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the concentration of AA in venison, with a view to increasing colour stability and extending shelf life and from unpublished observations by these authors that it may be possible to achieve this from administering large single doses of AA before slaughter (J.M. Stevenson-Barry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 Feed was withdrawn 8 hours before AA was administered and fasting continued during the period of rumen and blood sampling (total 30 hours fasting).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

1.1. INTRODUCTION

Although deer farming is a new industry in the world, humans have a long history of game consumption. According to the archaeological evidence humans have been consuming game including venison, much earlier than today's meat such as chicken, beef, lamb and pork. It is only within the last 30 years that deer has been successfully introduced into the modern farming system in New Zealand as well as in Europe, USA and Australia. The breakdown of the New Zealand deer industry into numbers of hinds and stags and deer slaughtered per annum from 1993 to 1999 is given in Table 1.1. The New Zealand deer industry has grown more rapidly in comparison to deer industries in other countries, with 4500 registered farmers and 1.8 million farmed deer, which is the world's largest farmed deer population.

The highest demand for chilled venison is for the developing and expanding USA market. The shelf life of chilled venison is between 12-18 weeks at -1° C. based on Invermay work (Semen *et al* 1988 and 1989). The colour of chilled venison is one of the major factors in determining its attractiveness to consumers; colour deteriorates rapidly after 12 weeks of storage (Drew *et al.*, 1991) A longer colour shelf life would be advantageous to the marketing of fresh chilled meat. It has been observed by several workers that vitamin C as an antioxidant can prolong the shelf life of beef and its products (Hood, 1975; Shivas *et al.*, 1984; Okayama *et al.*, 1987; Mitsumoto *et al.*, 1991a ; Mitsumoto *et al.*, 1991b; Yin *et al.*, 1993; Lee *et al.*, 1999). Stevenson-Barry et al (1999) found that dipping venison steaks in solutions of vitamin C could similarly be used to improve colour stability and extend shelf life. They suggested that feeding treatments be devised to incorporate ascorbic acid into muscle.

This thesis investigates the effect of supplementary vitamin C in raising plasma vitamin C concentration, as part of a larger study involving the use of vitamin C to increase venison shelf life conducted by Stevenson-Barry et al (1999).

Table 1.1. The New Zealand National Deer Herd Slaughter Numbers (1993/94 to 1998/99)

June Years	93/94	94/95	95/96	96/97	97/98	98/99
			Herd Numbers			
Hind	900,000	862,000	914,000	1,050,000	1,200,00	1,280,000
Stag	512,000	533,000	563,000	610,000	590,000	573,000
Total	**1,412,000**	**1,395,000**	**1,477,000**	**1,660,000**	**1,790,000**	**1,853,000**
			Slaughter Numbers			
Hind	141,000	239,000	147,000	88,280	140,000	168,000
Stag	193,000	191,000	176,000	191,850	260,000	284,000
Total	**334,000**	**430,000**	**323,000**	**280,130**	**400,000**	**452,000**

Source- New Zealand Game Industry Board (NZGIB), Statistics Summary, 2000

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CHAPTER 2

LITERATURE REVIEW

2. VENISION PRODUCTION FROM FARMED DEER IN NEW ZEALAND

2.1. PROGRESS OF DEER INDUSTRY IN NEW ZEALAND

Deer are not native to New Zealand and mostly European red deer (*Cervus elaphus*) were introduced here for sport from England and Scotland in the latter half of the 19th century (and watipi in the early 20th century). They multiplied rapidly due to the warmer climatic condition in New Zealand and went wild. They spread and developed well in the native bush and forest in both islands of New Zealand. A small number of the tropical species of deer such as rusa (*Cervus timorensis*) and sambar (*Cervus unicolor*) were introduced into the North Island. Deer were considered pests in the 1940s and 1950s because of their negative impact on the environment and native forests.

The export of feral deer products started in the 1960s, changing a pest into an export earning industry. In early 1970s, industry pioneers recognised the value of this animal and captured them for farming. From that time onward the deer industry spread throughout New Zealand. The development of the deer farming industry in New Zealand from its inception 30 years ago has progressed remarkably, with 4500 registered deer farmers and 1.8 million animals. These consist of 85% European red deer with the remainder being elk (*Cervus elaphus canadensis*), elk/red deer crossbreds, fallow deer (*Dama dama*) and a small number of other species (Barry and Wilson 1994). New Zealand has the largest global farmed deer population and also is one of the most advanced deer farming industries in the world and accounts for over 45% of the total global population (Table 2.1). New Zealand was most probably one of the last countries on earth to feel the tread of deer feet, yet they were the first to initiate a viable deer farming industry with modern processing facilities and strong marketing operations needed to sustain an export industry of venison and velvet.

Table 2.1. Total number of farms and deer population of the major deer farming countries (as at June1999)

Country	Farm Numbers	Farmed deer Population
Australia	1,700	40,000
Denmark	650	31,000
France	907	58,000
Germany	4,500	104,000
Great Britain	4,500	104,000
Ireland	600	61,000
Sweden	570	26,000
US	NA	250,000
Canada	1,700	100,000
China	NA	500,000
Russia	NA	400,000
Korea	NA	100-200,000
Taiwan	NA	36,000
Australia	1,200	200,000
New Zealand	4,500	1.8 million
Total		3,860,000

(From the web site- http://ourworld.compuserve.com)

2.2. FORMATION OF NEW ZEALAND GAME INDUSTRY BOARD (NZGIB) AND MARKETING OF NEW ZEALAND VENISON

As a result of the formation of the NZGIB in the mid 1980s the marketing sector (venison, velvet and other products) strengthened in New Zealand. Before the creation of NZGIB the export of venison and other products were small and the

industry lacked the capital to finance major market thrusts. Now the NZ deer industry has grown in number, gained experience over the years and has become a strong export market-led industry that differs from the NZ cattle and sheep industries. The New Zealand Game Industry Board's function is to assist the orderly development of the deer industry. This includes; market research, branding marketing strategy for venison and quality assurance programmes.

2.2.1. Market research

This is to analyse the present and future markets and also to determine what the market requires and when. This information will enable the development of production systems to fulfil the market requirement. The idea behind this is to have a market-led instead of a production driven deer industry. This type of marketing strategy is a very strong feature of the deer industry in New Zealand.

2.2.2. Branding marketing strategy for venison

Another feature is the development of the branding market strategy. The branded product ''Cervena'' strategy is designed to develop new markets. Cervena is natural tender venison, produced from New Zealand farmed-raised deer produced in a clean, green, nuclear-free environment. The deer are raised naturally, no steroids are used and antibiotics are given only as a remedy to injury. All Cervena deer are three years old or under to ensure consistent size, flavour, texture and taste of the meat. At present Cervena is targeted in USA and Canada, and in New Zealand and Australia. This brand is promoted as a high value product to be served in expensive restaurants and hotels. The creation of the Cervena appellation in 1990 under NZGIB auspices was initiated to strengthen the marketing strategy in North America and Australasia. Returns from the USA have seen slow but steady growth and stable price for Cervena. Consumption of farmed raised venison from New Zealand in the USA has increased 120% since 1991. About 85% of all venison served in restaurants in the US comes from New Zealand. The trademark Cervena TM is protected globally and this cannot be used by a deer industry of any other country.

The trademark "Zeal" is designed for existing commodity markets, such as Germany. The idea of this is to differentiate New Zealand farm produced venison from feral venison and from farmed venison produced by other countries. Other marketing plans in Europe include the "New Zealand" brand launched in late 1999 to penetrate the top quality hotels and restaurants in the same way as Cervena has done in the USA.

2.3. EXPORT MARKET FOR NEW ZEALAND VENISON

New Zealand venison is in demand in a range of countries (Table 2.2). Germany is the deer industry's largest single market for New Zealand venison (frozen + chilled) and accounts for 62.5 million NZ$ (43% of total export earning from venison) in 1999, followed by Belgium and USA (Table 2.2). Germany is the dominant market for NZ frozen venison, whilst the USA, Germany and Belgium are the main markets for chilled venison. Financial returns from chilled venison are smaller than from frozen venison, but are steadily growing.

New Zealand dominates the international trade in venison, and is also the highest in terms of world venison production. The total venison production in the year to August 1999 in New Zealand was 15,176 tonnes (NZGIB 2000) (Table 2.3). The total deer industry export earnings were 179.2 million NZ$ during the same period, with venison comprising 73% by value of the total exports.

Table 2.2 Major export markets for frozen and chilled New Zealand venison in 1998 (September years) and 1999 (September years) (NZGIB Export Report 2000) Principal markets are underlined

	1998 Frozen		1999 Frozen	
	Kg	**NZ$**	**Kg**	**NZ$**
Belgium	1,366,923	13,567,962	1,076,05	9,319,465
Denmark	184,314	2,145,992	154,563	2,034,479
France	953,157	7,603,369	976,794	4,977,555
Germany	7,862,136	56,095,655	7,275,749	49,945,826
Japan	112,556	1,468,609	80,913	1,911,926
Netherlands	826,923	6,321,243	383,656	3,049,820
Sweden	942,048	6,222,577	657,251	4,822,171
Switzerland	327,309	3,296,520	173,457	1,773,417
USA	391,317	4,653,278	459,242	5,276,420
Other Countries	986,336	8,530,869	250,090	2,935,694
Total	13,953,019	109,906,174	12,391,311	90,314,145

	1998 Chilled		1999 Chilled	
	Kgs	**NZ$**	**Kgs**	**NZ$**
Belgium	550,835	8,504,320	450,553	6,440,159
Denmark	1,276	12,413	1,364	31,346
France	125,315	1,174,621	135,758	1,142,922
Germany	468,720	6,409,575	864,046	11,372,662
Japan	30,751	725,093	23,332	655,715
Netherlands	51,284	777,557	125,353	2,310,911
Sweden	0	0	0	0
Switzerland	83,385	1,603,653	133,024	2,460,375
USA	514,373	12,646,399	565,758	13,104,678
Other Countries	139,969	2,280,442	38,128	890,788
Total	1,965,908	34,134,073	2,455,368	39,956,464

Table 2.3. Total export volume and value of venison and other products (in the year to August 1999)

Product	**Volume**	**Value (NZ$million, FOB)**
Venison	15,176 tonnes	130.6
Co-products	1015 tonnes	11.6
Velvet	210 tonnes (dried)	25.9
Skins	575,579	11.1
Total		179.2

Source- New Zealand Game Industry Board, Export Summary, 2000

2.3.1.Chilled venison

Chilled venison is preferred to frozen venison by many consumers dining in up-market restaurants, and it is the New Zealand deer industry policy to increase this segment of the market. Therefore the shelf life for sea freight (most of the exporters prefer sea freight to Europe) should be at least 12 weeks at – 1° C, and 16 weeks would be preferred. Semen *et al.*, (1989) found that vacuum packaged venison with a low bacterial count at packaging stored at -1^0C can achieve a shelf-life of 12-18 weeks. However colour stability (i.e. the time taken to turn from red to brown after the vacuum package is opened and muscles cut into steaks) deteriorates with time in a vacuum package (Semen *et al.*1989; Drew *et al.*, 1991). These studies found that tenderness was acceptable but flavour tended to deteriorate from 12-18 weeks. If venison is cut and displayed at 0^0C, it has an acceptable shelf-life of 5 days after one week storage and two days after 18 weeks storage, losing approximately one day with each 6 weeks of storage. Recent studies conducted with venison at 5^0C indicate that even after just one week of storage in a vacuum package at -1^0C venison has an acceptable shelf-life of only about 2 days (Trout *et al.*, 1996 and Stevenson-Barry, unpublished data. After 12 weeks in a vacuum package, this display life decreases to approximately 0.5 days(Stevenson-Barry, data in patent application).

In the past, all exported New Zealand venison had to be frozen. It is now possible for more than 16% of total New Zealand venison to be shipped chill packed. According to the information from NZGIB, USA was the largest market for New Zealand chilled venison followed by Belgium and Germany (Table 2.2). The values for chilled venison in 1999 were $13.1 million, $11.3 million and 6.4 million for the USA, Germany and Belgium respectively (Table 2.2).

2.4. NUTRITIVE VALUE OF VENISON

The current situation is that meat consumption patterns are now perceived by most people as closely linked with healthy or unhealthy characteristic of the meat. Nutrient related health problems are due to the high consumption of fat, saturated fatty acids and cholesterol (NRC 1988). Red meat contains all these items therefore consumers are curious receive nutritional information about red meat. Therefore it is worthwhile to know the nutrient content of venison. Nutritional information for beef, pork, lamb and chicken is already available.

Studies have been carried out at Invermay Agricultural Research Centre to analyse the protein, fat, water, mineral, fatty acids and cholesterol (Drew and Seman, 1987) in venison from 10 yearling deer (loin and leg) compared with similar analyses from lamb (Chrystall and Winger, 1986). They found that venison leg and loin had very low fat and energy levels while it had very high levels of protein, minerals and water content compared to lamb (Table 2.4). Venison contains about 35% more protein than lamb and it also shows that lamb has four to five times more fat and close to twice the amount of metabolizable energy compared with venison (per 100g). (Table 2.4)

Mineral composition of venison in loin and leg cuts, was found in higher amounts compared to lamb loins (Drew and Semen, 1987) (Table 2.4). Iron content of venison was almost three times higher than that of lamb. Venison and lamb both had low calcium and magnesium (Table 2.4). Recommended daily allowances (RDA) per 100 g serving of venison provides 10% of copper, 38% of iron, and 18% of zinc

whereas 100 g of lamb contributes only 3% of copper, 15% iron and 15% of zinc (National Research Council).

Table 2.4 Major nutrient and energy content of venison and lamb (g/100 g ±SD)

	Venison (n=10)		Lamb (n=30)	
	loin	leg*	loin* *	leg* * *
Protein	24.7±1.09	23.8±0.74	17.4±0.63	18.4±0.81
Fat	3.3±0.53	3.0±0.26	18.2±3.00	13.6±3.65
Water	70.8±0.91	71.2±0.58	63.6±6.09	65.7±3.02
Iron (μg)	3820±740	3900±490	1403±87	
Calcium (mg)	5±2	3±0.5	14±6.5	
Magnesium (mg)	25±2	29±5	19±1.0	
Total Minerals (mg)	1.4±0.38	1.9±0.78	0.96±0.04	
ME (kj/100 g)	545	519	969	814

* Untrimmed M. *Semimembranosus*

* * Raam lambs (Chrystall and Wigner, 1986)

* * * Soft tissues from wether lambs (Chrystall and Winger 1986)

From Drew and Seman, 1987

Cholesterol content of venison is lower than that of most of the other meats as shown in Table 2.5 (Drew and Seman, 1987). Although comparison is difficult in cooked and uncooked meat, in general venison has a lower cholesterol content than beef and chicken (Drew and Seman 1987) (Table 2.5).

Table 2.5 Cholesterol content of venison and other meat

Meat Type	Portion	Cholesterol (mg/100 g)	Reference
Caribou	Cooked serving	111	USDA, 1979 unpubl.
Beef- shoulder	Cooked lean and fat	103	USDA, 1980 unpubl.
- rib	Cooked lean and fat	82	USDA, 1980 unpubl.
Chicken-dark &light meat	Cooked without skin	94	USDA, 1979
-breast	Cooked without skin	85	USDA, 1979
Elk	Cooked unspecified	81	USDA, 1979 unpubl.
Venison	Cooked unspecified	77	USDA, 1979 unpubl.
Venison- loin	Uncooked	66	Drew and Seman, 1987
Venison – leg	Uncooked	74	Drew and Seman, 1987

From Drew and Seman (1987)

Essential amino acids particularly leucine and lysine as well as thiamine content were high in venison compared to other meats (Uherova *et.al*, 1992). Venison has all the good attributes of red meat, being high in protein, iron and copper; low in saturated fatty acids, energy and cholesterol, very low in total fat and has a relatively high polyunsaturated: saturated fat ratio compared to other red meat (Drew and Seman, 1987) Therefore venison can be a healthy red meat with all these excellent qualities for the new millennium.

2.5. RELATIONSHIP BETWEEN COLOUR STABILITY OF MEAT AND FORMATION OF METMYOGLOBIN

One of the most important factors involving the consumer acceptance of meat is colour (Faustman and Cassens, 1989; Manu-Tawiah *et al.*, 1991). The major pigment of fresh meat is myoglobin and the appearance of muscle tissue is determined by the oxidation state of this pigment (MacDougall, 1977). The rate of meat discolouration during refrigerated display is extremely variable.

2.5.1. Myoglobin structure

Myoglobin is a monomeric, globular haem protein with a molecular weight of approximately 17 000. It possesses a haem prosthetic group, which is responsible for oxygen binding and confers an intense red or brown colour on the protein (Hood, 1984). The reactive haem group contains a ring of four pyrollic nuclei co-ordinated with a central iron atom. Bonding of the iron with the tetrapyrollic ring structure satisfies four of the six coordination position of the atom. The fifth is coordinated to an imidazole residue within the protein structure. The remaining sixth position is available to bind with high-field ligands including oxygen (Hood, 1984).

2.5.2. Derivatives of myoglobin and auto-oxidation reaction

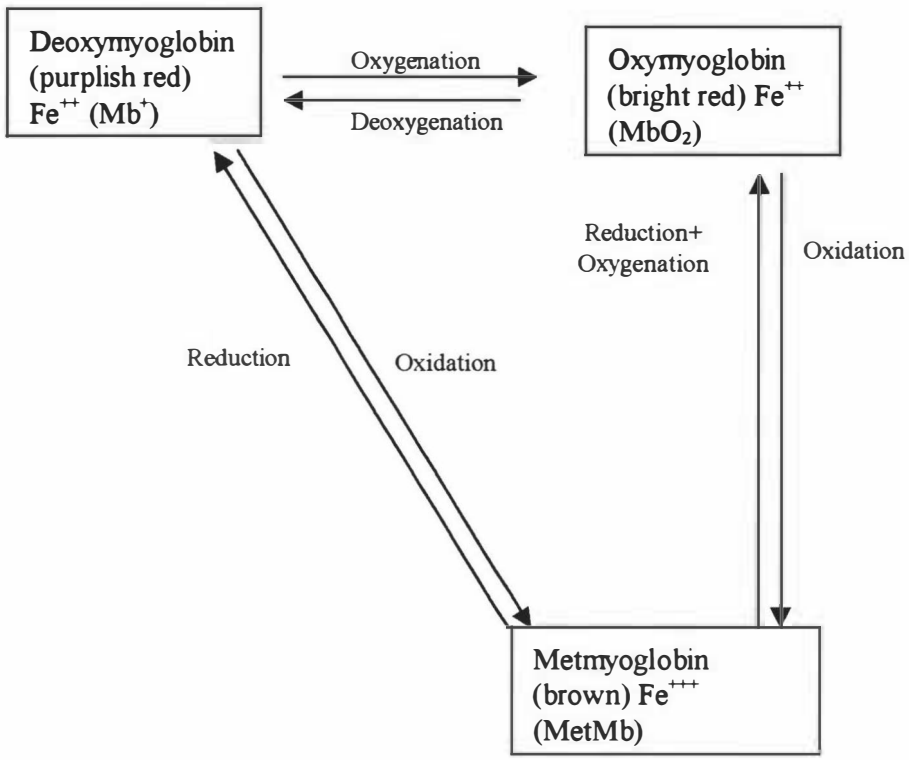
Fresh meat colour is judged by the relative amount and depth of the three derivatives of myoglobin- bright red oxymyoglobin (MbO_2), purple reduced myoglobin or deoxymyoglobin (Mb^+) and brown coloured metmyoglobin (MetMb). The colour of the meat is related to the relative amounts of these pigments (Hood and Mead, 1993). Due to exposure to air, reduced myoglobin combines with oxygen to give a surface layer of bright red MbO_2 (with iron on the haem group in its ferrous state) which is linked to the colour of fresh meat and consumers are considered to be attracted by this. When the meat is kept under refrigeration, the original bright red colour becomes greenish-brown and this discolouration of meat is not attractive to the consumer. This colour change is due to the spontaneous autoxidation of both $Mb^+$and MbO_2 to MetMb with iron on the haem group in its ferric state (Hood and Riordan,

1973; Faustman and Cassens, 1990; Renerre, 1990; Hood and Mead, 1993). The chemical changes that can occur to myoglobin during the development of the pigment in meat are given in Figure 2.1.

