THE INFLUENCE OF DIET AND INTAKE LEVEL ON HEPATIC AMMONIA METABOLISM AND UREAGENESIS BY THE OVINE LIVER

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ABSTRACT

The New Zealand agricultural industry is based on the efficient utilisation of fresh forages, a characteristic of which is a high soluble protein content. A large proportion of the ingested protein is highly soluble in the rumen. A significant proportion of the ingested N is removed from the rumen as ammonia with the bulk of this ammonia being removed from the venous blood by the liver for detoxification to urea. Hepatic urea-N production, or ureagenesis, typically exceeds the rate of hepatic ammonia-N extraction, consequently it has been suggested that the shortfall in N required for ureagenesis is contributed by amino acid-N (Parker *et al.* 1995; Lobley *et al.*, 1995).

This study tested the hypothesis that elevated hepatic ammonia extraction would require a concomitant increase in hepatic amino acid catabolism to supply the additional N required for ureagenesis.

In order to evaluate the level of rumen ammonia production and consequently the rates of hepatic ammonia extraction, ureagenesis and amino acid catabolism, the following feeding regimens were tested in sheep held indoors in metabolism crates in three separate experiments; Firstly, lucerne pellets (*Medicago sativa*) were compared with fresh white clover (*Trifolium repens)*, secondly fresh white clover was offered at either a low or high intake and finally the daily allowance of fresh white clover was fed in two 2 hour periods per day.

In each experiment, silicone based catheters were surgically inserted into the posterior aorta and the mesenteric (2), portal and hepatic veins. Following a ten day dietary adjustment period and a ten day nitrogen balance, the sheep were infused with *para*-aminohippurate (*p*AH) and $^{15}NH_4Cl$ *via* the mesenteric vein. The *p*AH was infused to allow the blood flow across the splanchnic tissues to be estimated, whilst the $^{15}NH_4Cl$ was infused to trace hepatic ammonia metabolism to urea. Blood samples were collected to determine the ammonia, urea, oxygen and amino acid concentrations in the mesenteric, portal and hepatic veins, as well as the posterior aorta.

Despite similar DM intakes, the nitrogen intake of the sheep fed fresh white clover was 60% higher ($P < 0.001$) than that of the same animals fed lucerne

pellets. The difference in rumen protein fermentation in these two contrasting diets resulted in higher ($P < 0.001$) rumen ammonia production in the animals offered fresh white clover. There was, however, only a trend ($P = 0.072$) toward elevated hepatic ammonia extraction in these animals and urea production was not significantly different to the animals fed lucerne pellets. Hepatic amino catabolism was not elevated in the sheep fed fresh white clover, nor was there a significant difference in the proportion of ME intake that was utilised for ureagenesis between the two groups.

In the second experiment the DM intakes of the two groups were different ($P < 0.001$), with the sheep offered the low intake of fresh white clover consuming 807 g DM/d whilst the high intake group consumed 1118 g DM/d. Even with these differences in intake, portal vein ammonia and urea concentrations were similar. Therefore the rate of hepatic ammonia extraction and urea production were also similar between the two intake groups. However, hepatic extraction of ^{15}N-ammonia was higher ($P = 0.033$) in the high intake group compared to the low intake group. There was no evidence to suggest that the level of hepatic amino acid catabolism increased with intake level, consequently the proportion of ME intake attributed to urea synthesis was similar for the two intake groups.

When the experimental animals were restricted to two 2 hour feeding periods per day the DM and N intake decreased by 31% from that of the low intake group in the second experiment. There was no significant effect of time after the onset of feeding on portal ammonia or urea concentrations, hepatic ammonia extraction or hepatic urea production. However portal ammonia concentration and consequently hepatic ammonia extraction and urea production tended to be higher 4-6 hours after ingestion of fresh white clover. However this trend was not observed when the ^{15}N tracer data was used to calculate the hepatic ammonia transfer rate. The ammonia, urea and amino acid hepatic transfer values in this experiment were largely comparable to those recorded for the low and high intake treatments in the second experiment.

In these studies, there was no evidence of elevated hepatic amino acid catabolism occurring in response to elevated rates of hepatic ammonia extraction and hence ureagenesis. Additionally there was no suggestion that ammonia provided both of the N atoms of the urea molecule.

It is concluded that the liver adapted to the changes in dietary nitrogen supply without incurring significant increases in the metabolic cost of ammonia detoxification to urea. However the nutritional challenges presented to the liver may not have been severe enough to induce measurable changes in hepatic ammonia metabolism. A possible mechanism to account for these observations may be that the liver adapted to the changes in nitrogen supply by altering the activity of the primary regulator of the rate of ureagenesis, carbamoyl phosphate synthetase (CPS*I*).

