QUANTITATIVE STUDIES ON THE DYNAMICS OF CARBON DIOXIDE AND BICARBONATE IN THE RUMEN

A thesis submitted for the degree of
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by

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Preface

The studies presented in this dissertation were completed by the author as a postgraduate student in the Department of Biochemistry and Nutrition, University of New England, Armidale, N.S.W., Australia. Assistance given by other persons is indicated in the list of acknowledgements. All references cited are included in the bibliography. The work is otherwise original.

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I certify that the substance of this thesis has not already been submitted for any degree and is not being currently submitted for any degree. I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

January, 1984 R.W. Dixon

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SUMMARY

1. The increase in fermentation rate which accompanies the feeding of more readily digested diets as compared with roughage diets fed to ruminants leads to large increases in gas (CO_2 and CH_4) and acid produced in the rumen. The latter results in a reduction in the buffering ability of rumen contents unless salivary flow or bicarbonate movement into the rumen increases with fermentation rate. Where the acidity of rumen fluid contributes to an increase in CO_2 produced by acidification of salivary HCO_3^-. The rate of gas production and degree of accumulation of HCO_3^- in the rumen are factors which dictate the severity of the condition of bloat.

   A study of the dynamics of the bicarbonate buffering system of the rumen with particular reference to the sources and routes of input of C into the HCO_3^-, H_2CO_3, CO_2 and CH_4 pools is required to fully understand the bloat syndrome.

2. Continuous infusion dilution techniques were used to:
   a) quantify the flow of C between the rumen fluid bicarbonate pool, blood bicarbonate pool and the rumen CO_2 and CH_4 gas pools under different rumen fluid pH conditions in sheep. Rumen fluid pH was manipulated by varying the grain content of diets.
   b) examine the fate of bicarbonate-C in rumen fluid when it leaves the rumen and passes into the omasum and abomasum.

3. During infusions of isotopes, there was considerable variability in the plateau SR of sampled pools leading to often high variations

in the flow of C between specific compartments and a relatively large SE.

4. Compartmental models consisting of 2, 3 or 4 pool models were used to describe the flow of C between total rumen fluid bicarbonate, blood bicarbonate and CO_2 and CH_4 gas pools in sheep.
   The mean IL for 3 pool models were 43.16, 185.00 and 48.14 gC/d for the rumen bicarbonate, blood bicarbonate and rumen CO_2 gas pools respectively and a mean rumen fluid pH of 6.58. The mean IL for 4 pool models were 58.90, 188.28, 64.97 and 16.28 gC/d for rumen bicarbonate, blood bicarbonate and rumen CO_2 and CH_4 gas pools respectively and a mean rumen fluid pH of 5.74.

   Analysis of compartmental models of C flow suggested that as rumen fluid pH decreases, there was:
   a) a large increase in the flow of C from rumen fluid bicarbonate to rumen CO_2 gas.
   b) a large increase in the production of CO_2 gas.
   c) an increase in the absorption of CO_2 gas across the rumen epithelium from the CO_2 gas pool into the blood bicarbonate pool.

5. Accompanying a decrease in rumen fluid pH, there was:
   a) a decrease in total rumen fluid bicarbonate and HCO_3^- directly related to the rumen fluid pH and a variable change in rumen fluid H_2CO_3.
   b) an increase in the proportion of CO_2 in the rumen gas pool.
   c) an increase in the proportion of propionate and a decrease in the proportion of acetate in rumen fluid.

   Rumen fluid pH had no effect on the concentration of bicarbonate

and H_2CO_3 in the blood nor the number of protozoa in rumen fluid.

At rumen fluid pH values around 5.5 and below, the concentration of bicarbonate in rumen fluid was too low for bicarbonate to be an effective buffer.

6. The infusion of $NaH^{14}CO_3$ into the omasum and abomasum resulted in a very rapid increase in ^{14}C detected in the blood. This suggested that the major route of loss of bicarbonate from the omasum and abomasum was by absorption into the blood and re-entry into the rumen via the saliva.

7. In order to test whether control over rumen pH could be attained, studies were designed to:
   a) test the response of rumen characteristics of sheep to Na-bentonite supplement when fed high concentrate diets.
   b) quantify (using isotopes) the flow of C between the rumen fluid bicarbonate, blood bicarbonate and rumen CO_2 gas pools in sheep fed a Na-bentonite supplement.

