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

RANJAN GURUSINGHE

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Master of Science in Nutritional Science at Massey University**

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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the concentration of AA in venison, with a view to increasing colour stability and extending shelf life and from unpublished observations by these authors that it may be possible to achieve this from administering large single doses of AA before slaughter (J.M. Stevenson-Barry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 Feed was withdrawn 8 hours before AA was administered and fasting continued during the period of rumen and blood sampling (total 30 hours fasting).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 administration 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 Confidentiality 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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TABLE OF CONTENTS

	Page
ABSTRACT.....	i
CONFIDENTIALITY AGREEMENT.....	vi
ACKNOWLEDGEMENT.....	vii
TABLE OF CONTENTS.....	ix
LIST OF TABLES.....	xv
LIST OF FIGURES.....	xix
LIST OF PLATES.....	xxiii
LIST OF ABBREVIATIONS.....	xxiv
 CHAPTER 1	
1.1. Introduction.....	1
1.2. References.....	3
 CHAPTER 2	
LITERATURE REVIEW.....	5
 2. VENISON PRODUCTION FROM FARMED DEER IN	
NEW ZEALAND.....	6
 2.1. Progress of Deer Industry in New Zealand.....	6
 2.2. Formation of Game Industry Board and Marketing of	
New Zealand Venison.....	7
2.2.1. Market research.....	8
2.2.2. Branding marketing strategy for venison.....	8
 2.3. Export Market for New Zealand Venison.....	9
2.3.1. Chilled venison.....	11

2.4. Nutritive Value of Venison.....	12
2.5. Relationship Between Colour Stability of Meat and Formation of Metmyoglobin.....	15
2.5.1. Myoglobin structure.....	15
2.5.2. Derivatives of myoglobin and auto-oxidation reaction.....	15
2.5.3. Colour stability of different types of meat.....	17
2.5.4. Factors involved in the discolouration of meat.....	18
2.5.4.1. Inter-muscular and inter-animal variability.....	20
2.5.4.2. Effect of pH	22
2.5.4.3. Effect of temperature.....	23
2.5.4.4. Effect of oxygen pressure.....	23
2.5.4.5. Type of packaging.....	25
2.5.4.6. Effect of freezing.....	25
2.6. Ascorbic Acid (Vitamin C) as an Antioxidant to Delay the Discolouration of Meat.....	26
2.6.1. Chemical structure of vitamin C.....	26
2.6.2. Functions of ascorbic acid in the body.....	27
2.6.3. Effect of ascorbic acid on colour stability of meat.....	28
2.6.4. Relevance to venison.....	35
2.7. Digestion and Absorption of Ascorbic Acid in Ruminants.....	35
2.7.1. Utilization and excretion of ascorbic acid by dairy cow.....	36
2.7.1.1. Effect of ascorbic acid administered intravenously.....	36

2.7.1.2. Effect of ascorbic acid injected subcutaneously.....	36
2.7.1.3. Destruction of ascorbic acid in the rumen.....	36
2.8. Techniques to Measure Digestion and Rumen Fractional Outflow Rate (FOR) of Ruminants.....	37
2.8.1. Marker technique to measure digestion and mean retention time(MRT) along the ruminal digestive tract.....	38
2.8.1.1. Continuous infusion with time sequence sampling to measure the flow of digesta.....	39
2.8.1.2. Continuous infusion with total sampling to measure rumen FOR and MRT.....	40
2.8.1.3. Single dose with time sequence sampling.....	40
2.8.2. Criteria of ideal digesta markers.....	41
2.9. Voluntary Feed Intake (VFI), Rumen Digestion and Rumen Fractional Outflow Rate (FOR) in Deer in Relation to other Ruminants.....	42
2.9.1. Voluntary feed intake and digestion of dry matter.....	42
2.9.2 Fractional outflow rate (FOR) of rumen digesta.....	44
2.10. The Hypothesis of Using Oral Ascorbic Acid (AA) Supplementation to Increase Plasma AA Concentration in Deer before Slaughter.....	46

