THE EFFECT OF SUPPLEMENTATION WITH ASCORBIC ACID UPON RUMEN METABOLISM AND PLASMA ASCORBIC ACID CONCENTRATION IN RED DEER (*Cervus elaphus*)

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ABSTRACT

RANJAN GURUSINGHE, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand. THE EFFECT OF SUPPLEMENTATION WITH ASCORBIC ACID UPON RUMEN METABOLISM AND PLASMA ASCORBIC ACID CONCENTRATION IN RED DEER (*Cervus elaphus*)

Six indoor experiments were conducted at the Massey University Deer Research Unit to study whether the blood plasma ascorbic acid (AA) concentration in farmed red deer (*Cervus elaphus*) could be raised, using a single oral or intraruminal administration of AA prior to a simulated slaughter situation. The work arose from the suggestion by Stevenson-Barry *et al* (1999) that feeding treatments be investigated for increasing the concentration of AA in venison, with a view to increasing colour stability and extending shelf life and from unpublished observations by these authors that it may be possible to achieve this from administering large single doses of AA before slaughter (J.M. Stevenson-Barry personal communication). Ruminal degradation of ascorbic acid was also studied, to establish a mechanism of how the single dose technique increased plasma AA concentration and particularly to identify the site of AA absorption.

Seven ruminally fistulated male castrated red deer (average age 13 years) and three male castrated red deer fistulated in both the rumen and abomasum (average age 1.5-3.0 years) were individually fed chaffed lucerne hay *ad libitum* at 30 minute intervals throughout the experimental programme from July 1999 to February 2000. Animals were brought into metabolism cages one week before the administration of AA, orally or intraruminally. Feed was withdrawn 8 hours before AA was administered and fasting continued during the period of rumen and blood sampling (total 30 hours fasting). Ascorbic acid was administered as a 50:50 w/v suspension in water. Blood (jugular vein), rumen fluid and abomasal fluid samples were taken 15 minutes (min.) before each dose of AA and further samples were taken at 15 min., 30 min., 60 min., 2 hours (hr.) 4 h., 6 h., 8 h., 12 h., 16 h. and 22 h., after AA administration depending on the experiment. Voluntary feed intake (VFI) of individual deer was measured during the 3 days before

dosing with AA in all experiments. Rumen fluid and abomasal fluid pH values were also recorded in Experiments 3, 4, 5 and 6. The liquid phase marker chromium complex of ethylenediaminetetra-acetic acid (Cr-EDTA) was administered with and without AA given intraruminally in Experiment 6, to measure rumen liquid fractional outflow rate (FOR) and to calculate the proportion of AA dosed that flowed into the abomasum. The animals grazed perennial ryegrass/ white clover pastures for periods of 1 to 2 weeks between individual experiments.

1. Experiment 1 and 2 were conducted to determine an appropriate dose rate of orally/ intraruminally administered AA to obtain high concentration of AA in rumen fluid and blood plasma and to define an appropriate time interval between repeat doses of AA. A range of oral and intraruminal doses of AA were given in Experiment 1 to individual deer and 2.8 g AA /kg liveweight was identified as a suitable dose to increase plasma AA concentration. At the end of Experiment 2, it was concluded that the use of a single intraruminal dose of 2.7 g AA equivalent/kg liveweight with repeat doses being a minimum of 2-weeks apart should be used for the remaining four experiments in order to obtain repeatable concentrations of AA in rumen fluid and blood plasma. In Experiment 2, dosing with AA depressed VFI for 4 days after its administration.

2. In Experiment 3, six rumen fistulated deer were used in a 3x3 Latin square experiment to study the best bioavailability of 3 different types of AA namely pure ascorbic acid (AA), ethyl cellulose coated ascorbic acid (EC) and silicone coated ascorbic acid (SC) using a single high dose technique. Pure AA and the other two derivatives were administered at 2.7 g AA equivalent/kg liveweight intraruminally. It was observed that all three types of AA administered increased the rumen and blood plasma AA concentrations to a desirable level with the maximum concentrations in both sites occurring during 1 hr after administration, indicating that the rumen could be the main site of absorption. The area under the concentration vs. time curve (AUC), area under the curve corrected for baseline (AUCB) and maximum concentration (MAX) of AA in both rumen fluid and blood plasma were not significantly different between the three

formulations of AA, indicating that all three were degraded at a similar rate in the rumen and that their bioavailability was similar.