8. The inclusion of Na-bentonite in high concentrate diets of sheep resulted in an increase in rumen fluid pH. This was partially due to an increase in rumen fluid total bicarbonate concentration in sheep fed Na-bentonite supplement. However, when rumen fluid bicarbonate concentration was related to rumen fluid pH, the inclusion of Na-bentonite (pH 9.6), pH was maintained at higher levels at the same concentration of total bicarbonate. This indicates that the action of Na-bentonite was not solely to increase pH by increasing the bicarbonate concentration, but that it performed some other role which resulted in an increase in the

buffering ability of the rumen fluid.

9. An isotope modelling approach was used with sheep to determine whether there was any effect of two Na-bentonites (pH 5.5 and pH 9.6) on the flow of C in a system consisting of rumen fluid bicarbonate, blood bicarbonate and rumen CO_2 gas pools. No significant difference was found between the two Na-bentonites.

   Over the experimental period, there was a gradual day to day increase in rumen fluid pH which may have been due to the action of Na-bentonite accumulating in the rumen or to gradual increases in salivary buffering of the rumen.

10. The results of this study have confirmed that the feeding of high concentrate diets results in an increase in the amount of CO_2 produced that has to pass through the gas phase of the rumen, usually via the liquid phase. The feeding of high concentrate diets also results in a reduction in the bicarbonate buffering ability of rumen contents. With manipulation of the diet, it is possible to increase and stabilize the buffering capacity of the rumen.

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A.1 The compartments being considered.

Definition of terms and abbreviations

Tracer terminology

Definitions of the terminology used in this thesis for describing tracer dilution techniques based on Brownell et al (1968) and Nolan et al (1976).

Model: A representation of an hypothesis in which a formal structure for a system is proposed.

Biological system: A set of compartments of interest between which the transfer of defined substances occurs.

Compartment: A biologically distinguishable entity of a system throughout which the ratio of concentration of tracer to tracee is uniform at any given time.

Tracee: The defined substance whose movement and behaviour in the system is under study.

Tracer: An isotope-labelled form of the defined substance used to study the system having the basic requirements that it be identical to the tracee in all physical, chemical and biological properties and be negligible in quantity compared to the tracee. Its introduction to the system must cause no perturbation of the characteristics of the system.

Tracer dilution: The change in the ratio of tracer and tracee following the introduction of tracer into a compartment.

Tracer kinetics: The movement and behaviour of tracer in a system with time.

Rate constant: The fraction of the amount of the defined substance that leaves a given compartment per unit time.

Transfer quotent ($A_{i,j}$): The proportion of tracee in a secondary pool (i) arising from the primary pool (j).

Plateau: The estimated value of the asymptote which is approached by the ratio of tracer to tracee during a continuous infusion of tracer into a compartment at a constant rate.

Steady state: A condition of a system characterized by constancy with respect to concentrations and quantities and rates of flow of tracee during the experimental period.

Rate of flux: The rate at which all tracee enters and leaves a compartment which is in steady state. Rate of flux is made up of the irreversible loss and recycling rates.

Recycling rate: Rate of entry of a defined substance into a compartment which has previously been in that compartment.

Irreversible loss (entry rate): That fraction of tracee which leaves the compartment and does not return to the compartment during the experimental period.

Pool: Equivalent to a compartment of tracee which composes a biologically distinguishable entity used for kinetic analysis.

Pool size: The total mass of a defined substance distributed throughout all compartments within the biological system.

Primary pool: The pool or compartment into which the tracer is injected or infused.

Secondary pool: The pool(s) or compartment(s) into which tracer may be

detected after entry from the primary pool.

Half-life: Time required for loss and replacement of one-half of the tracee. Equals turnover time multiplied by 0.693.

Abbreviations

A_{ij}	:	proportion of secondary pool (i) derived from primary pool (j)
AMP	:	adenosine monophosphate
ATP	:	adenosine triphosphate
C	:	carbon
Cr-EDTA	:	chromium complex of ethylenediamine tetra-acetic acid
d	:	day
DM	:	dry matter
DOMI	:	digestible organic matter intake
F_{ij}	:	flow of material into pool i from pool j (g/d)
g	:	gram
g	:	gravity
hr	:	hour
I.D.	:	internal diameter
IL	:	irreversible loss (g/d)
i.u.	:	international unit
kg	:	kilogram
l	:	litre
M	:	molar (concentration)
mCi	:	millicurie
m.equiv	:	milliequivalents (concentration)
mg	:	milligram
min	:	minute

ml	: millilitre
mm	: millimetre
mm	: cubic millimetre
mmol	: millimole
muCi	: millimicrocurie
N	: nitrogen
nCi	: nanocurie
OM	: organic matter
SA	: specific activity
SD	: standard deviation
SE	: standard error
spp.	: species
SR	: specific radioactivity
STP	: standard temperature and pressure
uCi	: microcurie
um	: micrometre
VFA	: volatile fatty acid(s)