2.10.1. Rapid rumen liquid fractional outflow rate (FOR) in Deer.....	46
2.10.2. Large single dose of AA to saturate rumen degradation Mechanism.....	46
2.10.3. Use of ascorbic acid analogues.....	47
2.11. Conclusion and Future Research.....	50
2.12. References.....	53

CHAPTER 3

THE EFFECT OF SUPPLEMENTATION WITH ASCORBIC ACID UPON RUMEN METABOLISM AND PLASMA ASCORBIC ACID CONCENTRATION IN RED DEER (<i>Cervus elaphus</i>).....	65
3.1. INTRODUCTION.....	66
3.2. MATERIALS AND METHODS.....	67
3.2.1. Experimental Design.....	67
3.2.2. Animals.....	68
3.2.3. Feeding System.....	68
3.2.4. Experiment One.....	69
3.2.5. Experiment Two.....	70
3.2.6. Experiment Three.....	71
3.2.7. Experiment Four.....	72
3.2.8. Experiment Five.....	73
3.2.9. Experiment Six.....	74
3.2.10. Laboratory Analysis.....	75
3.2.10.0 Rumen and abomasal pH measurement	75
3.2.10.1. Preparation of blood, rumen fluid and abomasal fluid samples for ascorbic acid analysis.....	76
3.2.10.2. Ascorbic acid analysis method.....	76
3.2.10.3. Chromium (Cr) concentration analysis in rumen fluid and abomasal fluid.....	77
3.2.10.4. Total nitrogen and <i>in vitro</i> organic matter digestibility.....	78
3.2.10.5. Dry matter percentage.....	78
3.2.11 Statistical Analysis and Data Collection.....	78

3.3. RESULTS.....	80
3.3.1. Animal Liveweight.....	80
3.3.2. Diet Composition and Voluntary Feed Intake.....	81
3.3.3. Experiment 1.....	82
3.3.4. Experiment 2.....	87
3.3.5. Experiment 3.....	87
3.3.6. Experiment 4.....	92
3.3.7. Experiment 5.....	97
3.3.8. Experiment 6.....	99
3.4. DISCUSSION AND CONCLUSION.....	107
3.5.....REFERENCES.....	117

LIST OF TABLES

	Page
Chapter 1	
Table 1.1 The New Zealand national deer herd slaughter number (1993/94 to 1998/99).....	2
Chapter 2	
Table 2.1 Total number of farms and deer population of the major deer farming countries (as at June 1999).....	7
Table 2.2 Major export markets for frozen and chilled New Zealand venison in 1998 and 1999 (September years).....	10
Table 2.3 Total export volume and value of venison and other products (in the year to August 1999).....	11
Table 2.4 Major nutrients and energy content of venison and lamb (g/100g±SD).....	13
Table 2.5 Cholesterol content of venison and other meat.....	14
Table 2.6 The relationship between the colour stability and the formation of a MetMb layer at the surface of venison, beef and pork <i>L.dorsi</i> muscles during refrigerated storage at 5°C.....	18
Table 2.7 Changes in inhibitory effects on myoglobin oxidation from pork, beef and venison during refrigerated display.....	20
Table 2.8 Components of variance for discoloration from ten bovine Animals after 96 h storage at three different temperatures (0, 5 and 10°C).....	21
Table 2.9 Mean pH of beef muscle stored 10-20 days post-mortem at 0°C.....	22