Rumen pH decreased from approximately 7.0 to 5.0 units within one hour of administering each compound, increased to pH 6.0 after 4 hours and then progressively increased to approximately 7.0 units after 22 hours.

There were no significant differences in AUC, AUCB, MAX or rumen pH between the three time periods, confirming that the experimental procedures used gave repeatable results.

3. Due to low rumen pH levels (5.0) experienced in Experiment 3, Experiment 4 was conducted to investigate the rumen buffering effect after dosing with AA along with sodium bicarbonate ($NaHCO_3$) to see whether the rumen pH levels could be maintained at 5.5 or above (the lower end of the normal physiological range) during the course of the experiment. Seven rumen fistulated deer were used in a changeover design, in two periods. Four deer were intraruminally dosed with AA plus $NaHCO_3$ (10:1 ratio) and the remaining 3 deer were dosed with only AA; the sequence was reversed in the second period. An amount of 2.7 g AA/kg liveweight as used in Experiment 3. It was possible to maintain the rumen pH above 5.5 in the group of deer that received AA plus $NaHCO_3$, but the ascorbic acid concentrations in both rumen fluid and blood plasma were lower than for the group of deer that received AA only. Including $NaHCO_3$ increased rumen pH by approximately 1 unit during the first hour after dosing and by 0.7- 0.4 units thereafter. It was also observed that AUC and AUCB for rumen fluid were significantly lower for the AA plus $NaHCO_3$ group of deer than for AA group ($P<0.05$), indicating that increasing rumen pH had increased the rate of ruminal destruction of AA. The area under the concentration vs. time curve(AUC), AUCB and MAX of ascorbic acid in blood plasma were not statistically different between the two treatments ($P>0.05$), perhaps explained by $NaHCO_3$ increasing rumen liquid FOR and hence the amount of AA absorbed post-ruminally.

4. Experiment 5 was conducted to study the differences in AA concentrations in the rumen, abomasum and blood plasma after administration of AA via rumen and also to observe the differences in AA concentrations in blood plasma after dosing with AA via abomasum. Three deer, fistulated in both the rumen and abomasum were administered intraruminally with AA (2.7 g/ kg liveweight) in trial 1. In trial 2, three deer were given AA 0.75 g/kg liveweight via the abomasum.

Following intraruminal administration, it was observed that the AA concentration in the abomasum was much lower than that of rumen fluid. Mean AA concentration in blood plasma was very low when AA was given abomasally. Rumen adminstration of AA caused a rapid reduction in rumen pH (from 7.0 to 5.0 units) and a less rapid rise in abomasal pH (from 2.4 to 3.7 units). Abomasal administration of AA likewise caused an increase in abomasal pH but had no effect on rumen pH.

5. In Experiment 6, three deer fistulated in rumen and three deer fistulated in both the rumen and abomasum were used in two trials to measure the rumen fractional outflow rate (FOR) of liquid under normal conditions and after dosing with a large dose of AA into the rumen. In trial 1, all six deer were given Cr-EDTA (180ml, 2.77 mg Cr/ml water) via rumen fistula. In trial 2, all six deer were administered intraruminally the same dose of Cr-EDTA mixed with 2.7 g AA/kg liveweight. Rumen liquid FOR was low in the fasted deer (5.1 %/h) and was further reduced by administration of AA (3.5 %/hr; $p<0.05$), allowing more time for absorption from the rumen. It was calculated that 29% of the AA administered would flow out of the rumen between the time of dosing and infinity; however, as the half life of the solute marker in the rumen was approximately 20 hours, only half of the 29% (i.e. 14.5 of the dose) would flow out of the rumen in this time.

