whilst working as a research technician within the Food, Metabolism & Microbiology Section at AgResearch Grasslands and for his support as representative for the Institute of Food Nutrition and Human Health, Massey University.

I also wish to thank the many people who have participated in bringing this research to fruition and for helping to make the research experience so enjoyable during long hours in the surgery, the animal house, the laboratory and with gene annotation and statistical advice. Thank you all very much; Mr Jason Peters, Dr Nicola Schreurs, Mrs Kate Broadley, Mr Karl Fraser, Dr Geoff Lane, Dr Michael Tavendale, Mr Peter Schreurs, Ms Zaneta Park-Ng, Ms Deborah Simon, Mr Bruce Sinclair, Mr John Rounce, Dr Graeme Attwood, Dr Sue McCoard, Mr Steve Lees and Ms Bianca Knoch.

I am indebted to my parents Alan and Prue for their unfailing encouragement and support throughout my tertiary studies and in particular during the completion of this thesis. I could not have completed this research without the education you have provided me and your love and your guidance, thank you.

To my wife Lee who has been most inconvenienced by the constant distraction of this part-time student. Thank you for all the late nights, lost weekends and your love and support through this process, I could not have done it without you.

This research was funded by the New Zealand Foundation for Research Science and Technology as part of the "Foods that Delight" research program. I am also very grateful for the provision of funding in the form of an Animal Science Award from the New Zealand Society of Animal Production and Massey University Postgraduate support that has enabled the presentation of part of this research as a plenary lecture at the 2nd International Symposium on Energy and Protein Metabolism and Nutrition, Vichy, France, 9-13 September 2007.

TABLE OF CONTENTS

CHAPTER 2:

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

LIST OF TABLES

CHAPTER 1:

CHAPTER 2:

LIST OF FIGURES

CHAPTER 1:

CHAPTER 2:

LIST OF ABBREVIATIONS

pH	hydrogen potential
BW	bodyweight
CA	California
cDNA	complementary DNA
cm	centimetre(s)
CP	crude protein
d_2	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)
g	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
m/z	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_2O	water
w	weight
WI	Wisconsin