The oxygenation of myoglobin is rapid and reversible, and the fraction of the pigment in the oxygenated form increases with high oxygen concentration (Forrest *et al.*, 1975). However, MetMb is stable, and is changed to Mb^{+} very slowly, by enzyme-mediated reactions known as metmyoglobin reduction activity (Ledward, 1985). Muscles vary largely in metmyoglobin reduction activity and the muscles which have high methmyoglobin reduction activity such as *longissimus dorsi*, are normally colour stable in air, and their red colour is stable 3 or 4 times longer than that of colour-unstable muscles of low metmyoglobin reduction activity, such as *psoas major* (O'Keeffe and Hood 1980-81). It was also reported by Moore and Gill (1987) that, reduction activity decreases during the storage of muscle. After a long period of storage of meat, the colour of initially colour-stable muscles is similar to that of those muscles, which were initially of relatively poor colour stability.

It was suggested by Renerre and Labas (1987) that the discolouration rate of meat depends on the depth to which oxygen can penetrate into the tissue and initiate the oxygenate reaction of myoglobin. The rate of discolouration of meat according to Faustman and Cassens (1989) is believed to be associated with the effectiveness of oxidation processes and enzymatic reducing systems in controlling metmyoglobin levels in meat. The oxidative change of haem pigments (MetMb formation) may be initiated by bacterial, enzymatic or by lipolytic action or by any conditions that reduce the partial pressure of oxygen (Shivas *et al.*, 1984).

Figure 2.1 Chemical changes during the development of pigment in meat (From Judge M.D. *et al.*, 1989).



When MetMb comprises about 20% of the surface of meat, sales decrease by a factor of 2 and this is one of the major problems in the meat industry (Renerre and Mazuel 1985). It was noted that at a level of 20% MetMb the ratio of sales of bright red beef to discoloured beef was 2:1 and that there was a noticeable discrimination against discoloured meat, even at lower levels of MetMb (Hood and Riordan 1973).

2.5.3.Colour stability of different types of meat

The relationship between the rate of metmyoglobin formation in the surface layer of meat and the colour stability of beef, pork and venison muscles were studied (Gutzke *et al.*, 1997). They found that the colour stability of these meats was highly correlated to the formation of the MetMb layer of the meat (Table 2.6).

Colour stability of pork (159.7 h) and beef (143.6 h) was three times higher than that of venison (50.4 h). Formation of the MetMb layer (h) was also observed and a different trend was found in pork (144.0 h) and beef (90.0 h) compared to venison (28h). Gutzke *et al.*, (1997) concluded that differences in the colour stability of meat may be related to the rate of MetMb formation at the surface of muscles with this occurring much faster for venison than for beef or pork.

Table 2.6 The relationship between the colour stability and the formation of a metmyoglobin layer at the surface of venison, beef and pork (*Longissimus dorsi*) muscles during refrigerated storage at 5^0 C

	Colour stability versus formation of MetMb layer in meat	
Species	Colour stability of meat (i.e. display-life;h)	Formation of MetMb layer (h)
Venison	50.4 (6.2)	28.0 (8.1)
Beef	143.6 (5.1)	90.0 (8.1)
Pork	159.7 (4.4)	144.0 (8.1)

Terms in brackets are standard errors of least square mean

MetMb- methmyoglobin (From Gutzke *et al.*, 1997)

2.5.4. Factors involved in the discolouration of meat

As little information is available on this topic for venison, an account is given using other meats, notably beef, to illustrate the general principles. A final concluding section is then given, discussing the relevance of these findings to venison.

The rate of MetMb formation during storage on the meat is related to many intrinsic and extrinsic factors (Renerre 1990). The intrinsic factors are pH values, muscle type, animal age, breed, sex and diet, and the extrinsic ones are pre-slaughter treatments,

hot-boning, electrical stimulation and chilling mode. Apart from these, during retail display, physical factors such as temperature, amount of oxygen availability, type of lighting, microbial growth on surface of meat and storage pattern (air, modified atmosphere, vacuum) also influenced the saleable life of red meat. Oxidation of fresh meat to MetMb is affected by the reducing capacity of the muscle, oxygen availability (oxygen penetration and tissue respiration) and also the myoglobin autoxidation rate (Renerre and Labas, 1987).

The rate of discolouration of different meats during refrigerated display is extremely variable (Hood 1980). One explanation for this intrinsic difference is the variation in concentration or activity of reducing compounds or reducing enzymes or reducing systems present in meat (Reddy and Carpenter, 1991). The role of these reducing systems *in vivo* is to decrease the amount of any metmyoglobin formation and to maintain the myoglobin in its reduced form so that it can bind oxygen (Hagler *et al.*, 1979).

Trout *et al.* (1996) studied the *in vitro* antioxidative effects on purified myoglobin of high molecular weight (HMW) and low molecular weight (LMW) soluble fractions extracted from muscles from pork, beef and venison. They also studied how the antioxidative properties of these fractions changed with the time. (Table 2.7). It shows that the LMW fraction of myoglobin from all three animals produced similar inhibitory effects on myoglobin oxidation throughout seven days refrigerated display. They found that the inhibitory effect did not change with time ($p>0.05$) and it was between 36% and 43% in all species (Table 2.7). The results were different with the HMW fractions and there was a significant difference ($p<0.05$) in the inhibitory effect between the species, initially (20% for venison and 80% for both beef and pork) and the change in inhibitory effect over time. Compared to pork and beef, the inhibitory effect of the venison HMW fraction, decreased from 20% at day 0 to -40% at day 7 (Table 2.7)

Table 2.7 Changes in inhibitory effects on myoglobin oxidation from pork, beef and venison during refrigerated display

	Species	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
Inhibition %								
LMW*	Pork	40	38	37	38	36	35	36
	Beef	40	43	40	39	38	37	37
	Venison	34	36	35	34	34	33	34
Inhibition %-								
HMW**	Pork	80	55	55	57	60	38	-5
	Beef	80	55	53	57	47	38	-6
	Venison	20	30	7	3	-18	-20	-40

LMW*- low molecular weight, HMW**-high molecular weight

From Trout *et al.*, (1996)

2.5.4.1. Inter-muscular and inter-animal variability

It was stated by Hood (1980) that inter-muscular variability is the most important single factor, which controls the rate of discolouration in pre-packed beef. It varies from the stable *M. longissimus dorsi* to the unstable *M. psoas major*, the degree of discolouration of the latter muscle being almost eight times higher than the first one after 96 h storage at 0 °C. He studied the discolouration of four muscles namely *M. longissimus dorsi*, *M. psoas major*, *M. semimembranosus* and *M. gluteus medius*, after 96 h at 0°C, 5°C and 10°C and presented the results as percentage components of total variance. Results of an analysis of variance of $(K/S)_{572}/(K/S)_{525}$ (the ratio of K/S values at 572 nm and 525 nm gives a measure of the metmyoglobin content at the surface of the meat) data from four muscles are presented in Table 2.8. It shows that the muscle effect was most important and accounts for about 45% of the total variation. The effect of temperature was also highly significant ($p< 0.001$), accounting for 32.5% of the total variation (table 2.8). The animal effect was also highly significant ($p< 0.001$) but only accounting for 7.3% of the total variation. The intrinsic factor affecting muscle constitution, between animal variability is probably

the least understood (Lawrie 1985). The muscle×temperature interaction was a significant effect ($p < 0.05$), but represents only 3.5% of the total variation. Among nine muscles removed from 10 beef carcasses of different age, sex and breed showed that at 192h post-mortem, *M. longissimus dorsi*, *obliques externus* and *tensor fasciae latae* were the most stable ones, *M. semi-membranosus* was intermediate whereas *M. gluteus medius, supra-spinatus, triceps brachii caput longum, psoas major* and *diaphragma medialis* were the least stable (Renerre 1984).

Inter-animal variability is also an important intrinsic factor, which can significantly influence the discolouration rate between small groups of test animals (Renerre, 1990). Although a small percentage of the total variation observed by Hood (1980), under different experimental conditions, a highly significant ($p<0.01$) effect (wholly comparable to that in muscle) was observed on both colour characteristics and colour stability (Renerre, 1984).

Table 2.8 Components of variance for discolouration from ten bovine animals after 96 hr storage at 0, 5 and 10 °C

Effect	% of total variation	F-test significance
Animal	7.3	* * *
Muscle	45.5	* * *
Temperature	32.5	* * *
Muscle x temperature	3.5	*

* $p< 0.05$ *** $p< 0.001$

From Hood (1980).

2.5.4.2. Effect of pH.

The rate of fall of pH *post- mortem* and the ultimate pH value reached by a muscle can have an effect on rate of discolouration and the colour of a specific muscle (Renerre,1990). Rate and extent of pH fall are decided by intrinsic factors such as species, breed, muscle, breed and animal variability as well as by extrinsic factors such as environmental temperature and degree of stress (Lawrie 1985). High pH encourages microbial growth and significantly reduces the keeping quality of meat (MacDougall, 1982). High pH values of the meat increases the respiratory activity of the tissue, and, with a very tiny layer of red MbO_2, the underlying reduced myoglobin is more visually apparent and is more red-purple. When the rate of pH fall is very fast or when the ultimate pH is very low, the rate of Mb oxidation is enhanced and the formation of MetMb leads to a low colour intensity meat (Tarrant, 1982). Low pH causes denaturation of the globin moiety and dissociation of oxygen from the haem (Renerre, 1990).

Mean pH values for individual beef muscles vary from 5.58 for *M. gluteus medius* to 5.73 for *M. semimembranosus* (Table 2.9). Hood (1980) reported that more than 90% of pH values of all muscles showed a very small range of 0.4 units from 5.41 to 5.80.

Table 2.9 Mean pH of beef muscle stored 10-20 days post-portem at 0° C

Muscle	Ultimate pH	SE	N
M. longissimus dorsi	5.64	0.013	142
M. psoas major	5.69	0.010	137
M. gluteus medius	5.58	0.010	137
M. semimembranosus	5.73	0.009	125
M. semitendinosus	5.62	0.026	23
M. varsitus lateralis	5.65	0.016	22

From Hood (1980).

2.5.4.3. Effect of temperature

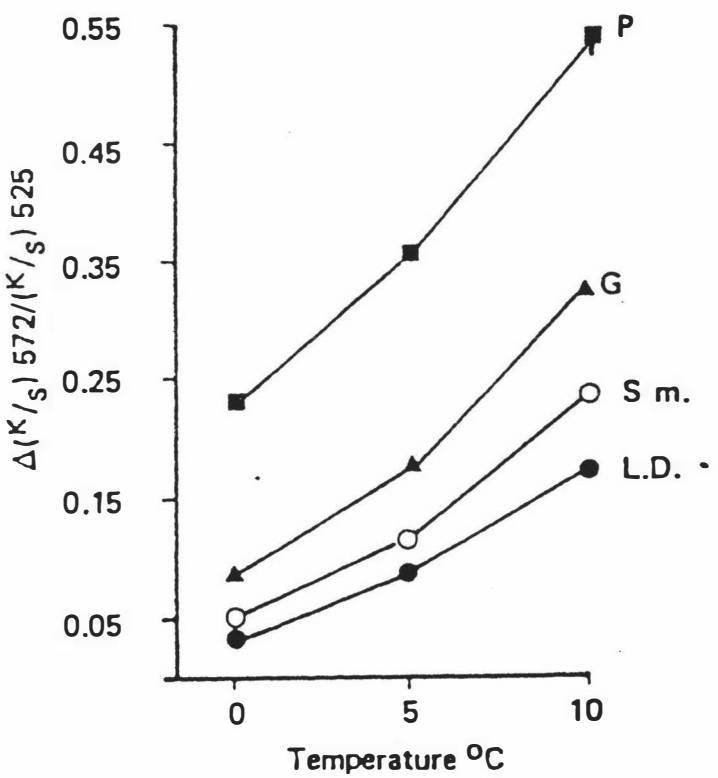
High temperature increases the colour instability of meat due to the increased effect on myoglobin oxidation and as a result the rate of methmyoglobin accumulation increases and also promotes bacterial growth. High temperatures will move the intermediate brown MetMb layer between MbO_2 and Mb closer to the surface and as a result MetMb becomes more visible with increasing display time. It was observed by O'Keefe and Hood (1980-1981) that the discolouration rate at 10°C is two to five fold higher than at 0°C. Other results have shown that the discolouration rate of packaged beef was nearly doubled at 5°C. Therefore pre-packaged meat should be stored closer to 0°C to obtain a longer shelf life. A lower temperature (0°C/5°C) improves colour and colour stability either by a brighter initial blooming and/or by a decrease in the fading rate (MacDougall 1982). Higher temperatures are also responsible for the loss of globin moiety's function of protecting the haem, resulting to in an increased tendency for autoxidation (Lawrie, 1985). A comparison of the discolouration [(K/S)572/(K/S)525 ratio, used as the measure of discolouration] for four muscles namely, *M. longissimus dorsi, M. psoas major, M. semimembranosus* and *M. gluteus medis*, after 96 h at 0°C, 5°C C and 10°C were studied (Hood 1980). The analysis of variance of the data (Table 2.8) showed that the effect of temperature was highly significant ($p< 0.001$) and accounted for 32.5% of the total variation in discolouration. *M. longissimus dorsi* was the most stable muscle, followed by *M. semimembranosus, M. gluteus medis* and finally *M. psoas* major, the discolouration being almost eight times higher in *M. psoas majo*r than in *M. longissimus dorsi* at 0°C after 96 h storage (Figure 2.2). Packaging of high pH meat under CO_2 with an O_2 scavenger gave better results than vacuum packaging and gives a shelf life up to 42 days and 48 h exposure in air with a more complete inhibitory effect on spoilage flora and a lesser myoglobin oxidation (Rousset and Renerre, 1990).

2.5.4.4. Effect of oxygen pressure

The bright red colour of fresh meat is determined by the depth of MbO_2, which depends on the rate of O_2 diffusion, O_2 consumption, and also by the O_2 pressure at the meat surface (Giddings, 1974). The rate of oxidation increases with decreasing

O_2 pressure. The maximum rate of MetMb occurred when the oxygen partial pressure was between 6-7.5 mmHg depending on pH and temperature (Georges and Stratman, 1952). Prevention of autoxidation can be met by the elimination of MbO_2, vacuum packaging in an O_2-impermeable wrap, which will help to decrease the effective O_2 tension to nearly 0. Alternatively, if meat is held under high O_2 containing atmosphere (above 30mmHg) autoxidation of Mb is minimized and does not depend on the partial pressure of O_2 (Taylor, 1985; Renerre, 1988).

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



P- *M. psoasmajor* G- *M. gluteus medis*

Sm- *M. semimembranosus* LD- *M. longissimus dorsi*

From Hood, 1980

2.5.4.5. Type of packaging

Meat is packaged in modified atmospheres with nitrogen, carbon dioxide, oxygen, alone or in mixtures (Taylor, 1985; Renerre, 1988). Use of gases such as nitrogen and/or carbon dioxide in retailing beef meat is not very important as they do not improve the red display colour but the advantage of oxygen is that it develops a thick layer of MbO_2 pigment at the surface, which is now exploited commercially. It was found that packaging in more than 50% O_2 improves colour shelf life, and this is more effective for muscles which are more prone to rapid discolouration (Renerre, 1990). When CO_2 and 0_2 combination were used, the colour enhancing effect of high O_2 concentration and the bacteriostatic effect of CO_2 can greatly preserve meat colour (Clark and Lentz, 1972). When 80:20% O_2-CO_2 combination was used, red meat keeps up to 15 days at +4°C without being spoilt Ledward (1983). A separate study suggested that a combination of 75-85% O_2 + 25-15% CO_2 is the most effective but off odours and rancidity have been observed (Taylor 1985). Regarding colour, combination of CO_2-O_2-N_2 was not efficient as CO_2-O_2, which gives the best colours with over 60% oxygen (Taylor 1985).

2.5.4.6. Effect of freezing

Although freezing is an excellent way of preserving meat when properly carried out, it can be associated with some deterioration of meat quality. The deterioration of frozen meat during storage and display leads to rejection of the product by the consumer if the meat is not bright red. This is a prominent discrimination among the consumers especially when fresh and frozen meat are displayed together. The colour of frozen meat is controlled by many factors such as freezing rate, the storage temperature, and the intensity of the light during display and method of packaging (MacDougall, 1982). Meat, which is frozen slowly, is normally bright in colour, whereas quick frozen meat in liquid nitrogen is unnaturally pale in colour. The attractiveness of frozen meat lasts for many months, if meat is stored in the dark. For example, at -18°C, the colour remains attractive for two months in the dark but it lasts only three days in the light (MacDougall, 1982).

2.6. ASCORBIC ACID (VITAMIN C) AS AN ANTIOXIDANT TO DELAY THE DISCOLOURATION OF MEAT

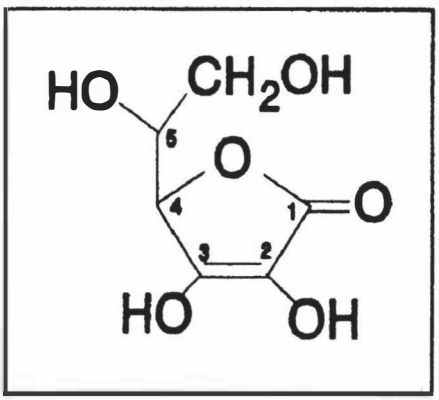
Many researchers have used ascorbic acid (AA) to extend the colour and shelf life of intact beef, lamb, pork or chicken cuts and ground meat (Greene *et al.*, 1971; Harbers *et al.*, 1981; Rikert *et al.*, 1957; Shivas *et al.*, 1984; Okayama *et al.*, 1987; Hood, 1975; Mitsumoto *et al.*, 1991(a,b and c); Liu *et at.*, 1994; Schaefer *et al.*, 1995; Lee *et al,* 1999). Very little work has been carried out to investigate the relationship between the colour of venison and AA (Stevenson Barry *et al.* 1999), therefore an account of the use of AA to improve the colour stability of other meats (mainly beef) is given in this chapter.

2.6.1. Chemical structure of vitamin C

"Vitamin C" is the general descriptor for all compounds exhibiting qualitatively the biological activity of ascorbic acid. The oxidized form of ascorbic acid is called "L-dehydroascorbic acid or dedydroascorbic acid". Vitamin C is a colourless, crystalline, water-soluble compound that has acidic and strong reducing properties. Although vitamin C is heat stable in acid solution it is easily decomposed in the presence of alkali. The destruction of vitamin C is enhanced by the exposure to light (McDonald *et al.*, 1995). In this thesis, vitamin C will be referred to as ascorbic acid (AA).

Figure. 2.3 Chemical structure of vitamin C

Essential features of the chemical structure:



I. 6-carbon ***lactone***
II. ***2,3-endiol*** structure

From Combs,Jr., G.F. (1991).

2.6.2 Functions of ascorbic acid in the body

Ascorbic acid (AA) is the most powerful reducing agent available to cells and is of general importance as an antioxidant, because of its high reducing capability (Friedrich, 1988). L-ascorbic acid is synthesized in almost all mammals except in humans, several other primates and guinea pigs (Horning, 1975). Calves and perhaps lambs do not synthesize AA until they are 3 weeks of age and meet their AA requirement from the diet during this period (Palludan and Wegger, 1984). Ascorbic acid is synthesized in the body from glucose, via glucuronic acid and gulonic acid lactone; the enzyme L-gulonolactone oxidase (which is the last step in AA synthesis)

is required for the synthesis of AA (Fig.2.4). In some animals, however, a deficiency of L-gulonolactone oxidase leads to the need for a dietary source of AA.

Figure 2.4 Ascorbic acid synthesis pathway

Galactose←——→Glucose——→ D-Glucuronic acid——→ L-Gulonic acid——→

L-Gulono-γ-lactone ——1→L-2-Oxogulono-γ-lactone—2——→Ascorbic acid

1. L-Gulonolactone oxidase
2. Spontaneous

From Rucker and Morris, (1977)

Ascorbic acid functions by contributing either one or two electrons to more oxidized neighbouring species. Ascorbic acid has an antioxidant function and it can reduce ferric (F^{+++}) to ferrous (Fe^{++}) iron and also react with the superoxide radical (O^{-}) and hydroxyl radical (OH^{-}) and scavenge singlet oxygen (Halliwell and Gutteridge, 1985).

Ascorbic acid also has many other functions such as involvement in electron transport reactions, synthesis of collagen proteins, microsomal hydroxylation reactions of drugs and steroid metabolism, tyrosine metabolism, metal ion metabolism, enhancing iron bioavailability, inhibiting histamine release and enhancing its degradation and it is also involved in the immune system in different ways (Rucker and Morris, 1997).

2.6.3. Effect of ascorbic acid on colour stability of meat

Several studies have been conducted to determine the effect of AA provided by three possible routes, although the relative efficacy of these routes has not been observed. Mitsumoto *et al.* (1991b) and Okayama *et al.* (1987) worked on fresh unground

meat, dipped in an aqueous solution of AA while Mitsumoto *et al.* (1991 a), Shivas *et al.* (1984), Benedict (1975) and Greene *et al.* (1971) exogenously added AA into ground beef. The third route involving intra-venous administration was investigated by Hood (1975). He administered 500 ml of 50% AA solution into young heifers 10 minutes before slaughter. Hood (1975) proposed that surface application of AA to intact muscle cuts may be less effective than antemortem infusion, as the reducing agent is not likely to be in intimate contact with MetMb which forms at the low partial pressures of O_2 below the surface of meat.