The pH values in both rumen and abomasal fluid (AbF) of deer did not appreciably change with time when Cr-EDTA was given alone. The mean rumen pH values of deer used in trial 2, showed a rapid decline after administration of AA mixed with Cr-EDTA and this was followed by an increase in AbF pH as found in Experiment 5. Normal pH

values were reached in rumen and AbF at 22 hours and 8 hours respectively after administration of AA intraruminally.

6. Overall it was concluded that the high AA single oral/intraruminal dose technique could be used to consistently increase the AUC, AUCB and MAX of AA concentrations in both rumen fluid and blood plasma. There was no significant difference between the three formulations of AA used (pure AA, EC and SC), probably due to similar rates of destruction of these 3 formulations by rumen bacteria, giving a similar bioavailability. Administration of AA into the rumen reduced the pH value during the initial period of one hour, which may have reduced the rate of AA destruction by the rumen micro-organisms, as indicated by the reduction in AUCB when rumen pH was raised by including $NaHCO_3$ with the AA administered. This is one of the reasons for suggesting that the main absorption site of AA occurred from the rumen and to a lesser extent from the abomasum and small intestines of deer. Other reasons include lower AA concentration in abomasal than rumen fluid, reduced liquid FOR from the rumen following the administration of a large dose of AA into the rumen and a calculated AA outflow of 14.5% of the dose during the first 20 h after administration.

Methods for improving the efficiency of the single large dose AA technique are discussed and recommendations for future work are given.

CONFIDENTIALITY AGREEMENT

The experimental work described in this thesis was conducted under a Confidentially Agreement between the New Zealand Pastoral Agricultural Institute Limited (AgResearch) and Massey University, signed on 21/06/1999. Under the agreement this work is embargoed from publication, and may not be shown to any third party without the approval of AgResearch, for a period of two years from the above date. R.Gurusinghe and T.N.Barry have signed statements accepting the above conditions. Both examiners of the thesis have also signed statements of confidentiality.

The process of administering a large dose of vitamin C to ruminants prior to slaughter to increase antioxidant properties of the meat is the subject of a pending patent to AgResearch Limited. The idea for this process originated from AgResearch and specially Dr Joanne Stevenson-Barry.

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LIST OF ABBREVIATIONS

AA	ascorbic acid
AAS	Atomic Absorption Spectrometry
AbF	abomasal fluid
APU	Animal Physiology Unit
AUC	area under curve
AUCB	area under curve corrected for baseline concentraion
C	centigrade
CHO	carbohydrate
CO_2	carbon dioxide
Cr-EDTA	chromium complex of ethylenediaminetetra-acetic acid
DDMI	digestible dry matter intake
DMD	dry matter digestibility
DM	dry matter
DMI	dry matter intake
DOMI	digestible organic matter intake
DOMD	digestible organic matter in the dry matter
EC	ethyl cellulose coated ascorbic acid
EtOH	ethyl alcohol
FAR	fractional outflow rate
FDPR	fractional disappearance rate
FDR	fractional degradation rate
Fe^{++}	ferrous
Fe^{+++}	ferric
FOR	fractional outflow rate
g	gram
GIT	gastro-intestinal tract
GM	*gluteus medius*

h	hour
HMW	high molecular weight
kg	kilogram
LL	*longissimus lumborum*
LMW	low molecular weight
M	muscle
MAX	maximum concentration
Mb^+	deoxymyoglobin
MbO_2	oxymyoglobin
MetMb	metmyoglobin
min.	minute
MRT	mean retention time
N	nitrogen
$NaHCO_3$	sodium bicarbonate
NRC	National Research Council
NZ	New Zealand
NZGIB	New Zealand Game Industry Board
O_2	oxygen
OMI	organic matter intake
OM	organic matter
OMD	organic matter digestibility
OMD	organic matter digestibility
PM	*psoas major*
RC	Rovimix C coated with silicone
RDA	recommended dietary allowance
RF	rumen fluid
RO	rumen outflow
SA	sodium ascorbate
SC	silicone coated ascorbic acid
SD	standard deviation
SEM	standard error mean

$T_{1/2}$	half life
UK	United Kingdom
USA	United States of America
VFI	voluntary feed intake