THIS THESIS IS DEDICATED TO MY GREAT GRANDMOTHER
FLOSSY HOLYOAKE

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AA	Amino acids
Ala	Alanine
APE	Atoms percent enrichment
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
ATP	Adenosine tri-phosphate
AV	Arterio-venous difference
BF	Blood flow
BF_a	Blood flow in the hepatic artery
BF_h	Blood flow in the hepatic vein
BF_m	Blood flow in the mesenteric vein
BF_p	Blood flow in the portal vein
bwt	Body weight
C_a	Concentration *p*AH in the posterior aorta
C_h	Concentration *p*AH in the hepatic vein
Cit	Citrate
cm	Centimetre
C_p	Concentration *p*AH in the portal vein
CPS*I*	Carbamoyl-phosphate synthetase *I*
Cys	Cysteine
d	Day
DM	Dry matter
DTT	Dithiothreitol
E_a	APE in the posterior aorta
EC	European commission
EDTA	Ethylenediaminetetra-acetic acid
E_h	APE in the hepatic vein
E_p	APE in the portal vein
FHE	Fractional hepatic extraction rate
$Flow_h$	Blood flow in the hepatic vein
$Flow_p$	Blood flow in the portal vein
FSR	Fractional synthesis rate

g	Gram
G	Gravity
GCMS	Gas chromatography-mass spectrometry
GIT	Gastro-intestinal tract
GLDH	Glutamate dehydrogenase
Gln	Glutamine
Glu	Glutamate
Gly	Glycine
GTP	Guanosine tri-phosphate
h	Hour
$H^{15}NT$	Hepatic ^{15}N transfer
Hepatic extraction $rate_h$	FHE based on venous SRA
Hepatic extraction $rate_{ha}$	FHE based on arterial SRA
His	Histidine
HMT	Hepatic mass transfer
HPLC	High performance liquid chromatography
I_r	Infusion rate of the *p*AH
ID	Internal diameter
Ile	Isoleucine
ILR	Irreversible loss rate
IR	Infusion rate
IU	International units
kg	Kilo gram
kJ	Kilo joule
l	Litre
IR_a	SRA infusion rate
L	Lucerne pellets
Leu	Leucine
Lys	Lysine
M	Maintenance level of intake
MBq	Mega becquerel
MDV	Mesenteric drained viscera
ME	Metabolisable energy
Met	Methionine

mg	Milligram
min	Minute
MJ	Mega joule
ml	Millilitre
μl	Microlitre
mm	Millimetre
mmol	Millimole
μm	Micrometer
μmol	Micro mole
mol/l	Mole per litre
mRNA	Messenger ribo-nucleic acid
m/z	Molecular weight
N	Nitrogen
NAD(P)	Nicotinamide adenine dinucleotide phosphate
$NAD(P)^+$	Nicotinamide adenine dinucleotide phosphate - oxidised form
NADPH	Nicotinamide adenine dinucleotide phosphate - reduced form
NAG	N-acetyl glutamate
nm	Nano-meter
nmol	Nanomole
NPN	Non-protein nitrogen
OM	Organic matter
Orn	Ornithine
P	Probability
P	Portal
*p*AH	*Para*- aminohippurate
PDV	Portal drained viscera
*p*H	Measure of acidity or alkalinity
Phe	Phenylalanine
Post. aorta	Posterior aorta
Pro	Proline
PVC	Poly-vinyl chloride
Q_a	SRA flux of in the posterior aorta

Q_h	SRA flux of in the hepatic vein
Q_p	SRA flux of in the portal vein
R^2	Correlation coefficient
RDP	Rumen degradable protein
Ser	Serine
SE	Standard error
sec	Second
SED	Standard error of the difference
SRA	Specific radio-activity
SRA_a	SRA in the posterior aorta
SRA_h	SRA in the hepatic vein
SRA_p	SRA in the portal vein
TBDMS	N-*tert*, butyldimethylsylil derivative
Thr	Threonine
tRNA	Transfer ribo-nucleic acid
Trp	Tryptophan
Tyr	Tyrosine
UDP	Undegradable protein
Val	Valine
VFA	Volatile fatty acid
WC	White clover
W:V	Weight : volume
W:W	Weight : weight
Z	Generic symbol for metabolites
Z_a	Generic metabolite concentration in the posterior aorta
Z_h	Generic metabolite concentration in the hepatic vein
Z_m	Generic metabolite concentration in the mesenteric vein
Z_p	Generic metabolite concentration in the portal vein