Hood (1975) studied effects on MetMb concentration in four beef muscles namely, *M. psoas major, M. gluteus medius, M. semimembranosus and M. longissimus dorsi* from 10 beef cattle, intravenously injected pre-slaughter with sodium ascorbate. This was compared with similar muscles from 10 control cattle, at 0°C and 5°C. His observation was similar to the results obtained from Liu *et al.*, (1994). Meat from sodium ascorbate treated cattle had significantly better colour stability, particularly *M. psoas major* and *M. gluteus medius* (Table 2.10). Accumulation of MetMb pigment, and thus the rate of colour deterioration, was reduced in ascorbate treated meat at 5°C compared to that of meat from control animals for three(*M. psoas major, M. gluteus medius, M. semimembranosus*) of the four muscles tested, after 6 days of storage (Table 2.10). The most important finding was that at this temperature, and at 6 days of storage time, the differences were more statistically significant (control versus ascorbate treated) especially in *M. psoas major* and *M. gluteus medius*, muscles which are highly unstable with respect to colour. Meat from *M. longissimus dorsi* normally has better colour stability and less discolouration problems compared to other muscles. This was proved in this experiment where the colour of *M. longissimus dorsi* from both control and treated meat was still acceptable after 6 days storage, even at 5°C (Table 2.10).

In another study, Mitsumoto and his co-workers (1991a) studied the effect of dipping in AA solution, vitamin E solution and their combination on pigment and lipid stability in raw ground beef during 7 days illuminated display at 4°C(Figures 2.5 and 2.6). They found the oxidation of pigments and lipid were greatest in controls

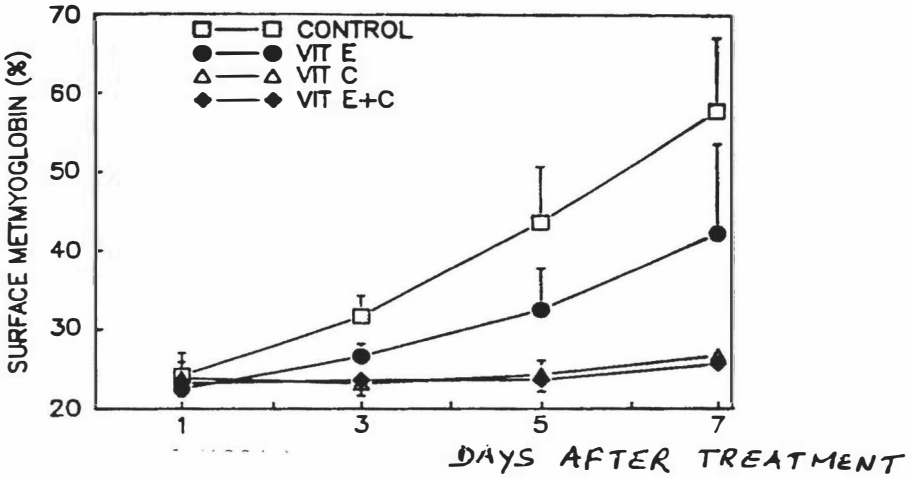
whereas vitamin E treated meat showed moderate oxidation of lipid and pigments. Ascorbic acid treated meat showed low pigment and lipid oxidation. Vitamin E and AA combined showed the lowest oxidation of pigment and lipid.

Table 2.10. Effect of ascorbate treatment on metmyoglobin accumulation in four beef muscles at two (0° and 5°C) temperatures. Ten beef cattle (approximate liveweight 360 kg) in each treatment were injected with a 50% w/v solution of sodium ascorbate 5-10 minutes before slaughter via jugular vein.

			Storage time (days)		
			2	4	6
Muscle	Temperature (0° C)	Treatment	Mean $(K/S)_{572}/(K/S)_{525}$		
M. psoas major	0	Ascorbate	.06	.08	.10
		Control	.09	.16	.23
	5	Ascorbate	.09	.10	.14
		Control	.23	.33	.40
M. gluteus medius	0	Ascorbate	.04	.03	.04
		Control	.03	.05	.07
	5	Ascorbate	.05	.05	.09
		Control	.08.	.16	.28
M. semimembranosus	0	Ascorbate	.03	.02	.02
		Control	.01	.02	.03
	5	Ascorbate	.04	.04	.08
		Control	.04	.07	.17
M. longissimus dorsi	0	Ascorbate	.03	.02	.03
		Control	.01	.02	.03
	5	Ascorbate	.05	.04	.12
		Control	.04	.05	.11

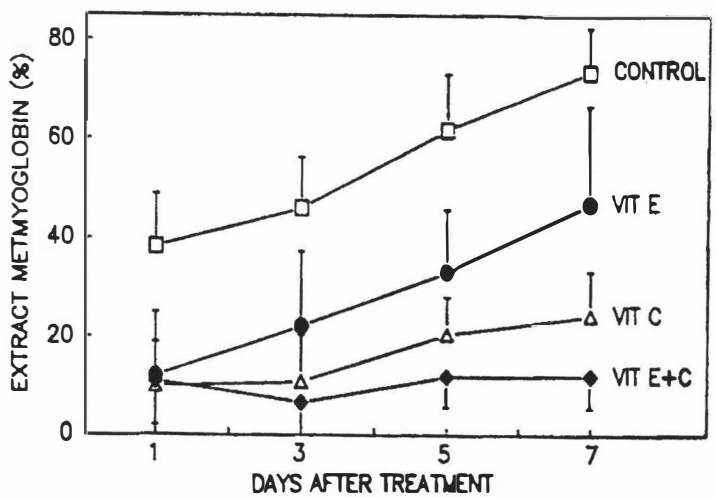
From Hood (1975)

Figure 2.5 Relationship between time and surface MetMb percentage in raw ground beef, dipped in vitamin E and ascorbic acid solution during 7 days at 4^0 C (standard error bars are indicated)



From Mitsumoto *et al.* (1991a)

Figure 2.6 Relationship between time and extract metmyoglobin percentage in raw ground beef dipped in vitamin E and ascorbic acid solution during 7 days at 4^0 C (standard error bars are indicated)



From Mitsumoto *et al.* (1991a)

Liu *et al.* (1994) conducted some valuable studies to determine if antemortem supplementation of AA and vitamin E individually and in combination decreased the rates of myoglobin oxidation to MetMb in beef during retail display. In their work, vitamin E was provided by dietary supplementation to finishing cattle and AA infused into the jugular vein.

In their first experiment, AA concentrations in muscle were elevated due to AA infusion (Table 2.11). Maximum AA concentration in plasma and muscle increased with increasing infusion of sodium ascorbate. Maximum plasma concentration was observed by Liu and his co-workers (1994) within the first few minutes after infusion was completed and followed by disappearance of plasma AA (Fig. 2.7 and 2.8). They also observed that the AA concentration in the neck muscle was much higher than any other muscle (Table 2.11), perhaps due to this being closest to the infusion site. They suggested that a large dose (500 g) of AA was not suitable due to some adverse behavioural and carcass quality effects. In their second experiment they found that the largest increase in muscle concentration and meat colour stability occurred in samples collected 10 minutes after the intravenous infusion of AA. It was also observed that there were no differences of distribution of AA among three major muscles, namely *psoas major* (PM), *gluteus medius* (GM) and *longissimus lumborum* (LL). This may be due to rapid and uniform distribution of ascorbate in the muscles after the infusion of AA. They found that the muscle colour response to AA treatment was PM>GM>LL and also observed higher temperature resulted in more rapid discolouration (Table 2.12). Their unpublished data on vitamin E research has indicated a looser relationship between alpha tocopherol concentration and colour stability of meat. It is still unknown, whether vitamin E and AA are complementary, additive or synergistic with regard to the colour stability of meat.

Table 2.11: Preliminary ascorbic acid concentrations (μg/g fresh muscle) as influenced by intravenous infused dose, muscle and post-mortem sampling time

Infused sodium Ascorbate (g)	Muscle	Post-mortem sampling time 3 h	24 h
0	Neck	17.8	20.2
	Psoas	15.3	-
	Longissimus	15.1	-
300	Neck	88.8	90.6
	Psoas	40.5	-
	Longissimus	45.0	-
500	Neck	493.8	76.8
	Psoas	47.6	-
	Longissimus	84.9	-

From Liu *et al.*, (1994).

Table 2.12 Colour display life of beef (days) at 0°C and 10°C as influenced by intravenous administration of ascorbic acid (300g) before slaughter.

Infusion	Time of slaughter after AA administration	LL* 0°C	LL* 10°C	GM* * 0°C	GM* * 10°C	PM* * * 0°C	PM* * * 10°C
Saline	10 min.	7	2	-	1	3	0
Ascorbate	10 min.	10	5	-	3	9	4
Ascorbate	2.5 hr	9	2	-	2	5	1
Ascorbate	5.0 hr	8	2	-	1	4	1

LL*- *longissimus lumborum* GM* *- *gluteus medius* PM* * *- *psoas major*

From Liu *et al.*, (1994)

Figure 2.7 Plasma ascorbic acid concentration before and after infusion of 100g sodium ascorbate (50% solution) via jugular vein into two Holstein steers (600kg liveweight) (From Liu *et al.*, 1994)

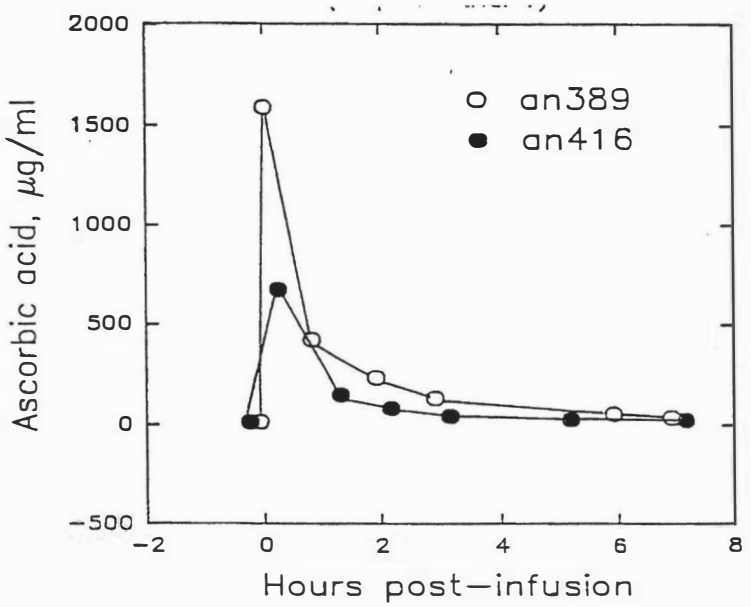
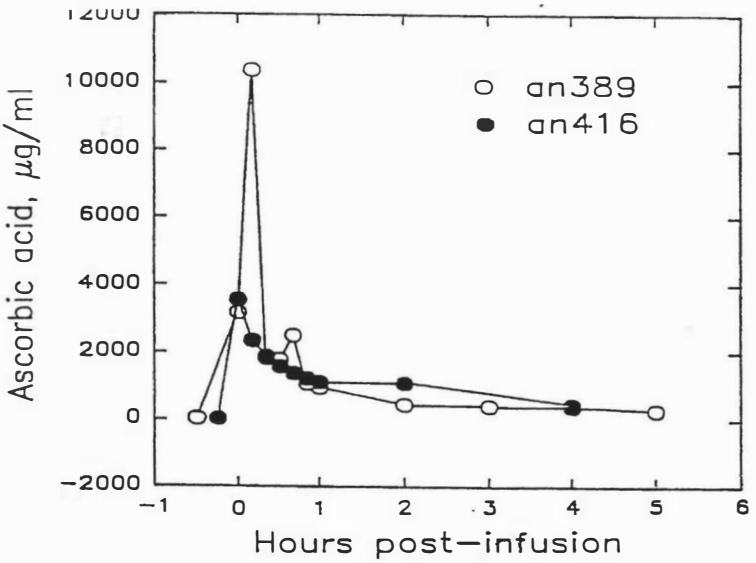


Figure 2.8 Plasma ascorbic acid concentration before and after infusion of 300g sodium ascorbate (50% solution) via jugular vein into two Holstein steers (600kg liveweight) (From Liu *et al.*, 1994)



2.6.4. Relevance to venison

Venison discolours more rapidly than beef and pork (Trout and Gutzke, 1995; Trout *et al.* (1996). One of the reasons for this variation is that different muscles contain different levels of anti-oxidative and pro-oxidative compounds, which control the *in vivo* oxidation rate of myoglobin. Another reason may be that venison contains more iron than of beef and pork.

The information related to AA and the colour stability of venison is very limited. Stevenson-Barry *et al.* (1999) recently studied whether additional levels of anti-oxidants would improve venison colour stability. Venison steaks were dipped for 10 sec. in 70% ethyl alcohol (EtOH); 70% EtOH with 3% L-ascorbic acid (AA); 70% EtOH with 8% DL-α-tocopherol: control samples received no dip treatment. These venison samples were then placed on plastic trays over wrapped with oxygen permeable film and kept at 5°C ±0.5°C for 10 days. Dipping venison steak in either EtOH or 8% vitmain E in EtOH had no significant effect on colour stability, whereas dipping venison in AA in EtOH significantly improved venison colour stability, in a similar manner to beef (Okayama *et al.* 1987).

2.7. DIGESTION AND ABSORPTION OF ASCORBIC ACID IN RUMINANTS

Little work has been done on the digestion and absorption of AA in ruminants. This is mainly due to the rapid and pronounced destruction of ingested AA in the rumen, which was reported as early as 1940 (Knight *et al.* 1941). In addition to this, AA in feed is unstable and broken down especially by the presence of heavy metal ions. The amount of AA required to enhance the immune system of ruminants may be much higher than that provided in the normal diet for nutritional needs (McDonald *et al.*, 1995). Therefore supplementation of AA is beneficial for ruminants to enhance resistance against infectious diseases (McDonald *et al.*, 1995).

2.7.1. Utilization and excretion of ascorbic acid by the dairy cow

Knight and his co-workers (1941) conducted studies on the utilization and excretion of AA in dairy cows. They determined the effects of AA administered intravenously and subcutaneously, through a rumen fistula (intraruminally) and also orally.

2.7.1.1. Effect of ascorbic acid administered intravenously

The intravenous injection of AA produced a dramatic increase in its concentration in milk and urine as well as in the blood (Knight *et al,* 1941). Urinary excretion accounted for over ninety per cent of the total AA injected. These results were similar to the work conducted by Richmond *et al.* (1940) in which AA was injected into goats.

2.7.1.2. Effect of ascorbic acid injected subcutaneously

In order to study the differences between the direct administration of AA (intravenous) into the blood stream and an indirect route, Knight and his co-workers (1941) used the subcutaneous method. Results of subcutaneous administration were similar to those of intravenous injections, but AA concentrations in the blood, milk and urine after subcutaneous administration increased less rapidly than in the case of intravenous injections.

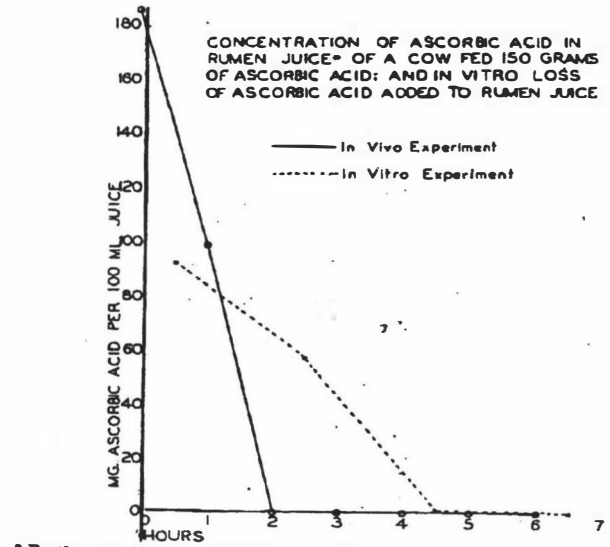
2.7.1.3. Destruction of ascorbic acid in the rumen

Rumen fistulated cattle were used to investigate the possible destruction of AA in the rumen (Knight *et al.* 1941). Rapid and pronounced destruction of AA in the rumen was observed by removal and analysis of samples of rumen contents at regular intervals after feeding supplements of AA and after insertion of AA directly into the rumen. The disappearance of AA from rumen contents is shown in Figure 2.9, in which 150 g of ascorbic acid was fed in the diet (Knight *et al.*, 1941). This graph shows slower destruction of AA in the *in vitro* experiment as compared to the *in vivo* experiment.

Riddell and Whitnah (1938) gave a different interpretation where the very soluble AA was rapidly absorbed in the rumen. They studied the fate of AA in the rumen

contents of a cow with a rumen fistula and in a steer at slaughter. Concentration of AA in rumen contents was found to be less than one tenth the AA concentration in green ryegrass fed twelve hours earlier. Their explanation was that the rapid disappearance of AA from the rumen might have been due to the rapid absorption.

Figure. 2.9 Concentration of ascorbic acid in rumen fluid of a cow (500 kg liveweight) fed 150 g of ascorbic acid in the diet and the concentration of ascorbic acid in an *in vitro* rumen system



From Knight *et al.* (1941)

2.8. TECHNIQUES TO MEASURE DIGESTION AND RUMEN FRACTIONAL OUTFLOW RATE (FOR) IN RUMINANTS

The measurement of digestion along the gastro-intestinal tract (GIT) of ruminants is an important area of study, to understand the amounts and proportions of the particular nutrients absorbed. The reason is that, unlike non-ruminants, for ruminants the difference between the amounts of a particular nutrient consumed and the output of that nutrient in the faeces may not reflect its absorption. The absorption from within any part of the GIT can be measured as the differences between inflow and outflow of that particular section. Marking some of the food with a chemical tracer can follow the passage of food

particles through the GIT. To estimate the passage of indigestible residues through the whole tract, these particles are recovered in faeces and counted, and their mean retention time (MRT) can be calculated. Forage with highly lignified cell walls has 50-80 hours long retention time whereas more rapidly digested food, like immature pasture herbage or concentrate diets have shorter retention times (30-50 hours) (McDonald *et al.*, 1995). Fractional outflow rate (FOR) and MRT can also be measured in individual segments of the GIT.

Tracer or marker substances can be used to measure the passage rate of either liquid or solid digesta out of the rumen. This is commonly expressed as the rumen fractional outflow rate (FOR), defined as the proportion of the total rumen content that leaves and flows into the abomasum per hour. Typical values for liquids and rumen solids are 0.084 and 0.043 per hour respectively (McDonald *et al.*, 1995). Mean retention time (MRT) can be calculated as 1/FOR.

2.8.1. Marker techniques to measure digestion and MRT along the ruminal digestive tract

Types of marker techniques may be considered in relation to the method of administration and to the method of sampling. The marker can be administered continuously at a constant rate or as a single dose. A small sample may be taken from a section of GI tract at successive times or the animal can be slaughtered and the whole of the digesta in any part or all sections of GI tract may be taken (total sampling). There are four combinations of marker techniques and these are given below.

1. Continuous infusion with time-sequence sampling (primarily for digesta flow measurement)
2. Continuous infusion with total sampling (to determine FOR and MRT)
3. Single dose with time-sequence sampling (to determine FOR and MRT)
4. Single dose with total sampling

Method number one is used mainly to measure digesta flow rates but, if samples are taken after the infusion is stopped, this method can also be used to estimate the volume

and the MRT for the section of the GI tract into which the infusion is made. It can also measure the MRT for compartment into which the infusion was given and for all sections of the GI tract distal to the point of infusion.

Continuous infusion with total sampling technique is primarily used to study water and electrolyte transaction along the GI tract and to measure FOR and MRT. This method also provides the measurement of MRT for the compartment into which the infusion was given and for all sections of the GI tract distal to the point of infusion.

The third method mentioned above, single dose with time-sequence sampling, is mainly used to estimate the MRT in segments of the GI tract and rates of flow from mixing compartments (reticulo-rumen, abomasum, caecum).

The fourth is mainly used for rabbits and not suitable in ruminants, as many animals need to be slaughtered to provide single estimates of FOR and MRT.

To study the progress of digestion within the GI tract, two markers are required, one to mark the liquid phase and one mark the particulate (or solids) phase. In the examples the ^{51}Cr complex of ethylenediaminetetra-acetic acid (Cr-EDTA) was used to mark the liquid phase and lignin was used to mark the particulate phase (Downes and McDonald 1964).