Table 2.10	Effect of ascorbate treatment on metmyoglobin Accumulation in four beef muscles at two (0°C and 5°) temperatures.....	30
Table 2.11	Preliminary ascorbic acid concentrations (µg/g fresh muscle) as influenced by infused dose, muscle and post-mortem sampling time.....	33
Table 2.12	Colour display life of beef (days) compared to hue angle.....	33
Table 2.13	Voluntary intake and digestible intake of dry matter and organic matter together with their apparent digestibilities (%) and total rumen pool size of deer, goats and sheep fed on lucerne hay during summer and winter.....	43
Table 2.14	Fractional outflow rate (FOR, %/h) of Cr-EDTA and lignin from the rumen of deer, goats and sheep fed on lucerne hay at <i>ad libitum</i>	44
Table 2.15	Effect of season upon rumen digestion in castrate red deer Stags fed chaffed lucerne hay.....	45
Table 2.16	Means and standard deviations of plasma ascorbic acid concentration, AUC and AUCB in sheep given 4 g ascorbic acid equivalent daily doses of several ascorbic acid formulations and in control sheep.....	48
Table 2.17	Means and standard deviations for AUC and AUCB during 0 to 7 h (µg/ml) for sheep given single oral and intra-duodenal administration (4 g) of several ascorbic acid formulations.....	48

Chapter 3

Table 3.0	Amount of pure ascorbic acid administered orally (O) and abomasally (A) in Experiment 1 (Trial 1 and 2).....	69
Table 3.1	Two 3x3 Latin square design for Experiment 3, period 1, 2 and 3.....	71
Table 3.2	Experimental 4 design (period 1 and 2).....	72
Table 3.3	Initial and final liveweight and number of deer used in each experiment.....	80
Table 3.4	Composition of chaffed lucerne fed to deer during Experiment 1, 2, 3, 4, 5 and 6.....	80
Table 3.5	Mean Voluntary Feed Intake (VFI) of deer in the 3 days immediately before dosing with ascorbic acid (kg as DM basis).....	82
Table 3.6	Nutritional effects in Experiment 3; Area under curve (AUC), area under curve corrected for baseline concentration (AUCB) and maximum concentration (MAX) of ascorbic acid in rumen fluid during 0 to 22 h in deer given single intraruminal doses of ascorbic acid (AA), ethyl cellulose coated ascorbic acid (EC) and silicone coated ascorbic acid (SC) at 2.7 g AA equivalent/kg liveweight.....	90
Table 3.7	Nutritional effects in Experiment 3; Area under curve (AUC), area under curve corrected for baseline concentration (AUCB) and maximum concentration (MAX) of ascorbic acid in blood plasma during 0 to 22 h in deer given single intraruminal doses of ascorbic acid (AA), ethyl cellulose coated ascorbic acid (EC) and silicone coated ascorbic acid (SC) at 2.7 g AA equivalent/kg liveweight.....	90
Table 3.8	Period effect of Experiment 3: Area under curve (AUC), corrected for baseline concentration (AUCB) and maximum concentration (MAX) of ascorbic acid in rumen fluid and in blood plasma in period 1, 2 and 3 during 0-22 h in deer.....	92

Table 3.9	Nutritional effect in Experiment 4; Area under curve (AUC), area under curve corrected for baseline concentration (AUCB) and maximum concentration (MAX) of ascorbic acid in rumen fluid during 0 to 8 hr in deer given single intraruminal doses of AA at 2.7 g /kg liveweight either alone or mixed with NaHCO ₃ at 10:1 ratio.....	96
Table 3.10	Nutritional effect in Experiment 4; Area under curve (AUC), area under curve corrected for baseline concentration (AUCB) and maximum concentration (MAX) of ascorbic acid in blood plasma during 0 to 8 hr in deer given single intraruminal doses of AA at 2.7 g /kg liveweight either alone or mixed with NaHCO ₃ at 10:1 ratio.....	96
Table 3.11	Period effect in Experiment 4; Area under curve (AUC), area under curve corrected for baseline concentration (AUCB) and maximum concentration (MAX) of ascorbic acid in blood plasma and rumen fluid in Trial 1 and 2.....	97
Table 3.12	Experiment 6; Trial 1 and Trial 2. Mean rumen Cr-EDTA fractional outflow rate (FOR; %/hr) and rumen volume for 6 deer given single intraruminal doses of 180 ml Cr-EDTA (2.77mg Cr/ml water), either alone or mixed with 2.7 g AA /kg liveweight.....	103
Table 3.13	Mean and standard error values of rumen fractional disappearance rate, fractional outflow rate, fractional degradation rate + fractional absorption rate, FOR/FDPR ratio, half life values for FDPR and FOR for six deer given a single intraruminal dose of AA in Experiment 6, Trial 2 ...	106
Table 3.14	Mean and standard error values of rumen outflow of ascorbic acid in six deer in Experiment 6, trial 2.....	106
Table 3.15	A comparison of nutritional treatments on rumen fractional outflow rate (FOR) measured with chromium EDTA and rumen mean retention time (MRT) of liquid in deer fed lucerne chaff. Data in the present investigation for fasted deer (without ascorbic acid and with ascorbic acid administration) are compared with the data of Dominigue et al. (1991) for fully fed deer.....	114