2.8.1.1. Continuous infusion with time sequence sampling to measure the flow of digesta

In this technique the animals are prepared with canulae at the points along the GI tract past which flow is to be measured. Partition of digestion between the stomach, small intestine and large intestine requires canulae in the rumen, abomasum and terminal ileum of each animal. Alternatively, if no specific information on rumen digestion is required, canulae in the abomasum, proximal duodenum and terminal ileum could be used. Normally, the use of the duodenum is avoided as this part of the GI tract has a role in the regulation of neural and endocrine functions. The selected marker can then be administered either in feed or as an infusion into the rumen. When marker concentration

at any given sampling point remains constant, that means the equilibrium has been met. At this time, flow rate past each sampling point can be calculated as the infusion rate divided by the marker concentration. In this calculation it is assumed that the concentrations in the sample of all constituents of digesta, including the marker, are the same as their concentration in digesta flowing pass the sampling point.

However, as digesta consists of two phases, it is difficult in practice to obtain samples containing not only particulate matter but also dissolved substances in the same proportions as are present in the digesta flowing past the canula (Hogan 1964 and Hogan and Weston 1967). To overcome this problem, two markers are used, one of which remains in solution and the other marker which is intimately associated with the particulate matter (Hogan and Weston 1967). The flow of each phase can only be accurately estimated if the markers associate exclusively and are distributed uniformly throughout the phase they are marking.

2.8.1.2. Continuous infusion with total sampling to measure rumen FOR and MRT

In this technique it is necessary to establish equilibrium throughout the GI tract by the continuous administration of marker either in the feed or directly into the rumen through a fistula. Equilibrium is confirmed by constant marker concentration in the faeces. Total rumen contents are either then removed through the fistula or the animal is then killed and the GI tract is removed and divided into sections as soon as possible, or the total rumen contents are quantitatively removed through a large fistula. Then the rumen MRT and other various sections of the GI tract can then be measured. The solutes and particulate matter behave independently and the MRT can then be calculated for each phase. This method cannot be used to detect the flow rate of organic constituents other than recognizable plant materials such as lignin in digesta, particularly in the proximal half of the small intestine, due to marked shedding of epithelium that occurs at death.

2.8.1.3. Single dose with time sequence sampling

This technique is used to provide estimates of volume and fluid flow from the rumen, abomasum, and caecum and it can also measure the MRT of markers in these organs (Faichney 1975; Warner and Stacy 1968; Hecker 1971) In this technique, after a dose of marker into an organ, MRT is calculated from the disappearance curve using the following equation.

$$C = C_o . e^{-kt} \quad (1)$$

Where C is the marker concentration at time t, C_o is the marker concentration at t_o, ie equilibrium concentration, k is the dilution rate (or FOR) and is the reciprocal of marker MRT. Volume of distribution of the marker (V) is calculated by dividing the dose of marker given by its concentration at t_o (ie C_o).

Single dose with time sequence sampling may also be used to estimate the MRT of markers in the whole GI tract or sections of it (Balch 1950; Blaxter *et al.*,1956; Castle 1956; Coombe and Kay, 1965; Faichney 1975).

2.8.2 Criteria of ideal digesta markers

It is important to know how far an ideal marker will fulfil the needs of the particular technique before selecting the markers. The criteria of an ideal marker has been reviewed by some workers (Engelhardt 1974; Kotb and Luckey 1972) and is summarized as,

a. It must be strictly non-absorbable
b. It must not affect the GI tract or its microbial population
c. It must be physically similar to or intimately associated with the material it is to mark
d. Its method of estimation in digesta samples must be specific and sensitive and it must not interfere with other analyses.

None of the markers available fully meet all these criteria (Engelhardt 1974). A small proprtion of Cr-EDTA (approximately 5%) is absorbed and excreted in the urine (Downes and McDonald 1964 ;Weston and Hogan 1967). There are possibilities that gastro-intestinal parasites may increase the absorption of markers such as 51Cr-EDTA, but this marker is the best currently available for marking the liquid phase of digesta. Cr-EDTA moves with the liquid phase of digesta and does not associate with particulate matter (Downes and McDonald, 1964). Lignin is regarded as a suitable marker for the particulate digesta phase (Faichney, 1980).

2.9. VOLUNTARY FEED INTAKE (VFI), RUMEN DIGESTION AND RUMEN OUTFLOW RATES (FOR) IN DEER IN RELATION TO OTHER RUMINANTS

Much work has been carried out to study comparative digestion in deer, goats and sheep (Milne *et al.*, 1978; Fennesy *et al.*, 1980; Watson and Norton 1982; *Doyle et al.*, 1984; Alam et al. 1985; Domingue *et al.*,1991). Deer show seasonal variation of VFI, with high values in summer and low values in winter (Milne *et al.*, 1978; Fennessy *et al.*, 1981; Kay 1985), which could potentially affect their digestive processes. Domingue and co-workers (1991) studied the comparative digestive process in deer, sheep and goats in summer and winter for the first time in New Zealand. Their results are given below in detail.

2.9.1. Voluntary Feed Intake (VFI) and digestion of Dry Matter (DM)

Domestic sheep showed no seasonal differences ($p>0.05$) in voluntary dry matter intake (DMI), organic matter intake (OMI), digestible dry matter intake (DDMI), digestible organic matter intake (DOMI) and apparent digestibilities of DM and OM (Table 2.13). Deer had higher voluntary DMI, OMI, DDMI and DOMI (by 30%) during summer compared to winter (Table 2.13) (Dominigue *et al.*, 1991), associated with an increase in total rumen pool (DM +liquid) size ($P<0.001$) of 51%. Rumen contents of deer had significantly higher concentrations of DM than that of sheep and goats ($p<0.001$), during summer as well as in winter. Goats showed an increase in the voluntary intake of DM (20%) and OM (14%) from winter to summer, but it was not significant ($P>0.05$). The apparent digestibilities of DM ($P< 0.01$) and OM ($P<0.001$) decreased in goats from winter to summer as shown in Table 2.13. This resulted in no changes in DOMI in goats from winter to summer. The total rumen pool (DM+liquid) in goats increased by 27% ($P<0.01$) from winter to summer (Dominigue *et al.*, 1991).

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.

		Deer	Goats	Sheep	SEM
Voluntary intake (g/kg W0.75 per day)					
DM	S	62.5	68.7	52.2	3.20
	W	46.7	57.4	54.8	4.24
OM	S	56.6	62.2	47.2	2.90
	W	45.7	54.7	52.0	5.52
Digestible DM	S	35.6	38.6	28.2	0.88
	W	27.4	36.9	31.6	1.77
Digestible OM	S	34.2	36.4	27.0	1.79
	W	27.0	35.4	31.0	1.73
Apparent digestibility					
DM	S	0.57	0.56	0.54	0.0044
	W	0.55	0.62	0.56	0.0078
OM	S	0.60	0.58	0.57	0.0046
	W	0.59	0.65	0.60	0.0075
Total rumen pool (g/kg W0.75/ day)	S	289	340	275	17.5
(DM+liquid)	W	191	268	307	13.4

S-Summer, W-Winter

From Dominigue *et al.*,1991

2.9.2. Fractional Outflow Rate (FOR) of rumen digesta

Dominigue *et al.* (1991) found that rumen water FOR (marked by Cr-EDTA) was much faster in deer than that in sheep and goats, in both summer and winter (Table 2.14). Particulate matter FOR (as measured with lignin) was lower in summer than winter in deer, in contrast to the findings for sheep and goats. This indicates that rumen outflow rate in deer reduced during summer, especially for particles, meaning that particulate MRT (1/FOR) in deer is longer in summer, allowing more time for microbial attack and explaining why digestibility does not fall as VFI increases in deer during summer. The ratio FOR Cr.-EDTA: FOR lignin was much higher in deer than in sheep and goats ($P<0.001$), indicating that water left the rumen at higher rates relative to particles in deer compared to sheep and goats. Particulate matter in the rumen has to be reduced to below the critical particle size (passage through a 1 mm sieve) to have a high probability of passing through the reticulo-omasal orifice and leaving the rumen of sheep, deer and goats (Dominique *et al.*, 1991).

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 *ad libitum*

FOR (% /h)		Deer	Goats	Sheep	SEM
Cr-EDTA	S	15.8	10.8	10.4	0.54
	W	16.3	9.6	10.3	0.56
	S/W	0.97	1.13	0.99	0.062
Lignin	S	2.77	3.66	3.32	0.163
	W	3.47	3.47	3.29	0.142
	S/W	0.81	1.04	1.03	0.050
Cr-EDTA/ Lignin	S	5.97	3.07	3.24	0.308
	W	4.77	2.82	3.12	0.110

S-summer W-winter (From Domingue *et al.*, 1991)

Increased MRT in summer compared to winter in red deer fed chaffed lucerne hay was confirmed by Freudenberger *et al.* (1994), who also showed that the effect was independent of the summer increase in VFI (Table 2.15). These authors also confirmed that apparent DM digestibility did not change between seasons in red deer, despite the summer increase in VFI.

Table 2.15 Effect of season upon rumen digestion in castrate red deer stags fed chaffed lucerne hay

	Winter *ad lib*	Summer *ad lib*	Summer restricted
Voluntary food intake:			
Organic matter (g/kg $W^{0.75}$/d)	53	77	56
Apparent digestibility:			
Organic matter	0.640	0.632	0.642
Fibre	0.436	0.394	0.421
Rumen mean retention time (h):			
Liquid	6.1		8.1
Particulate	31.7	37.5	36.0
Rumen ammonia:			
Concentration(mg NH_3-N/l)	193	191	218
Pool size (mg/g N intake)	20.4		23.9
Irreversible loss rate (mg N/g N intake)	522		649
Rumen volatile fatty acid:			
Concentration (mmol/l)	82	97	91
Pool size (mmol/100g DMI)	29	37	39

From Freudenberger *et al.*, 1994

2.10. THE HYPOTHESIS OF USING ORAL ASCORBIC ACID SUPPLEMENTATION TO INCREASE PLASMA ASCORBIC ACID CONCENTRATION IN DEER BEFORE SLAUGHTER

Although low oral doses of AA to sheep (4g/day to 50 kg sheep) produced negligible increases in plasma AA concentration and area under the plasma concentration vs. time curve (AUC) (Hidiroglou *et al.*, 1997), AgResearch, Invermay have found that giving a single high oral dose of AA to deer (100 or 200 g in 200 or 500 ml liquid to 70 kg deer) can elevate the plasma AA levels for up to 3 hours (J Stevenson-Barry personal communication). There are some reasons to accept this hypothesis, particularly with deer, which are given below.

2.10.1 Rapid rumen liquid Fractional Outflow Rate (FOR) in deer

Rapid degradation of AA in the rumen (Knight 1941) may be one of the reasons that gives a low AA plasma concentration following a small oral dose of AA in ruminants. Ascorbic acid is water-soluble and the FOR of liquid from the rumen into the abomasum is about 60% higher for deer (16%/h) than that of sheep and goats (10%/h) (Table 2.14) (Domingue *et al* 1991). Therefore, the rapid rumen liquid FOR in deer may increase wash out of AA into the abomasum and reduce the rumen degradation. The result would be higher plasma AA concentration in deer.

2.10.2. Large single dose of AA to saturate rumen degradation mechanisms

Another possibility is that large single oral doses of AA may saturate the rumen degradation mechanisms, allowing absorption of AA from the rumen, as found by Barry and Manley (1985) for large single oral doses of the amino acid methionine in sheep

Two experiments were conducted by Barry and Manley (1985) using Romney wether sheep fed on forage kale. In Experiment 1, twenty-four sheep were given oral supplements of 4.0g methionine twice per week for 10 weeks. The remaining twenty-four sheep were given an equal amount of control solution comprising the suspension agent. In their second experiment, four rumen fistulated sheep were fed on kale at hourly intervals for 3 weeks. The forage kale contained 11.4 g S-methyl-L-cysteine /kg dry

matter and the ratio, readily fermentable: structural CHO was high at 2.9. The group of sheep given oral methionine supplementation had higher plasma concentration of methionine and cysteine than control animals. It was suggested that the responses to oral methionine supplementation can be explained through SMCO (which is structurally similar to methionine) reducing methionine degradation in the rumen, and through a significant portion of methionine escaping rumen degradation due to the high rumen liquid FOR measured on this diet (16%/h)

Similarly, high single doses of AA may saturate the rumen degradation mechanisms, at least for a short period after dosing, allowing absorption from the rumen to take place, whilst the high rumen liquid FOR in deer (16%/h) may result in some AA escaping rumen degradation and flowing into the abomasum.

2.10.3 Use of ascorbic acid analogues

Several stable AA preparations, namely ethyl cellulose coated ascorbic acid (EC), Rovimix Stay-C (SC), sodium ascorbate (SA) and Rovimix C coated with silicone (RC) have been prepared for use in animal feeds

Hidiroglou and co-workers (1997) compared the bioavailabilities of different formulations of AA following either multiple or single dosing in sheep weighing 45-50 kg. They also compared oral and duodenal supplementation of AA to determine the degradability of AA in the rumen. In their Experiment 1 they used fifty, one year old sheep in the multiple oral dosing of different formulations of AA. Five different formulations of pure AA, EC, SC, SA and RC were fed daily at the rate of 4g of AA equivalent for 28 days. Blood samples were taken at 0, 4 and 7 h and 3, 7, 10, 14, 17, 21, 24 and 28 day following the dosing of AA. Experiment 2 was conducted to investigate the effect of single oral administration of five different formulation of AA and intraduodenal supplementation of AA. A single dose of each formulation equivalent to 4g of AA was given to five sheep in Experiment 2. Blood samples were taken at 0, 10 and 30 min., once every hour up to 7 h. after AA supplementation. The results obtained from these experiments are given in Tables 2.16 and 2.17.

Table 2.16 Means and standard deviations for plasma ascorbic acid concentration (μg/ml), AUC and AUCB in sheep given 4 g ascorbic acid equivalent daily doses of several ascorbic acid formulations and in control sheep

Group	Plasma		AUC		AUCB	
	Mean	SD	Mean	SD	Mean	SD
AA	8.19	1.03	233	28	34.1	13.7
EC	6.83	1.14	195	31	20.7	7.9
SC	7.48	0.72	213	16	27.8	10.0
SA	7.70	0.95	222	23	23.7	13.9
RC	10.60	2.08	304	61	47.2	25.2
Control	7.38	1.53	207	43	0.0	8.1

AUC = area under the plasma AA concentration vs. time curve, AUCB= area under the plasma AA vs. time curve corrected for basal concentration, AA= ascorbic acid fine powder, EC= ethyl cellulose coated ascorbic acid, SC= Rovimix STAY-C, SA=sodium ascorbate, RC= Rovimix coated with silicone.
(From Hidiroglou *et al.*,1997)

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

Pathway	Product	AUC		AUCB	
		Mean	SD	Mean	SD
Oral	AA	2.46	0.38	0.35	0.28
	EC	2.42	0.44	0.15	0.07
	SC	2.03	0.55	0.20	0.12
	SA	2.08	0.23	0.09	0.18
	RC	2.28	0.46	0.38	0.17
Intra-duodenal	AA	4.60	1.37	2.58	1.31

AUC = area under the plasma AA concentration vs. time curve, AUCB= area under the plasma AA vs. time curve corrected for basal concentration, AA= ascorbic acid fine powder, EC= ethyl cellulose coated ascorbic acid, SC= Rovimix STAY-C, SA=sodium ascorbate, RC= Rovimix coated with silicone (From Hidiroglou *et al.*,1997)

The results from Table 2.16 where they used daily supplementation of different formulations of AA show that only RC produced a small increase in plasma AA concentration, whilst all five treatments had higher AUCB than the control group. In Table 2.17, where the animals were given a single oral dose of AA, there were no differences between the five formulations, but the group of animals which received intra-

duodenal supplementation of pure AA had significantly higher AUC and AUCB values compared with oral supplementation of the same form of AA. This indicated that there was no increase in plasma AA concentration or AUCB after a single oral dose of five different formulation of AA when given at 0.08 g/kg live weight. Only multiple daily oral supplementation of RC significantly increased both plasma concentration and AUCB values. One reason for higher AA plasma concentration in daily oral supplementation may be due to partial saturation of the rumen degradation mechanisms, resulting in some absorption from the rumen.

In a separate study conducted in horses (463 kg±36kg, n= 6), it was found that oral ascorbyl palmitate (20 g equivalent AA; 0.04 g/kg liveweight) gave both highest plasma concentration and AUC for AA, when compared to a similar dosage of ascorbyl stearate and formulated AA (Snow and Frigg, 1987). Studies in chickens gave no differences in plasma AA concentration after supplementation of crystalline L-ascorbic acid and L-ascorbyl-2-polyphosphate in broiler chicken diets and in drinking water (Pardue *et al.*, 1993). Johnston and Luo (1994) found that there were no differences in relative bioavailability to humans of three commercially available vitamin C tablets namely AA, ester-C and AA with bioflavonoids.

2.11.CONCLUSION AND FUTURE RESEARCH

The demand for chilled venison exports from the New Zealand Deer Industry is increasing compared to the demand for frozen venison in the world market. This is most visible in the USA market where the demand for chilled venison grew by 10% in volume from 1997 to 1999. Sale of venison in the chilled form means that the industry must pay increased attention to factors that influence shelf life, including colour stability.

Nutrition related health problems such as coronary heart disease are currently causing much public concern, and can develop due to the over consumption of fat, saturated fatty acids and cholesterol. Beef and lamb as normal red meats are rich in these items. According to Drew and Semen (1987), the venison loin and leg is very high in protein and iron and very low in fat, energy and cholesterol (Table 2.4 and 2.5), compared with other livestock meats.

The relationship between the colour stability of meat and the formation of metmyoglobin as well as the factors involved in the discolouration of meat, notably beef have been well studied and documented. Major conclusions are given below.

- Rate of discolouration of different types of meat during refrigerated display at 4^0 C is extremely variable (Hood 1980)
- Oxidation of bright red fresh meat (MbO, with iron on the haem group in its ferrous state) to MetMb (brown, iron in its ferric state) is affected by the reducing capacity of the muscles, oxygen availability (oxygen penetration and tissue respiration) and also due to the auto oxidation rate (Renerre and Labas, 1987)
- Rate of discoloration of meat is believed to be associated with the efficiency of oxidation processes and enzymatic reducing systems in controlling MetMb levels in meat. (Faustman and Cassens, 1989)
- Colour stability of pork (159.7 h) and beef (143.6 h) is three times longer than that of venison (50.4 h) and the differences in the colour stability of meat may be related to the rate of MetMb formation at the surface of the muscle during refrigerated display. (Gutzke *et al.*, 1997). The colour stability of meats was highly correlated to the

formation of MetMb layer of meat (Table 2.6) (Gutzke *et al.*, 1997). Therefore the shelf life of venison is known to be less than that for beef and pork. One reason for this may be that venison has high iron content than other red meats.

- Several studies have been conducted and concluded that discoloration of beef and its products can be minimized by the use of AA (and Vitamin E) with their powerful antioxidant activity (Lee *et al.*, 1999; Liu *et al.*, 1994; Yin *et al.*, 1993; Mitsumoto *et al.*, 1991a; Mitsumoto *et al.*, 1991b; Mitsumoto *et al.*, 1991c; Okayama *et al.*, 1987; Shivas *et al.*, 1984; Benedict *et al* 1975; Hood, 1975.)

However little information is available in relation to venison. Stevenson-Barry *et al.* (1999) reported that dipping venison steaks in AA or vitamin E solutions improved the colour stability of venison. These results were similar to the conclusions of Okayama *et al.* (1987) with beef. Based upon these results, Stevenson-Barry *et al*, (1999) concluded that oral administration should be investigated for increasing the AA concentration in venison muscle.

Scientists have not used oral administration of AA in the past to study the improvement of colour in ruminant meats, due to its rapid ruminal degradation (Knight *et al.*, 1941; Black and Hidiroglou, 1996). However, intravenous administration of AA before slaughter in beef animals has produced the following major conclusions.

- Maximum plasma AA concentration was observed within the first few minutes after the infusion was completed and was followed by a sharp decline in plasma AA concentration (Liu *et al.*, 1994)
- In animals slaughtered 10 minutes after intravenous AA administration, the infusions increased AA concentration in a range of muscles. Maximum increase in AA concentration was observed in the neck muscle (due to this being the closest to the infusion site) (Liu *et al.*, 1994).
- Ante-mortem administration (intravenous or oral route) of AA may be better method, than dipping meat in AA solutions as it allows delivering AA in its reduced form to the entire body (Hood 1975).

• Intravenous infusion of AA 10 minutes before slaughter reduced metmyoglobin formation on the surface layer of beef(Hood 1975).

• It was also concluded that better meat colour stability occurred in samples obtained from the animals, which were slaughtered 10 minutes after the infusion of AA. (Liu *et al.*, 1994), resulting in longer shelf life.

• Although the administration of AA via the intravenous route gave a substantial increase in plasma AA concentration (Liu *et al.*, 1994), this route of administration of AA and the dipping method may not be accepted by regulatory authorities or consumers for commercial application.

A single oral supplementation of stable AA in sheep did not result in a significant increase in plasma AA concentration (Hidiroglou *et al.*, 1997) where they used a low amount of AA (0.08 g/kg LW). Work recently carried out at AgResearch Invermay, which is currently under patent application, has shown that giving large single oral doses of AA to deer (100 g or 200 g to 70 kg animals) can be successfully used to increase plasma AA concentration; this is currently being evaluated by Agresearch as a pre-slaughter treatment for increasing the colour stability of venison and hence for increasing venison shelf life (J.M. Stevenson-Barry personal communication). The mechanism of how the AA is absorbed from the GIT tract in this delivery method is unknown.

The hypothesis that a large single dose of AA may saturate the rumen degradation mechanisms and hence allow absorption from the rumen and also that the rapid rumen liquid FOR in deer (compared to other ruminants) would increase rumen outflow and hence intestinal absorption needs experimental testing. Both mechanisms could potentially contribute to the observed increase in plasma AA concentration in the 3 h period following dosing.

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CHAPTER 3

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

3.1. INTRODUCTION

There has been a substantial growth in the New Zealand deer industry during the last decade. Total export returns from the deer industry have increased from $100 million for the year 1991 to $177 million in the year 1999. Venison production has shown major export growth, with returns increasing from $ 60 million in 1991 to $ 130 million in 1999. The projected export earnings from venison in 2004 will be $ 272 million (MAF; Sonza Venison Report 2000).

The demand for chilled venison has been increasing rapidly compared to that for frozen venison in recent years, due partly to the success of the Cervena programme in the USA. NZGIB forecast that the demand for chilled venison will continue to rise in the near future. Therefore the New Zealand Deer Industry has to look into new technologies to increase the shelf life of chilled venison.

Venison contains a higher concentration of iron compared to that in beef and lamb (Drew and Seman 1987) and this leads to faster discolouration of venison than beef and lamb. Gutzke and co-workers (1997) found that pork (160 h) and beef (144 h) had three times longer shelf life than venison (50 h), due to better colour stability (Table 2.6). It has also been well documented that the intravenous administration of a large amount of AA (500 ml, 50%w/v sodium ascorbate) 10 minutes before slaughter in beef heifers delayed the colour changes and prolonged the shelf life of beef, due to its antioxidant activity (Hood 1975).

Stevenson-Barry *et al* (1999) showed that dipping venison steaks in solutions of AA dramatically improved colour stability, which would be expected to increase shelf life. These authors suggested that feeding treatments should be evaluated for increasing AA concentration in venison muscle and then showed that giving large single oral doses of AA (100 or 200 g to 70 kg deer) could be used to elevate plasma AA concentration for up to 3 hours. (J.M. Stevenson-Barry personal communication).

Objectives of the present study were to evaluate different formulations of AA for increasing plasma concentration and to determine the mechanism of how these large single doses of AA successfully increased plasma AA concentration.

3.2. MATERIALS AND METHODS

3.2.1 Experimental Design

Six experiments were conducted, involving ten fistulated deer given single large doses of AA or its derivatives such as ethyl cellulose coated ascorbic acid (EC) and silicone coated ascorbic acid (SC). All compounds were mixed as a suspension or slurry in distilled water (1:1 ratio) just before commencing each experiment. All experiments were conducted at Massey University Deer Unit, between 13/07/1999 and 16/02/2000. All the animals were housed indoors in individual metabolism cages (1.87m^2), as described by Milne *et al.* (1978). The temperature inside the Deer Unit was controlled by using three exhaust fans and the maximum temperature was 26°C. Deer were weighed before commencing and after completing each experiment. The animals were put into the cages one-week prior to the commencement of each experiment, in order to accustom them to the surroundings and to attain voluntary feed intake (VFI). They were returned to grazing perennial ryegrass/ white clover pasture after each experiment except in Experiment 2, where the animals were kept in the cages for a further two weeks, and in Experiments 5 and 6 for a further 1 week, until the next experiment (trial) commenced.

Animals were cared for during the whole period of experimentation according to the guidelines provided by the Director, Animal Welfare Science and Bioethics Centre, Massey University. Some of the experiments involved changeover designs, where deer were given different AA formulations in different time periods. The purpose of this was to determine the bioavailability of the different formulations by the best available methods; this does not imply that repeat dosing of animals should be considered as a practical proposition.

3.2.2 Animals

Seven ruminally fistulated castrate male red deer (liveweight 100-173 kg; average age 13 years) and three castrated male red deer fistulated in both the rumen and abomasum (liveweight 100-138 kg; age 1.5 to 3.0 years) were used in the experiments.

Rumen fluid was withdrawn through perforated metal probes suspended through the fistula and covered with synthetic polyester fibre (mesh size 80 micron; Swiss Screens, Australia) and then placed on ice. Blood was withdrawn from the jugular vein using green top vacutainer tubes (Beckton Dickinson, USA) and immediately placed on ice.

3.2.3. Feeding System

All the deer were individually fed chaffed lucerne hay *ad libitum* at 30 minute intervals to guarantee steady state conditions. Lucerne hay obtained from the same source was used in all experiments. Chaffed lucerne was fed *ad libitum* for one week before dosing and taking samples for AA analysis in all experiments, unless stated otherwise in the experimental design. Feeding was stopped for 8 hours before dosing with AA and its derivatives in all six experiments, followed by a further fast of 22 hours during which samples were taken (total fast 30 h.). This was designed to duplicate an 8 hour fasting before deer arrival at the commercial slaughter plant, followed by up to 22 hours of further fasting before slaughter. Voluntary feed intake of each animal was determined in all experiments for the last 3 days before fasting commenced.

3.2.4. Experiment 1

Objective: To determine a suitable dose rate of orally administered AA in deer to obtain desirable levels of AA in plasma and rumen fluid (RF). Two trials were conducted.

Table 3.0 Amount of pure ascorbic acid administered orally (O) and abomasally (A) in Experiment 1 (Trial 1 and 2)

Deer No	Trial 1 (g)	Trial 2 (g)
1	200 (O)	400 (O)
2	300 (O)	200 (O)
3	400 (O)	100 (A)
4	100 (A))	

O- Oral administration

A- Abomasal administration

Trial 1:

Three rumen fistulated deer (No.1, 2 and 3) and one abomasally fistulated deer (No. 4) were used. Deer No. 1, 2 and 3 were given oral doses of 200 g, 300 g and 400 g of pure AA respectively. Deer No. 4 was given 100 g of pure AA via abomasal fistula (Table 3.0). All three rumen fistulated deer were sampled from the rumen and blood (jugular vein), whilst blood samples were taken from the abomaslly fistulated deer. Samples were taken 15 minutes (min.) before each dose of AA and 0 hour (h) and 15 min., 30 min., 60 min. and 2 h after each dosage to determine the AA concentrations in blood plasma and rumen fluid.

Trial 2:

Two rumen fistulated deer (No.1 and 2) and one abomasally fistulated deer (No. 3) were used. Trial 2 started two days after trial 1. Deer No. 1 and 2 were given oral doses of 400 g and 200 g pure AA respectively. Deer No. 3 was dosed with 100g of pure AA via abomasal fistula. Deer No. 1 and 2 were sampled from the rumen and blood whilst blood samples were taken from deer No. 3 (abomasally fistulated deer) 15 minutes before each dose of AA and 15 min., 30 min., 60 min., 2 h., 3 h., 4 h., 5 h., 6 h., 7 h. and 8 h. after each dose to determine the AA concentrations in blood plasma and rumen fluid.

3.2.5. Experiment 2

Objective: To define an appropriate time interval between doses of AA in order to obtain repeatable AA concentrations in blood plasma and rumen fluid of deer

Trial 1:

Six ruminally fistulated deer were given 400g of pure AA via rumen fistula (plate 3.1). Blood and rumen fluid samples were taken from them 15 minutes before each dose of AA and 15 min., 30 min., 60 min., 2 h., 3 h., 4 h., 5 h., 6 h., 7 h. and 8 h. after each dose to determine the AA concentrations in blood plasma and rumen fluid. All six ruminally fistulated deer used in Trial 1 were kept in cages for two weeks before commencing Trial 2. Voluntary feed intake (VFI) was measured for 3 days before dosing and for 7 days after dosing.

Trial 2:

The deer were all dosed with the same amount of pure AA (400 g) via rumen fistula and blood and rumen fluid samples were taken at 0 h., 15 min., 30 min., 60 min., 2 h., 3 h., 4 h., 5 h., 6 h., 7 h. and 8 h. as in trial 1. VFI was measured for 3 days before dosing and for 4 days after dosing.

3.2.6. Experiment 3

Objective: To identify the type of AA derivative that was most practically acceptable to raise plasma AA concentration over a prolonged period of time.

Six rumen fistulated deer were used in a 3x3 Latin square experiment, investigating three forms of AA given over three periods. The three forms of AA used were pure ascorbic acid (AA), ethyl cellulose coated ascorbic acid (EC; 97% ascorbic acid) and silicone coated ascorbic acid (SC; 96% ascorbic acid). Two Latin squares were used as shown in Table. 3.1.

Table 3.1. Two 3x3 Latin square design for Experiment 3, period 1, 2 and 3

Deer No.	Period # 1	Period # 2	Period # 3
1	AA	SC	EC
2	SC	EC	AA
3	EC	AA	SC
4	EC	SC	AA
5	SC	AA	EC
6	AA	EC	SC

2.7 g ascorbic acid/kg body weight were given to all six deer
EC (Ethyl cellulose coated ascorbic acid – 97% ascorbic acid)
SC (Silicone coated ascorbic acid- 96% ascorbic acid)

Ascorbic acid and its derivatives were administered intraruminally at 2.7 g AA equivalents /kg LW, which was deemed to be an appropriate dose based on the results of Experiments 1 and 2. Two weeks were allowed between repeat doses, (ie. periods) comprising one week of grazing followed by one week in the cages and fed lucerne chaff *ad libitum*, as determined in Experiment 2. Blood and rumen fluid were taken from all six deer (No. 1, 2, 3, 4, 5 and 6) 15 minutes before each dose of AA, SC and EC and 15

min., 30 min., 60 min., 2 h., 4 h., 6 h., 8 h., 12 h., 16 h. and 22 h. after each dose to determine the AA concentrations in blood plasma and rumen fluid. Rumen pH values were also determined at the Deer Unit soon after taking the samples.

3.2.7. Experiment 4

Objective: It was observed in Experiment 3 that some of the animals showed rumen pH values of less than 5.5, which is the bottom end of the normal Physiological range for rumen pH (Kusmartono 1996). Therefore Experiment 4 was designed to observe the rumen buffering effect of adding sodium bicarbonate to AA to see whether rumen pH levels could be maintained above 5.5 during the course of the experiment.

Table 3.2 Experiment 4 design (Period 1 and 2)

Deer No.	Period 1	Period 2
1	AA+$NaHCO_3$	AA
2	AA+$NaHCO_3$	AA
3	AA+$NaHCO_3$	AA
4	AA+$NaHCO_3$	AA
5	AA	AA+$NaHCO_3$
6	AA	AA+$NaHCO_3$
7	AA	AA+$NaHCO_3$

Seven rumen fistulated deer were used in a changeover design, involving two periods, as shown in Table 3.2. Four rumen fistulated deer in group A (deer No. 1, 2 3 and 4) were given pure AA mixed with sodium bicarbonate ($NaHCO_3$) at 10:1 ratio via rumen fistula

in period 1, whilst group B animals (deer No. 5, 6 and 7) were given only AA via rumen (Table 3.2). An amount of 2.7 g AA /kg body weight was administered intraruminally as a single dose to all deer. All seven deer were sampled from the rumen and blood, 15 minutes before each dose of AA and 15 min., 30 min., 60 min., 2 h., 4 h., 6 h. and 8 h. after each dose to determine the AA concentration in rumen fluid and blood plasma. Rumen pH values also determined soon after taking rumen fluid at the above times. At the conclusion of period 1, the deer were turned out to graze for one week and then individually fed lucerne chaff *ad libitum* during period 2 for a further one week. The deer were then changed over between nutritional treatments and the experimental protocol repeated.

3.2.8 Experiment 5

Experiment 5 had two trials.

Objective (Trial 1): To observe the differences in AA concentrations in the rumen, abomasum and blood plasma after dosing pure AA via the rumen

Objective (Trial 2): To observe differences in ascorbic acid concentrations in plasma after dosing of pure AA via abomasum

Trial 1:

Three deer, fistulated in both the rumen and abomasum, were dosed with an amount of 2.7 g AA/kg body weight, as administered in Experiments 3 and 4.

All deer were sampled from the rumen and blood, 15 minutes before each dose of AA and 15 min., 30 min., 60 min., 2 h., 4 h., 6 h., 8 h., 12 h., 16 h. and 22 h after each dose. Abomasal fluid samples were also taken at all times except at 15 minutes and 30 min after dosing with AA, when samples could not be obtained due to lack of flow into the abomasum. Rumen and abomasal pH values were also determined.

All three deer were kept in the cages until Trial 2 started two weeks later, to observe any physiological disturbances.

Trial 2:

The same three deer used in Trial 1 were given pure AA via the abomasum. An amount of 0.75g AA/kg body weight was administered to all three deer.

Blood samples were taken from all three deer, 15 minutes before each dose of AA and 15 min, 30 min., 60 min., 2 h., 4 h., 6 h., 8 h., 12 h., 16 h. and 22 h. after each dose to determine the AA concentration in blood plasma. Rumen and abomasal fluid samples were also taken to observe the pH values (There was no ascorbic acid analysis in rumen fluid and abomasal fluid in trial 2). As in trial 1, there was no sampling of abomasal fluid at 15 min and 30 min after AA was administered to each deer.

3.2.9. Experiment 6

Objective: The first objective was to measure the rumen fractional outflow rate (FOR) of liquid and rumen fractional disappearance rate (FDPR) of AA in fasted deer after dosing a large amount of AA into the rumen, using the single dose and time-sequence sampling method (Faichney, 1975). It was assumed that AA was associated with the liquid phase of digesta and hence left the rumen with the same FOR as Cr-EDTA. A second objective was to calculate the proportion of the AA dose given that flowed out of the rumen and into the abomasums. Two trials were conducted.

Three deer fistulated in the rumen and three deer fistulated in both the rumen and abomasum were used in both trials. Feeding was stopped for 8 hours before dosing with Cr-EDTA, a standard liquid phase marker used in digestion studies. Cr-EDTA solution was made up using the method of Binnerts *et al.* (1968), to give a final Cr concentration of 2.77 mg/ml. Three litres of Cr-EDTA solution was prepared and adjusted to a final pH of 7.0, for use in both trial 1 and trial 2. All six deer were dosed with 180 ml. Cr-EDTA via rumen fistula, supplying 498.6 mg of Cr, in each of the two trials.

Trial 1:

Rumen samples from all six deer and abomasal fluid from three (abomasally fistulated deer) deer were taken, 15 minutes before dosing with Cr-EDTA and 15 min., 30 min., 60 min., 2 h., 4 h., 6 h., 8 h., 12 h., 16 h. and 22 h. after each dose of Cr-EDTA to determine the Cr concentration in the rumen and abomasum fluid. Rumen and abomasal fluid pH values were also determined at these times. All six deer were kept in the cages until trial 2 started one week later.

Trial 2:

In trial 2, all six deer were given pure AA mixed with 180 ml of Cr-EDTA (2.77 mg Cr./ml of water) via rumen fistula. An amount of 2.7 g AA/kg body weight was administered with Cr-EDTA to each deer.

All six deer were sampled from the rumen, blood and abomasum (three abomasally fistulated deer) 15 minutes before each dose of AA and Cr-EDTA and 15 min., 30 min., 60 min., 2 h., 4 h., 6 h., 8 h., 12 h., 16 h. and 22 h. after each dose to determine the concentration of Cr and AA in rumen and abomasal fluid and AA concentration in blood plasma. The pH values of rumen and abomasal fluids were also measured as in trial 1. No abomasal samples could be obtained 15 min and 30 min after dosing, due to lack of flow.

3.2.10 Laboratory Analysis

3.210.0 Rumen pH measurement

Rumen pH values in experiments 3, 4 5, 6 and abomasal pH values of deer in experiments 5 and 6 were measured using FE 257 Micro pH Meter (EDT instruments Ltd, Dover Kent ct16 2aa, UK). These pH values were determined soon after taking the samples (Plate 3.2). Before taking these values the pH meter was standardized each time using pH solutions 0 and 7.0.

3.2.10.1 Preparation of blood, rumen fluid and abomasal fluid samples for ascorbic acid analysis

Blood samples (10ml) were centrifuged at 2500 rpm for 20 minutes and plasma was separated. Rumen and abomasal samples were also centrifuged before de-proteinization. Blood plasma, rumen fluid and abomasal fluid were then immediately deproteinized with 4% W/V metaphosphoric acid in the ratio of 1:9 and mixed well using a Vortex. An amount of 900 µl, 4% metaphosphoric acid was added to 100 µl of plasma, rumen fluid and abomasal fluid in Eppendorf tubes (triplicate) and stored at –75°C.

3.2.10.2. Ascorbic acid analysis method

Ascorbic acid was determined using the Boehringer Mannheim L-Ascorbic acid analysis kit Cat No. 409 677. The analysis and the reading were carried out using ELISA plates rather than cuvettes in a spectrophotometer. Samples were read on an Anthos Labtec 2001 96 well ELISA plates reader. The results were calculated using AssayZap, a curve fitting programme supplied by Biosoft (Cambridge, UK).

Ascorbic acid analysis using Boehringer kit

Reagents:

Metaphosphoric acid 4% W/V

4g metaphosphoric acid was made up to 100 ml in a volumetric flask. This was used for diluting standards and samples. Kit reagents were used as supplied.

Into a 96 well microtitre plate 100 µl sample, 100 µl reagent 1 (buffer, substrate) and 10 µl colour reagent were pipetted in duplicate for each sample/standard. Plates were incubated for 30 minutes at 37°C. As the reaction was light sensitive, filters were kept in a light proof incubator. The resulting colour was read using a 540 nm primary and 620 nm secondary. The results were plotted in AssayZap using a linear standard curve option and then exported as a CSV file to read into Excel.

Preparation of standards for ascorbic acid analysis:

20mg Analar AA was weighed and made up to 10 ml in 4% W/V metaphosphoric acid. This stock was distributed in 0.50ml aliquots and stored frozen at –80°C. Standards were diluted from the stock to give a concentration range from 3.12 to 100µg/ml. A fresh set of standards was used for each analysis.

3.2.10.3 Chromium (Cr) concentration analysis in rumen fluid and abomasal fluid

3.2.10.3.1 Preparation of Cr-EDTA solution

Cr-EDTA solution was made up using the method of Binnerts *et al.* (1968), which gave a final Cr concentration of 2.77 mg Cr/ml. 42.6g of pure chromium Trichloride ($CrCl_3$ $6H_20$) was weighed into a 3 litre beaker and dissolved in 600 ml deionised H_20. 60 g di-sodium ethylenediaminotetraacetic acid (EDTA) was dissolved likewise in 600 ml deionised H_20 and added to the first solution. The mixed solution was boiled gently for one hour. After cooling, the small excess of EDTA was neutralised with about 12 ml 1M $CaCl_3$. pH was then adjusted using 1N NaOH to a final pH of 7.0 and the solution made up to 3 litres. This preparation was stored at 4°C.

3.2.10.3.2 Preparation of rumen fluid and abomasal fluid samples for Cr. analysis

Samples removed from the rumen and abomasum were centrifuged at 15,000 rpm (high-speed centrifugation) for 10 minutes. The supematants (15 ml) were stored in labelled glass tubes at 4°C until they were used for chromium analysis.

Atomic Absorption Spectrometry (AAS) was used to analyse the chromium concentration in rumen fluid and abomasal fluid of deer, without acid digestion. Standards for the calibration curve were made up in rumen fluid removed from deer before Experiment 6, trial 1 commenced. An amount of 100 ml rumen fluid was taken from all six deer used in Experiment 6 on two occasions, pooled and centrifuged at

15,000 for 10 minutes and stored at 4°C. Cr-EDTA from the solution infused was added to the supernatant to prepare the standard curve.