LIST OF FIGURES

Chapter 2

Figure 2.1 Chemical changes during the development of pigment in
 meat..... 17

Figure 2.2 Effect of temperature on methmyoglobin accumulation in
 four bovine muscles from ten experimental animals 24

Figure 2.3 Chemical structure of vitamin C..... 27

Figure 2.4 Ascorbic acid synthesis pathway.....28

Figure 2.5 Relationship between time and surface metmyoglobin
 percentage in raw ground beef, dipped in vitamin E
 and ascorbic acid during 7 days at 4⁰ C31

Figure 2.6 Relationship between time and extract metmyoglobin
 percentage in raw ground beef dipped in vitamin E
 and ascorbic acid during 7 days at 4⁰ C 31

Figure 2.7 Plasma ascorbic acid concentration before and after
 infusion of 100 g sodium ascorbate via jugular vein into two
 Holstein steers (600 kg liveweight).....34

Figure 2.8 Plasma ascorbic acid concentration before and after
 infusion of 300 g sodium ascorbate via jugular vein into two
 Holstein steers (600 kg liveweight)..... 34

Figure 2.9 Concentration of ascorbic acid in rumen fluid of a cow fed
 150 g of ascorbic acid in the diet and concentration of
 ascorbic acid in an *in vitro* rumen system.....37

Chapter 3

- Figure 3.1 Experiment 1; Trial 1. Rumen fluid (A) and blood plasma (B) ascorbic acid concentration during 0 to 2 h in a deer given single oral doses of 400 g ascorbic acid.....84
- Figure 3.2 Experiment 1. Blood plasma ascorbic acid (AA) concentration in one deer (during 0 to 2 h in Trial 1 and 0 to 8 hr in Trial 2) given 100 g AA by abomasal fistula (0.82 g AA/kg liveweight) on two occasions two days apart..... 85
- Figure 3.3 Experiment 2. Mean rumen fluid (A) and blood plasma (B) ascorbic acid (AA) concentrations during 0 to 8 h in six deer given single doses of 400 g ascorbic acid (AA) through rumen fistula on two occasions two weeks apart.. 86
- Figure 3.4 Experiment 3; Mean AA concentration in rumen fluid (A) and blood plasma (B) during 0 to 22 h in 6 deer given single doses of AA, EC and SC through rumen fistula at 2.7 g AA equivalent /kg liveweight. Mean values of AA, EC and SC were obtained from 6 deer in three trials, using two 3x3 Latin squares.....89
- Figure 3.5 Experiment 3; Mean pH values in rumen fluid during 0 to 22 h in 6 deer given single doses of AA, EC and SC through rumen fistula at 2.7g AA equivalent/kg liveweight...91
- Figure 3.6 Experiment 4; Mean pH values in rumen fluid during 0 to 8 hr in seven deer given single intraruminal doses of AA at 2.7 g/kg liveweight, either alone or mixed with NaHCO_3 at 10:1 ratio.....94
- Figure 3.7 Experiment 4; Mean concentration of ascorbic acid (AA) in rumen fluid (A) and in blood plasma (B) in 7 deer given single intraruminal doses of ascorbic acid (AA) at 2.7 g/kg liveweight, either alone or mixed with NaHCO_3 at 10:1 ratio..... 95
- Figure 3.8 Experiment 5; Trial 1. Mean concentrations of ascorbic acid (AA) in rumen and abomasal fluid (A) and in blood plasma (B) during 0 to 22 h in 3 deer given single intraruminal doses of AA at 2.7 g/kg.....98