3.2.10.4 Total Nitrogen and in vitro organic matter digestibility

Two samples of feed offered (A and B) in each experiment were prepared on a weekly basis by pooling 200g/day. An amount of 100g each from samples A and B was freeze dried and ground to pass through a 1mm diameter sieve for laboratory analysis. The total N and *in vitro* organic matter digestibility (OMD) were analysed at the Nutrition Laboratory, Massey University. Total nitrogen (N) was determined by the Kjeldahl method, organic matter (OM) by ashing samples for 16 hr at 550°C and *in vitro* organic matter digestibility (OMD) by the enzymatic method of Roughan and Holland (1977).

3.2.10.5. Dry matter percentage

Samples of " pooled" feed residue were taken each day, whilst samples of feed offered were taken every second day; dry matter (DM) percentage was determined in triplicate at the Animal Physiology Unit (APU), Massey University on the same day that the samples were taken. Dry matter percentage of residue and feed offered were determined by placing 100 g of samples in an oven for 24 h. at 100 °C.

3.2.11. Statistical Analysis and Data Collection

Data in Experiments 2 and 5 were expressed as the mean values with the standard error of the mean (SEM). Voluntary feed intake data in Experiment 2 was analysed using Repeat Measures Analysis of Variance (SAS System, REML Estimation). Voluntary feed intake on each day following AA dosing was compared with VFI for 3 days before dosing, for both Trial 1 and 2 data. Animal liveweight was also tested as a co-variate.

In Experiments 3 and 4, area under the concentration vs. time curves (AUC) was calculated using the Trapezoidal Rule and expressed both as AUC and AUC corrected for pre-dosing baseline values (AUCB). Maximum concentration (MAX) was also estimated. AUC, AUCB and MAX were then analysed by Analysis of

Variance (Genstat 5 Release 4.1; Law Agricultural Trust, Rothamsted Experimental Station UK), with period within animal as the block structure, and AA treatment as the treatment structure. Main effects of nutritional treatments were calculated by averaging over all time periods, whilst main effects of periods were calculated by averaging over nutritional treatments.

In Experiment 6, data for Cr concentrations were fitted to the following equation and k derived for each animal in each period as the slope of $\log_e$ Cr concentration vs. time. All post-dosing values for Cr concentration were first corrected for pre-dosing background values.

$$C = C_0 e^{-kt} \qquad (1)$$

C is the marker concentration at time t, C_o is the marker concentration at t_o, i.e. equilibrium concentration; k is the dilution rate (or FOR). Distribution volume of Cr-EDTA in the rumen was then calculated as the dose Cr given divided by its concentration in rumen fluid at time zero (C_0). The later was obtained as the antilog of the intercept in the plot of $\log_e$ Cr concentration versus time. Data for rumen liquid volume and rumen liquid FOR were then analysed by Analysis of Variance; in the case of rumen volume, animal liveweight was also tested as a co-variate.

Fractional disappearance rate (FDPR) of AA from the rumen in Experiment 6 was calculated as the slope (i.e. k value) of the $\log_e$ rumen AA concentration versus time curve, using Equation 1. All post-dosing values were first corrected for pre-dosing background values. Fractional disappearance rate (FDPR) of AA from the rumen is the sum of the following components,

- fractional degradation rate in the rumen (FDR)
- fractional absorption rate from the rumen (FAR)
- fractional outflow rate from the rumen (FOR)

As ascorbic acid is a solute, it is assumed that it would leave the rumen with the same FOR as Cr-EDTA. The amount of dosed AA that left the rumen and flowed into the intestines (rumen outflow; RO) was then calculated using Equation 2 (G.J. Faichney personal communication).

$$RO(g) = Dose(g) \times FOR/FDPR \quad (2)$$

The combined total of FDR and FAR was calculated as

$$FDR + FAR = FDPR - FOR \quad (3)$$

Without the use of isotope labelling, it is not possible to separate FDR and FAR.

The time for half the dosed AA to disappear from the rumen or flow out of the rumen ($T_{1/2}$; i.e. half life) was calculated using Equation 4.

$$T_{½}(h) = \log_e 2 / FDPR \text{ or } FOR = 0.6931 / FDPR \text{ or } FOR \quad (4)$$

3.3. RESULTS

3.3.1. Animal Liveweight

The mean initial and final liveweight of deer with the standard error (SEM) and the number of deer used in each experiment are given below in Table 3.3.

Table 3.3. Initial and final liveweight and number of deer used in each experiment

Experiment No.	Number of Deer (n)	Initial Liveweight (kg) (Mean±SEM)	Final Liveweight (kg) Mean±SEM)
1	4	137.3±6.05	132.1±6.27
2	6	130.8± 6.60	129.7±7.53
3	6	124.2± 7.17	125.4±7.71
4	8	131.0± 6.96	129.6±6.22
5	3	104.8±15.19	101.8±14.38
6	6	133.7±11.99	131.7±11.75

3.3.2 Diet Composition and Voluntary Feed Intake

The mean values for Nitrogen (N) concentration, Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD), Digestible Organic Matter in the Dry Matter (DOMD) and ash percentages in the diet (chaffed lucerne) are given in Table 3.4 and showed little variation between experiments.

The mean voluntary feed intake (VFI) of deer in each experiment was calculated for the last 3 days before dosing with AA and is given in Table 3.5. These data were collected from June 1999 (winter) to February 2000 (summer). Whilst VFI increased from winter to summer, as would be expected in farmed red deer, it was relatively constant for the individual trials within each experiment (Table 3.5).

Table 3.4. Composition of chaffed lucerne fed to deer during Experiments 1, 2, 3, 4, and 5 and 6

Analyses	Mean (n=13)	SEM
N (%/DM)	3.66	0.068
DMD (%)	66.06	0.606
DOMD (g/100g DM)	62.48	0.486
OMD (%)	69.23	0.594
Ash (%/DM)	7.65	0.094

Table 3.5. Mean Voluntary Feed Intake (VFI) of deer in the 3 days immediately before dosing with ascorbic acid (kg as dry matter basis)

Experiment No	Trial No.	Number of Deer	Mean VFI (kg DM/day)	SEM
1	1	4	2.384	0.1789
	2	3	2.045	0.1367
2	1	6	2.276	0.0976
	2	6	2.449	0.0621
3	1	6	2.410	0.1217
	2	6	2.568	0.1189
	3	6	2.413	0.4046
4	1	7	2.519	0.0986
	2	8	2.581	0.0950
5	1	3	2.837	0.1591
	2	3	2.891	0.1511
6	1	6	2.836	0.1972
	2	6	2.765	0.1762

DM- dry matter

3.3.3. Experiment 1

Initially, difficulty was encountered in reproducing the results obtained at Agresearch Invermay, where single oral doses of 100 and 200 g AA had increased plasma AA concentration in deer for up to 3 h after dosing (J.M. Stevenson-Barry personal communication). Single oral doses of 100 g AA given to the deer at Massey University failed to increase plasma AA concentration. It was felt that the difference was probably related to the deer used at Massey University being heavier than those used at Invermay. Single oral doses of 200, 300 and 400 g AA were then each given to individual deer, with the objective of identifying a suitable dose that gave similar rumen and plasma AA

profiles to those obtained at Invermay. This was achieved with the 400 g dose of AA (Figure 3.1 A and B), which worked out at 2.8 g AA/kg liveweight, similar to the highest dose given at Agresearch Invermay (200 g to 70 kg deer; J.M.Stevenson-Barry, personal communication). Lower values for AA concentration were obtained in both rumen fluid and blood plasma in trial 2, probably due to adaptation to AA degradation by rumen organisms, as trial 2 was conducted two days after trial 1.

Blood plasma AA concentrations were low after administrating 100 g of AA (0.8 g AA/kg liveweight) by abomasal fistula and were similar in both Trial 1 and 2, which were conducted two days apart (Figure 3.2). At the end of Experiment 1, it was decided to settle with a dose rate of 2.7-2.9 g AA/kg liveweight for the remaining experiments in order to produce a high AA concentration in blood plasma and in rumen fluid. It was also decided for reasons of animal ethics and practical reasons to use intraruminal administration of AA due to physiological disturbances experienced after high oral administration. For abomasal administration a suitable dose was 0.8g AA /kg liveweight, this being the maximum amount of AA suspension that could be administered as a single dose into this organ.

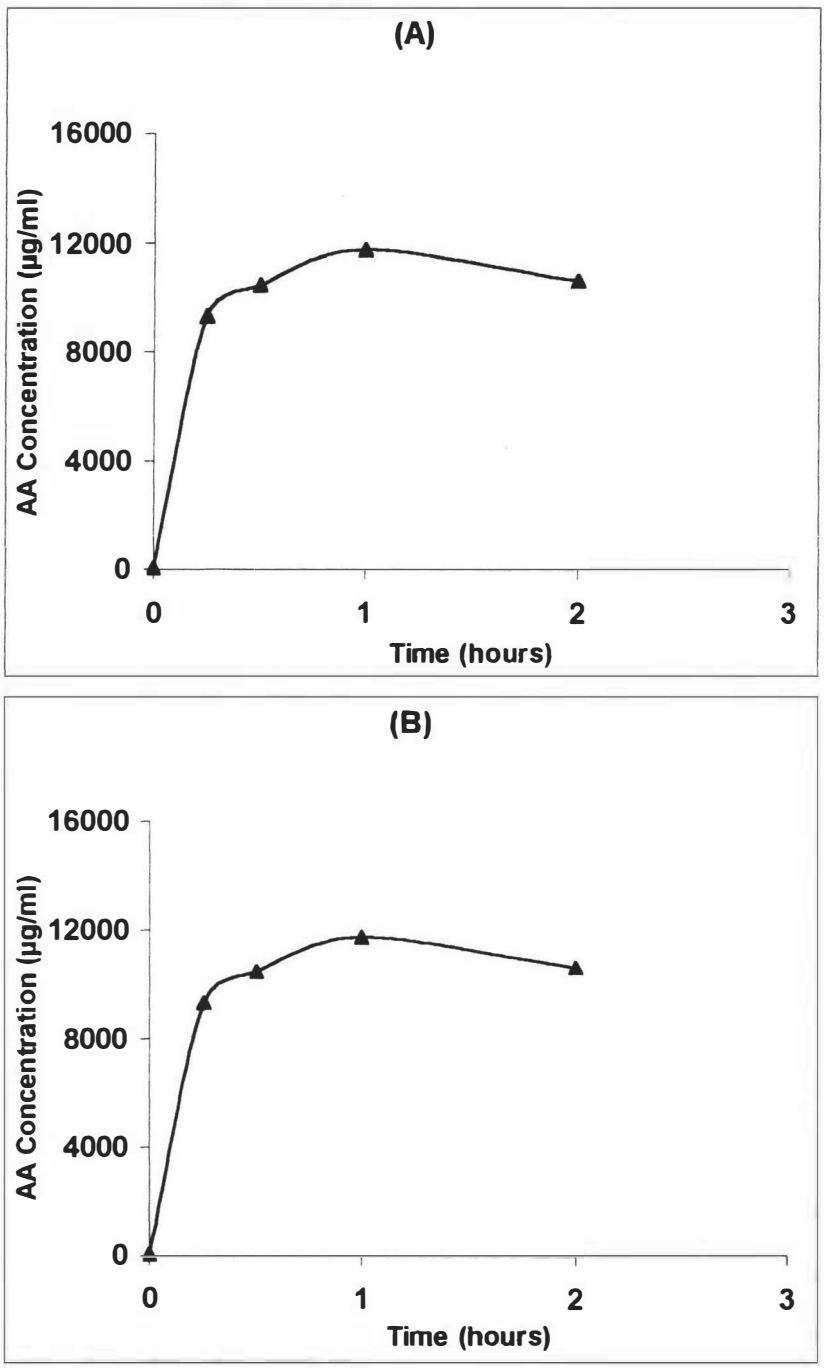


Figure 3.1. Experiment 1; Trial 1. Rumen fluid (A) and blood plasma (B) ascorbic acid concentration during 0 to 2 h in a deer given a single oral dose of 400 g ascorbic acid (2.8 g/kg liveweight)

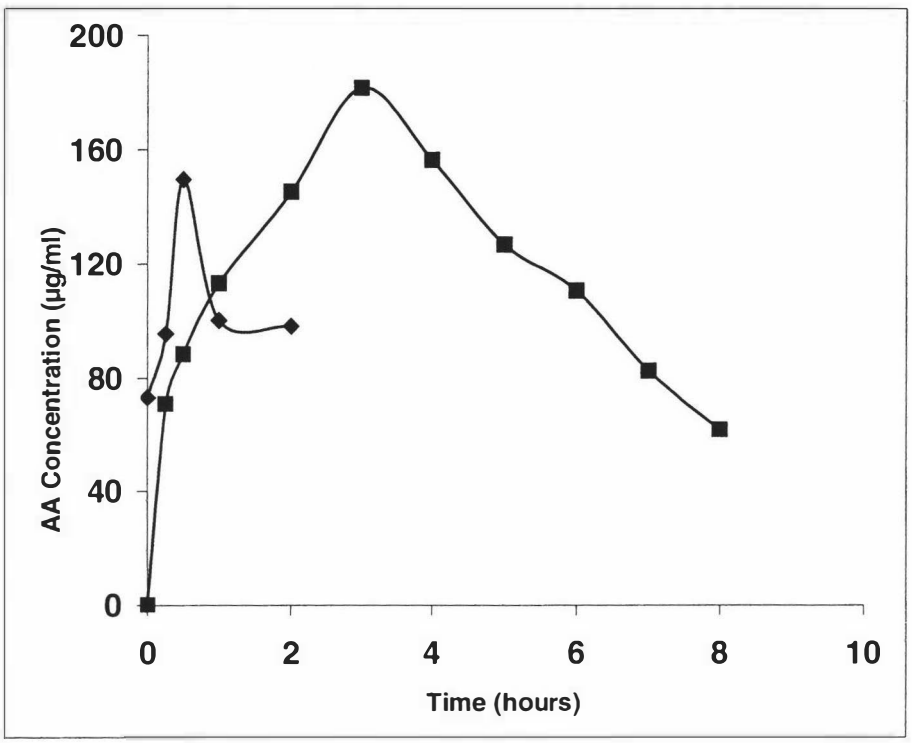


Figure 3.2. Experiment 1. Blood plasma ascorbic acid (AA) concentration in one deer, (during 0 to 2h in Trial 1 and 0 to 8 h in Trial 2) given 100 g AA by abomasal fistula (0.82 g AA/kg liveweight) on two occasions two days apart. —◆— Trial 1; —■—Trial 2.

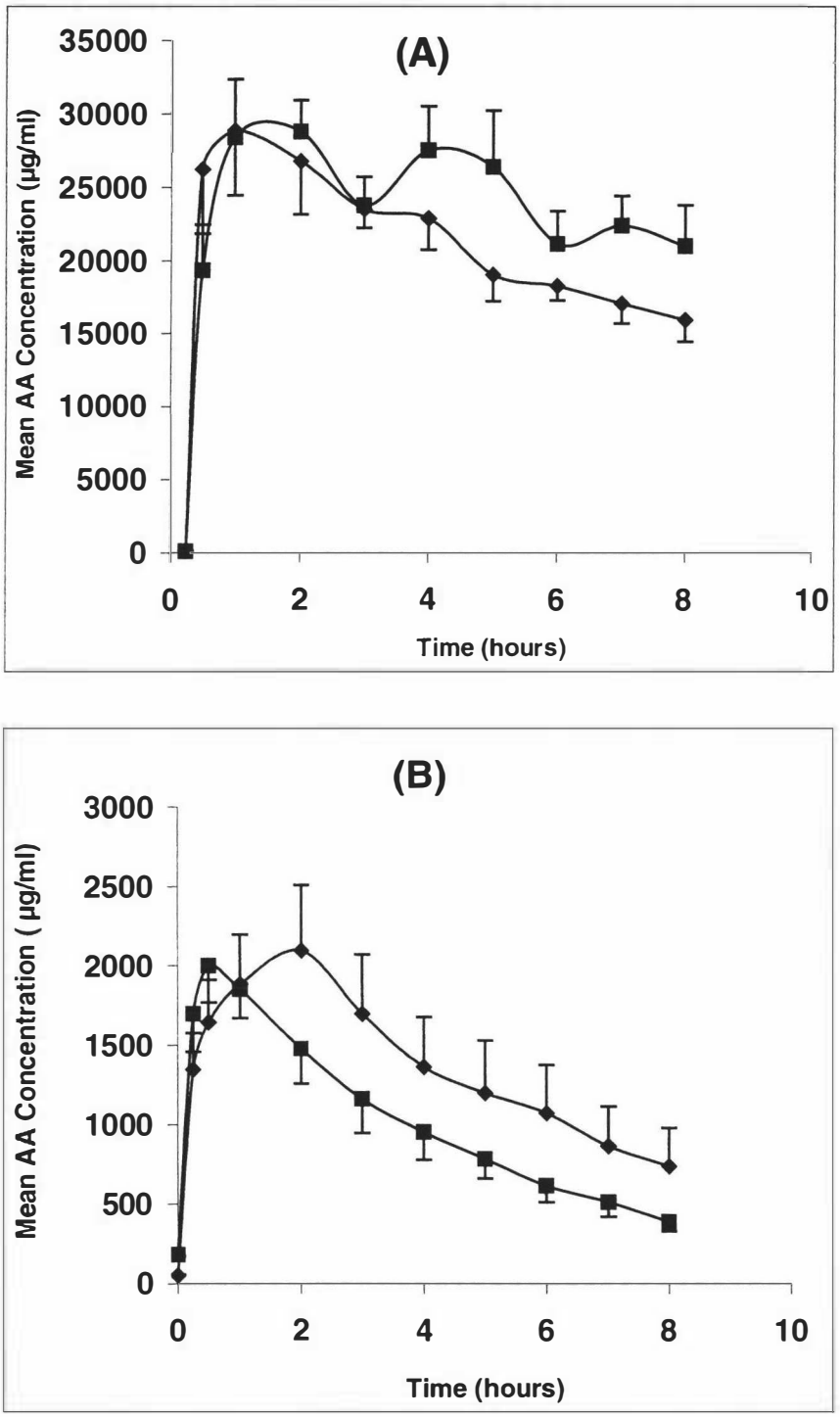


Figure 3.3. Experiment 2. Mean rumen fluid (A) and blood plasma (B) ascorbic acid (AA) concentrations during 0 to 8 h in six deer given single doses of 400 g ascorbic acid (AA) through rumen fistula on two occasions two weeks apart. —◆— mean Trial 1; —■— mean Trial 2; T⊥ standard error (SE) bars.

3.3.4. Experiment 2

Ascorbic acid concentrations in both rumen fluid and blood plasma after large single intraruminal doses (400g AA-2.7g/kg liveweight) of AA in Trial 1, remained high and these values were similar in Trial 2 which was conducted two weeks later (Figure 3.3;A and B). In trial 1, relative to the 3 days before dosing, VFI was depressed by 1.21 kg DM during the first day after dosing ($P<0.001$), by 0.87 kg DM on day 2 ($P<0.001$), by 0.59 kg DM on day 3 ($P<0.05$) and by 0.38 kg DM on day 4 ($P<0.10$); thereafter VFI was not significantly different from pre-treatment values (2.06 kg DM/day). In trial 2, VFI was depressed by 1.57 kg DM on day 1 ($P<0.001$), 1.13 kg DM on day 2 ($P<0.001$), 0.84 kg DM on day 3 ($P<0.001$) and by 0.51 kg DM on day 4 ($P<0.05$), relative to pre-treatment values (2.25 kg DM/day).

For intraruminal administration it was therefore decided to use single doses of 2.7 g AA/kg liveweight. Data for 15 min or zero time rumen and plasma concentrations in trial 2 were at baseline levels two weeks after trial 1 and that interval is therefore appropriate, in future experiments in order to obtain repeatable concentrations of AA in blood plasma and rumen fluid and to allow time for VFI to recover.