Figure 3.9	Experiment 5; Trial 1. Mean rumen and abomasal fluid pH during 0 to 22 h in 3 deer (A) and 2 deer (B) given single Intraruminal doses at AA 2.7 g/kg.....	100
Figure 3.10	Experiment 5; Trial 2. Mean blood plasma ascorbic acid (AA) concentration during 0 to 22 h in 3 deer given single doses of AA at 0.75 g/kg liveweight through abomasal fistula.....	101
Figure 3.11	Experiment 5; Trial2. Mean rumen and abomasal fluid pH during 0 to 22 h in 3 deer given single doses of AA at 0.75 g/kg liveweight through abomasal fistula.....	101
Figure 3.12	Experiment 5; Trial 1 and 2. Decay in rumen concentration of the soluble marker Cr-EDTA during 0 to 22 h in deer given a single intraruminal dose of 180 ml of Cr-EDTA (concentration of Cr-EDTA, 2.77 mg Cr/ml of water). The Cr-EDTA was administered alone after an 8 h fast (A) or mixed with ascorbic acid (AA) at 2.7 g/kg liveweight after an 8 hr fast (B) using the same deer	102
Figure 3.13	Experiment 6; Trial 1 Mean pH values of rumen and abomasal fluid in 6 deer (three, fistulated in both rumen and abomasum and three rumen fistulated only) given single intraruminal doses of 180 ml, Cr-EDTA (concentration of Cr-EDTA, 2.77 mg Cr/ml of water), a standard liquid phase marker.....	102
Figure 3.14	Experiment 6; Trial 2. Mean rumen and abomasal fluid (A) and blood plasma (B) ascorbic acid (AA) concentrations during 0 to 22 h in 6 deer given single intraruminal doses of AA at 2.7 g/kg mixed with Cr-EDTA 180 ml (concentration of Cr-EDTA, 2.77 mg/ml water).....	104
Figure 3.15	Fractional disappearance rate of ascorbic acid from the Rumen of a typical deer given ascorbic acid (2.77 g AA/kg liveweight) mixed with 180 ml of Cr-EDTA (2.77 mg Cr/ml of water) via rumen fistula.....	105
Figure 3.16	Experiment 6; Trial 2. Mean rumen and abomasal fluid pH during 0 to 22 h in 6 deer given single intraruminal doses of AA 2.7g/kg mixed with Cr-EDTA 180 ml (concentration of Cr-EDTA, 2.77 mg/ml water).....	108

Figure 3.17 Mean AA concentration in blood plasma during 0-22 h in
 deer given single doses of AA at 2.7g AA equivalent/kg
 liveweight through rumen fistula in the present study and
 suggested blood plasma concentration during the same
 time of deer given single doses of an "ideal compound"
 by the same route..... 117

LIST OF PLATES

Plate 3.1 A ruminally fistulated deer was given ascorbic acid via rumen
fistula109

Plate 3.2 Rumen and abomasal pH values were determined at the Deer
Unit soon after taking the samples..... 109

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	<i>gluteus medius</i>

h	hour
HMW	high molecular weight
kg	kilogram
LL	<i>longissimus lumborum</i>
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	<i>psoas major</i>
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