3.3.5. Experiment 3

Nutritional effects

In Experiment 3, three formulations of ascorbic acid (AA, EC and SC) were compared with 2-week periods between dosing. Peak concentrations of ascorbic acid occurred in both rumen fluid and blood plasma approximately one hour after the intraruminal administration of all three forms of ascorbic acid (Figure 3.4;A and B). These peak concentrations were similar and then gradually declined for all three compounds (Figure 3.4; A and B). The area under curve (AUC), area under curve corrected for baseline

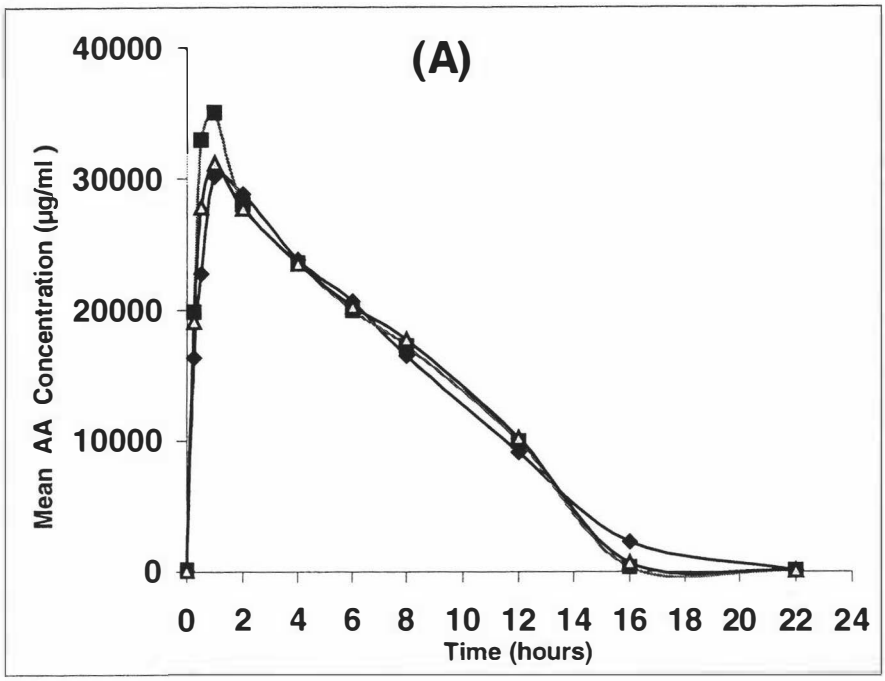
concentration (AUCB) and maximum concentration (MAX) of ascorbic acid both in rumen fluid and blood plasma were not significantly different between the three ascorbic acid treatments (P> 0.05; Table 3.6 and 3.7).

The mean pH values in rumen fluid of 6 deer were studied after single intraruminal doses of AA, EC and SC. Initial pH values before dosing with ascorbic acid were approximately 7.0 and declined to a value of approximately 5.0 units, 1h after AA administration. Then, there was a rapid increase in pH values in all three treatments to reach approximately 6.0 units 4 h after ascorbic acid administration and then gradually reaching normal rumen fluid value of 7.0 after 22 h. (Figure 3.5). There were no significant differences in rumen pH values between the three formulations of ascorbic acid (P >0.05) at any of the sampling times.

Period effects

Period effects of (Period 1, 2 and 3) AUC, AUCB and MAX of AA in plasma and rumen fluid in Experiment 3 during 0-22 h in deer given single intraruminal doses of AA, EC and SC are given in Table 3.8. These results show that, averaged over all AA formulations, there were no significant differences of AUC, AUCB and MAX of blood plasma and in rumen fluid between periods (p>0.05).

Statistical analysis of pH values at 0 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h 16 h and 22 h in three periods also showed that there were no significant period effects at any time after dosing with the different types of AA (p>0.05). Administration of AA depressed rumen pH in all samples taken up to and including 16 h after dosing (p<0.001), relative to pre-dosing values. Rumen pH was not significantly different after 22 h from pre-dosing values (p>0.05).



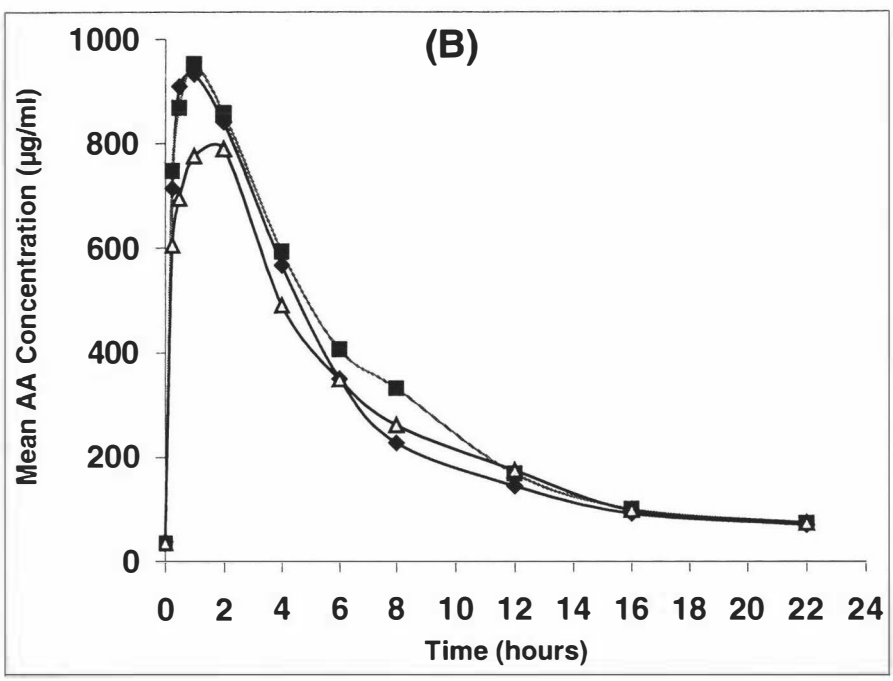


Figure 3.4 Experiment 3; Mean AA concentration in rumen fluid (A) and blood plasma (B) during 0 to 22 h in 6 deer given single doses of AA, EC and SC through rumen fistula at 2.7g AA equivalent/kg liveweight. Mean values of AA, EC and SC were obtained from 6 deer in three trials, using two 3x3 Latin squares. —◆—AA;—■—EC;—Δ—SC

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 2.7g AA equivalent/kg liveweight. n=6 deer/ treatment.

	Treatment			
	AA*	EC*	SC*	SED
AUC (μg/h/ml) **	12,120	12,191	12,160	1,090 (ns)
AUCB (μg/h/ml) **	11,998	12,065	12,052	1,096 (ns)
MAX (μg/ml) **	30,806	35,670	33,127	2,378 (ns)

*AA- pure ascorbic acid; EC- ethyl cellulose ascorbic acid; SC- silicone coated ascorbic acid
**AUC -area under curve; AUCB-area under curve corrected for base line; MAX- maximum concentration; ns- non significant ($p>0.05$)

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 2.7g AA equivalent/kg liveweight. n=6 deer/ treatment.

	Treatment			
	AA*	EC*	SC*	SED
AUC (μg/h/ml) ** (ns)	288	279	318	46.9
AUCB(μg/h/ml)** (ns)	244	238	274	47.6
MAX(μg/ml)**	958	820	954	160.4 (ns)

*AA- pure ascorbic acid; EC- ethyl cellulose ascorbic acid; SC- silicone coated ascorbic acid
**AUC -area under curve; AUCB-area under curve corrected for base line; MAX- maximum concentration; ns- non significant ($p>0.05$)

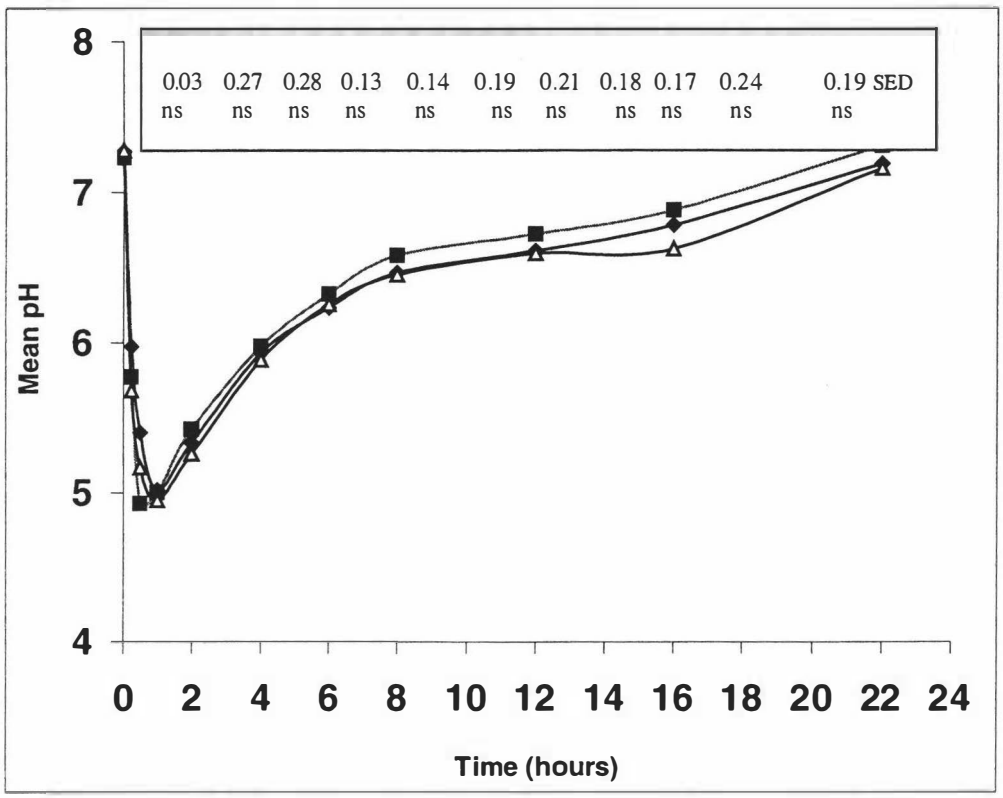


Figure 3.5. Experiment 3; Mean pH values in rumen fluid during 0 to 22hr in 6 deer given single doses of AA, EC and SC through rumen fistula at 2.7g AA equivalent/kg liveweight. —◆— AA; —■— EC; —Δ—SC ; SED, standard error of the difference between means; ns- non significant ($p>0.05$)

Table 3.8. Period effect of Experiment 3; Area under curve (AUC), area under curve 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.

	Period			
	1	2	3	SED
Rumen fluid ascorbic acid				
AUC (μg/h/ml)	11,527	13,007	11,937	1,090(ns)
AUCB (μg/h/ml)	11,403	12,867	11,845	1,096(ns)
MAX (μg/ml)	30,678	35,824	33,101	2,378(ns)
Blood plasma ascorbic acid				
AUC (μg/h/ml)	305	312	268	46.9(ns)
AUCB (μg/h/ml)	254	272	230	47.6(ns)
MAX (μg/ml)	989	930	813	160.4(ns)

ns- not significant $p>0.05$; *- $p<0.05$

3.3.6. Experiment 4

Nutritional effects

In Experiment 4, the mean rumen pH values were compared in seven deer after intraruminal administration of AA at 2.7 g/kg liveweight, either alone or mixed with $NaHCO_3$ at 10:1 ratio. Initial pH values before dosing with AA were approximately 7.0 and declined to a value of approximately 6.0 and 5.0, 1 h after administration of AA mixed with $NaHCO_3$ and AA alone respectively (Figure 3.6). Then there was a steady increase in pH values in both treatments up to 8 h after AA administration, when rumen

pH was 0.35 units higher for the AA plus $NaHCO_3$ group ($P<0.05$). Rumen pH in the AA plus $NaHCO_3$ group was consistently 0.7 to 0.4 units higher than for the ascorbic acid only group (Figure 3.6), with the difference attaining significance at $P<0.01$ for samples taken 1 to 6 h after dosing.

Ascorbic acid concentrations both in rumen fluid and in blood plasma were lower in deer dosed with AA mixed $NaHCO_3$ than in the deer that received ascorbic acid only (Figure 3.7; A and B). The area under curve (AUC) and area under curve corrected for baseline concentration (AUCB) for rumen fluid were significantly lower for the AA plus $NaHCO_3$ group than for AA only group ($P<0.05$; Table 3.9), whilst the maximum concentration (MAX) of AA in rumen fluid was not statistically significantly different between treatments ($P>0.05$; Table 3.9). AUC, AUCB and MAX of AA in blood plasma were all not statistically significantly different between the two treatments ($P>0.05$; Table 3.10).

Period effects

Period effects (Period 1 vs. Period 2) on AUC, AUCB and MAX of AA in plasma and rumen fluid in Experiment 4 during 0-8 h in deer dosed with single intraruminal doses of AA are given in Table 3.11. There were no significant differences of AUC, AUCB and MAX in rumen fluid between periods ($p>0.05$). MAX of blood plasma was also not significantly different between periods, but AUC and AUCB values of blood plasma were lower in Period 2 than in Period 1 ($p<0.05$), with this difference being difficult to explain.

Statistical analysis of rumen pH values at 0 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, and 8 h showed that there were no significant period effects at any time after dosing with AA ($p>0.05$).

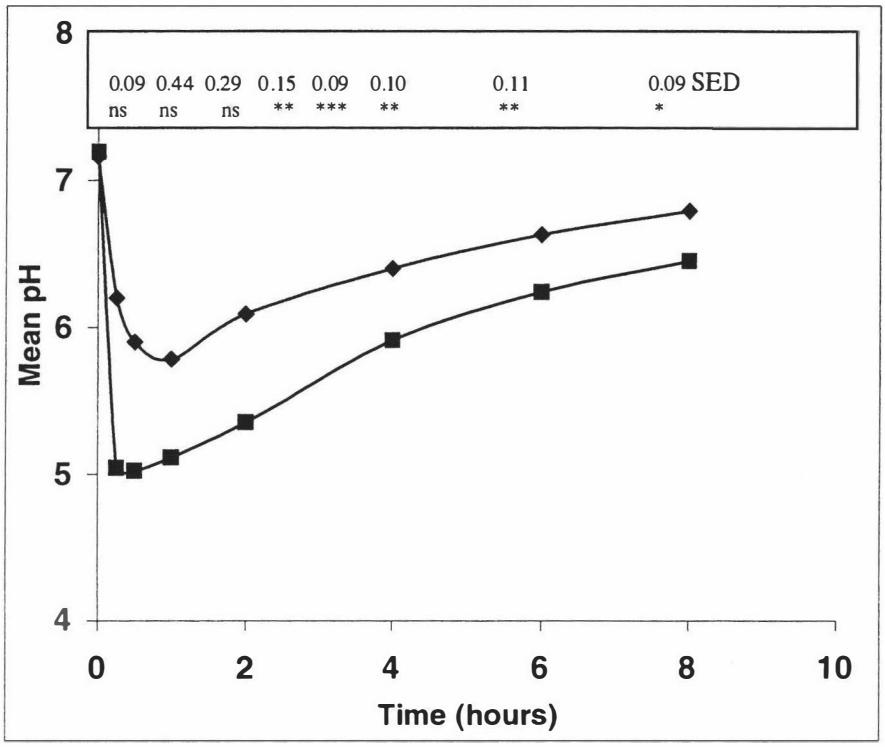


Figure 3.6. Experiment 4; Mean pH values in rumen fluid during 0 to 8hr in seven deer given single intraruminal doses of AA at 2.7 g/kg liveweight, either alone or mixed with $NaHCO_3$ at 10: 1 ratio. —◆— with $NaHCO_3$; —■— without $NaHCO_3$; SED- Standard Error of the Difference between means; ns- non significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p< 0.001$

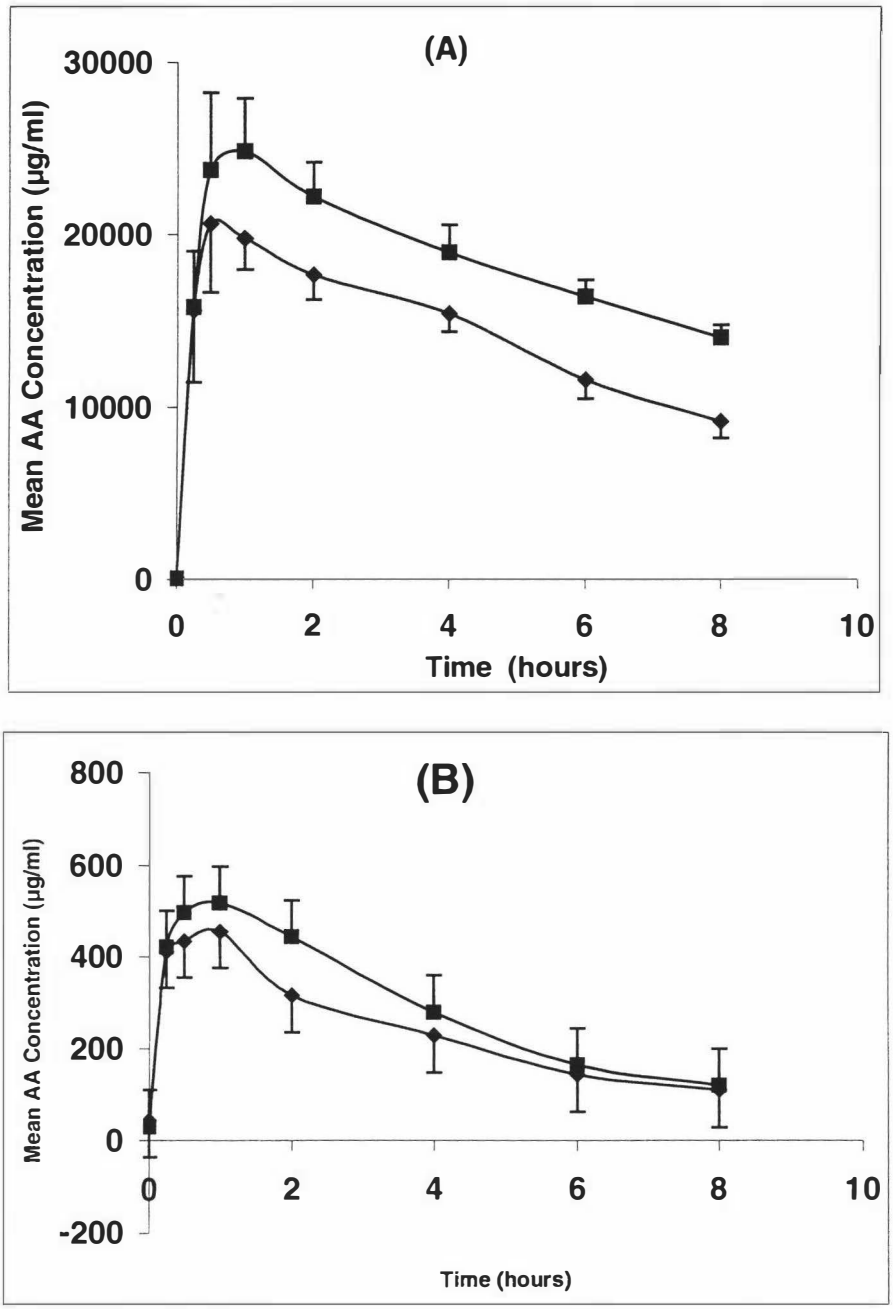


Figure 3.7. Experiment 4; Mean concentration of ascorbic acid (AA) in rumen fluid (A) and in blood plasma (B) in 7 deer given single intraruminal doses of ascorbic acid (AA) at 2.7g/ kg liveweight, either alone or mixed with $NaHCO_3$ at 10:1 ratio. —◆— mean AA with $NaHCO_3$; —■— mean AA without $NaHCO_3$; T⊥ standard error (SE) bars

Table 3.9. Nutritional effects in Experiment 4; Area under curve (AUC), area under curve corrected for baseline concentration (AUCB) and maximum concentration (MAX) of ascorbic acid (AA) in rumen fluid during 0 to 8 h in deer given single intraruminal doses of AA at 2.7g/kg liveweight, either alone or mixed with $NaHCO_3$ at 10:1 ratio.

	Treatment of AA		
	without $NaHCO_3$ [1]	with $NaHCO_3$ [1]	SED
AUC (μ/h/ml) [2]	18,942	14,690	990 **
AUCB (μg/h/ml) [2]	18,878	14,645	979 **
MAX (μg//ml) [2]	26,769	23,196	3,970 (ns)

$NaHCO_3$ [1] - Sodium bicarbonate
AUC [2] -area under curve; AUCB [2]-area under curve corrected for base line concentration ; MAX [2]- maximum concentration; * $p<0.05$; ** $p<0.01$; *** $p< 0.001$; ns- non significant ($p>0.05$)

Table 3.10 Nutritional effects 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 h in deer given single intraruminal doses of ascorbic acid (AA) at 2.7g/kg liveweight, either alone or mixed with $NaHCO_3$ at 10:1 ratio.

	Treatment of AA		
	without $NaHCO_3$ [1]	with $NaHCO_3$ [1]	SED
AUC (μg/h/ml) [2]	296	243	54.5 (ns)
AUCB (μg/h/ml) [2]	255	199	55.5 (ns)
MAX (μg//ml) [2]	516	470	99.0 (ns)

$NaHCO_3$ [1] - Sodium bicarbonate
AUC [2] -area under curve; AUCB [2]-area under curve corrected for base line concentration ; MAX [2]- maximum concentration; ns- non significant ($p>0.05$)

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 plasma and rumen fluid in Period 1 and 2.

	Period		
	1	2	SED
Rumen fluid ascorbic acid			
AUC (μg/h/ml)	17,056	16,575	999(ns)
AUCB (μg/h/ml)	16,995	16,528	979(ns)
MAX (μg/h/ml)	25,522	24,444	3,970(ns)
Blood plasma ascorbic acid			
AUC (μg/h/ml)	337	202	55.5(ns)
AUCB (μg/h/ml)	298	156	53.6 *
MAX (μg/h/ml)	628	358	99.0 *

ns- not significant $p > 0.05$; *- $p < 0.05$

3.3.7. Experiment 5

Mean concentrations of AA in rumen fluid and abomasal fluid in 3 deer after a large single intraruminal dose of AA (2.7 g/kg liveweight) in Trial 1, are shown in Figure 3.8A. Concentration of AA in abomasal fluid was consistently much lower than found in rumen fluid. Maximum mean AA concentration in blood plasma in Trial 1 was observed 15 min after the administration of AA and then there was a gradual decline to almost reach zero, 22 h after AA administration (Figure 3.8B).

Rumen fluid pH declined rapidly following intraruminal AA administration and this was followed by a sustained rise in abomasal pH (Figure 3.9A). Rumen pH was significantly

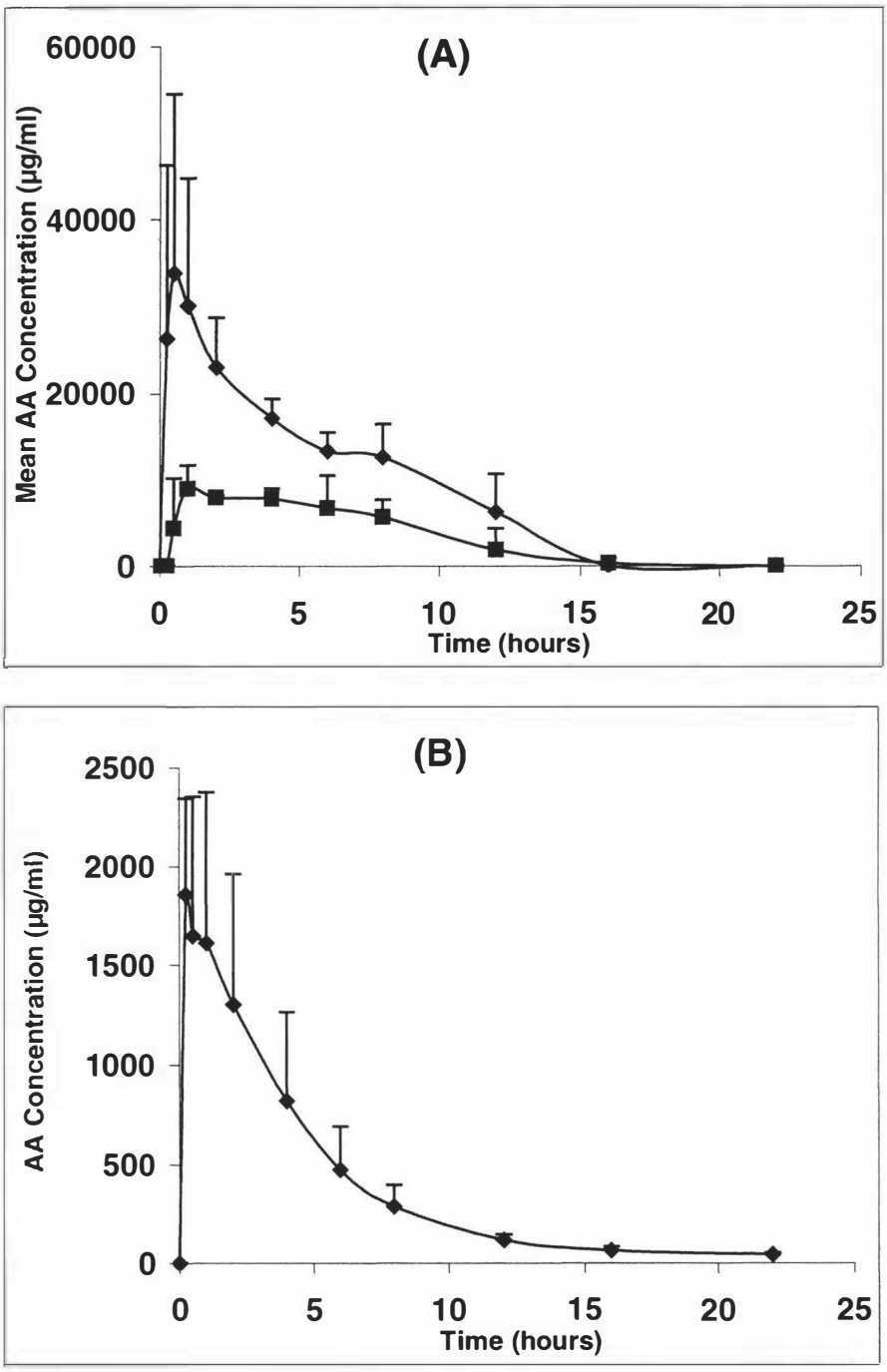


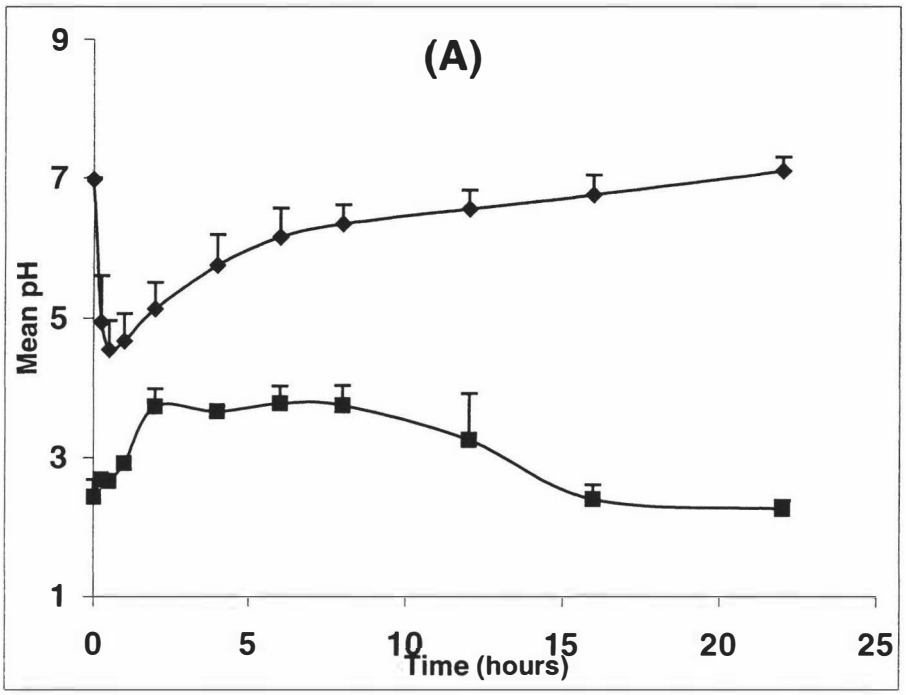
Figure 3.8. Experiment 5; Trial 1. Mean concentrations of ascorbic acid (AA) in rumen and abomasal fluid (A) and in blood plasma (B) during 0 to 22 h in 3 deer given single intraruminal doses of AA at 2.7g/kg, —◆— rumen fluid ; —■— abomasal fluid (A); —◆— blood plasma (B); ⊤ standard error bar.

lower than pre-dosing values in samples taken at 1 and 2 h ($p<0.0001$), 4 h ($p<0.01$) and 6 h ($p<0.05$) after dosing; thereafter values were not different from pre-dosing ($p>0.05$). Abomasal pH values were higher than pre-dosing at 2 and 4 h ($p<0.01$) and 6 h ($p=0.09$) and 8 h ($p<0.01$) after dosing with AA, after which values were not significantly different from pre-dosing values. One deer was observed to consistently have a lower rumen pH and a higher abomasal pH than the other two deer. Therefore it was not physiologically feasible to obtain data from this animal. However, the same trends with time were apparent when this animal's data was excluded (Figure 3.9B), but the amount of experimental variation was reduced.

In Trial 2, mean AA concentration in blood plasma after single doses of AA (0.75g/kg liveweight) via abomasal fistula was very low (Figure 3.10), and similar to that found in Experiment 1 (Figure 3.2). There was no change in the pH values of rumen fluid during 0 to 6 hours after AA administration, after which pH slowly increased to be 0.3 units above pre-dosing values ($p< 0.01$) by 22 hours after dosing. pH values of abomasal fluid increased to reach approximately 4.0 units 4 h. after AA administration and then declined to reach normal abomasal fluid value of 2.3 after 8 h (Figure 3.11). Abomasal pH values at 2 h ($p< 0.01$), 4 h ($p< 0.01$) and 6 h after dosing ($p=0.06$) were significantly higher than pre-treatment values; all other samples did not differ in pH from pre-treatment values.

3.3.8. Experiment 6

The linear regressions between $\log_e$ chromium (Cr) concentration in rumen fluid and time after giving a single intraruminal dose of the soluble marker Cr-EDTA alone (trial 1) and Cr-EDTA mixed with AA (trial 2) are shown in Figure 3.12 for a typical deer. Good linear relationships (slope= FOR) were obtained both in trial 1 and trial 2, with r^2 values of 85-98%. The mean rumen Cr-EDTA fractional outflow rates (FOR) were significantly different between group of 6 deer that received Cr-EDTA alone (Trial 1) and the same group of deer which were given AA mixed with Cr-EDTA in Trial 2 ($P<0.05$; Table 3.12). Adding AA at 2.7 g/kg liveweight reduced rumen liquid FOR.



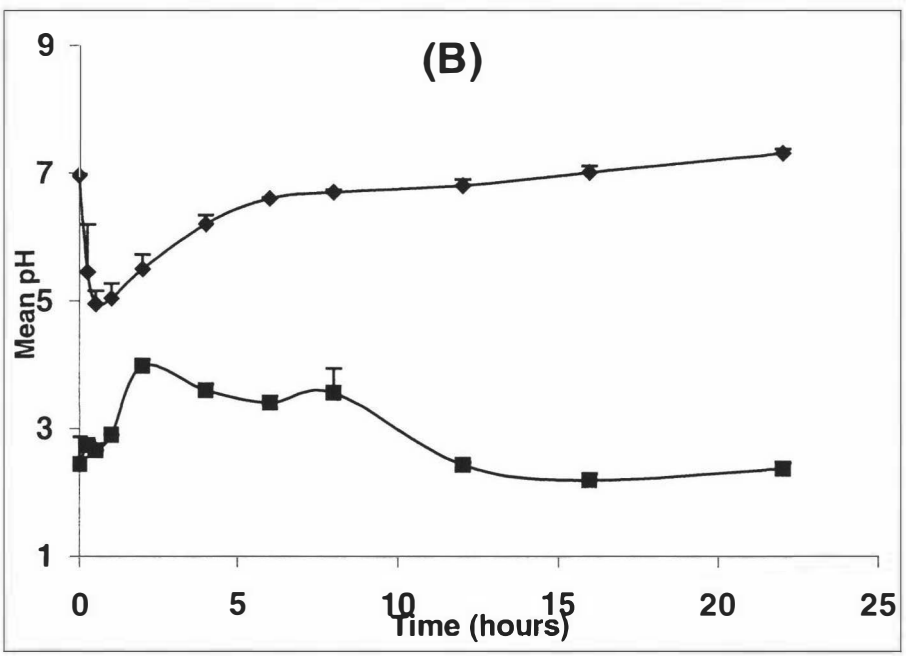


Figure 3.9 Experiment 5; Trial 1. Mean rumen and abomasal fluid pH during 0 to 22 h in 3 deer (A) and 2 deer (B) given single intraruminal doses at AA 2.7g/kg, —◆— rumen fluid; —■— abomasal fluid; ⊤ - standard error bar.

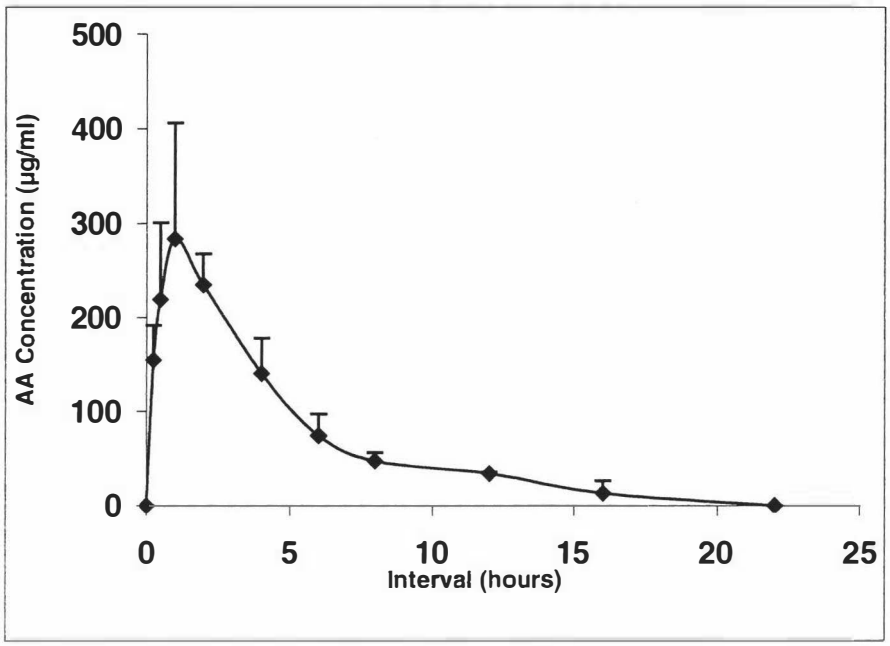


Figure 3.10 Experiment 5; Trial 2. Mean blood plasma ascorbic acid (AA) concentration during 0 to 22 h in 3 deer given single doses of AA at 0.75g/kg liveweight through abomasal fistula. —◆— blood plasma; ⊤ – standard error bar.

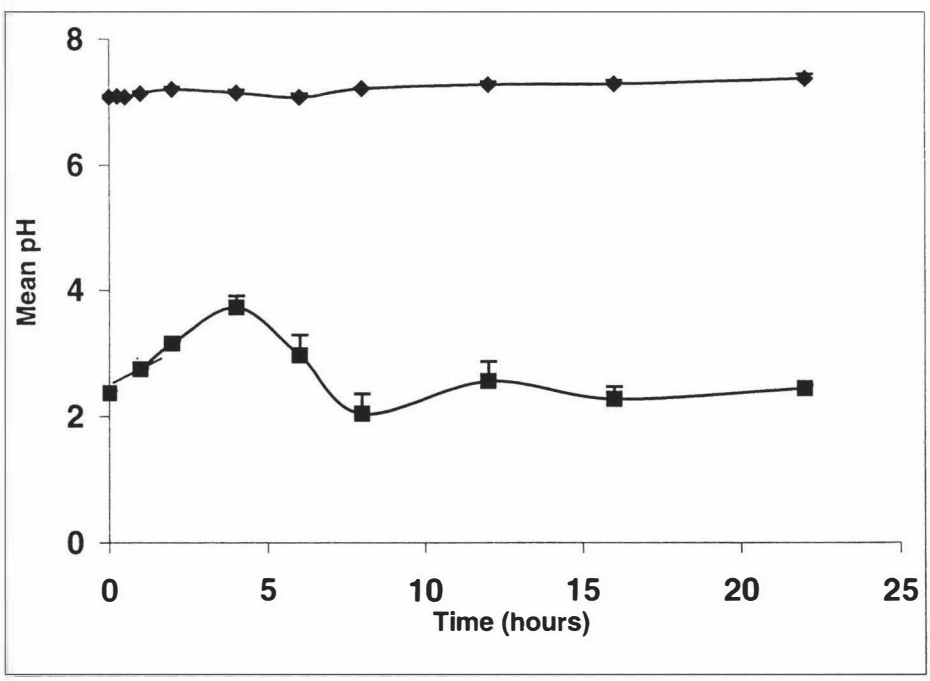


Figure 3.11. Experiment 5;Trial 2. Mean rumen and abomasal fluid pH during 0 to 22 h in 3 deer given single doses of AA at 0.75 g/kg liveweight through abomasal fistula. —◆— rumen fluid ; —■— abomasal fluid; ⊤ – standard error bar.

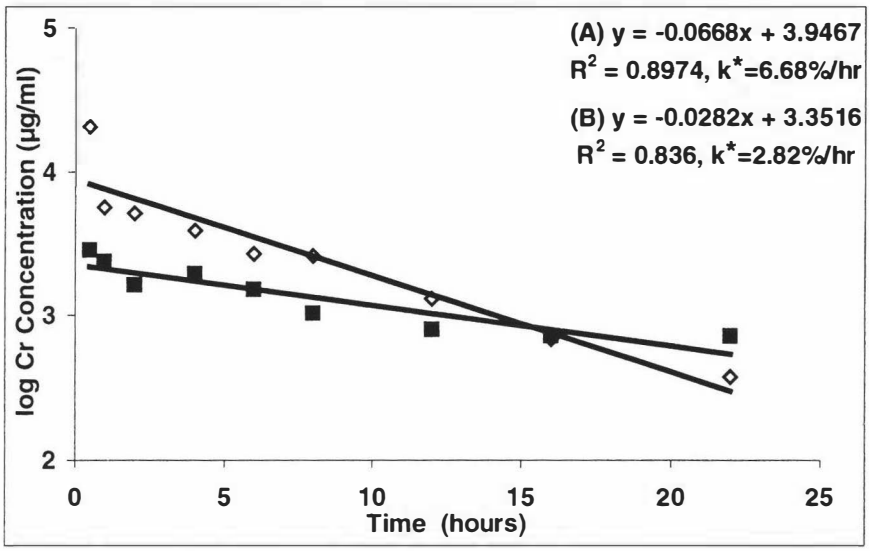


Figure 3.12. Experiment 6; Trial 1 and Trial 2. Decay in rumen concentration of the soluble marker Cr-EDTA during 0 to 22 hr in deer given a single intraruminal dose of 180 ml of Cr-EDTA (concentration of Cr-EDTA, 2.77mg Cr/ml of water). The Cr-EDTA was administered alone after an 8 h fast (A) or mixed with ascorbic acid (AA) at 2.7g/kg liveweight after an 8 h fast (B) using the same deer. —◇—Trial 1 (A); —■—Trial 2 (B); k*- Cr-EDTA rumen fractional outflow rate (FOR).

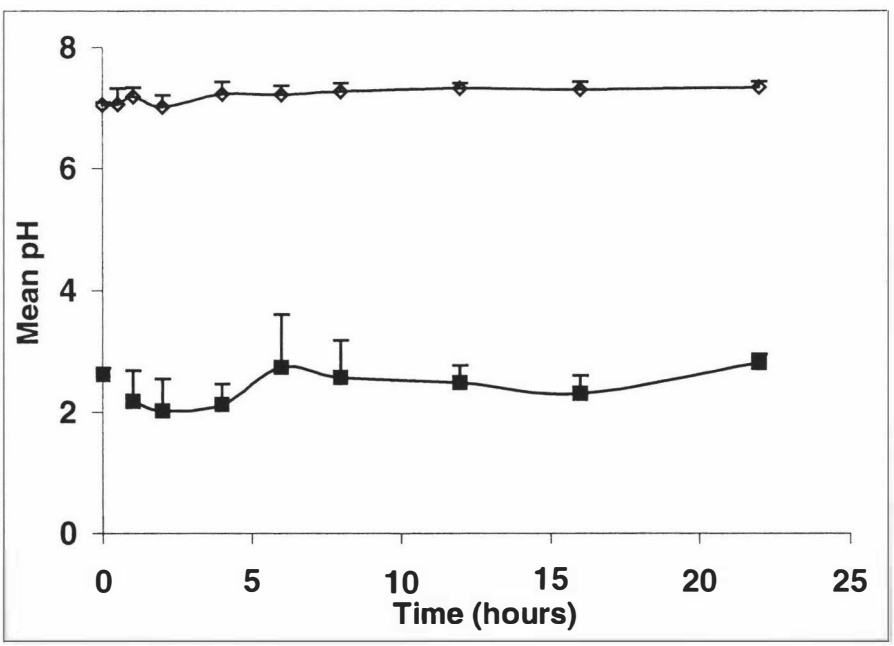


Figure 3.13. Experiment 6; Trial 1. Mean pH values of rumen and abomasal fluid in 6 deer (three, fistulated in both rumen and abomasum and three rumen fistulated only) given single intraruminal doses of 180 ml, Cr-EDTA (concentration of Cr-EDTA, 2.77mg Cr/ml of water), a standard liquid phase marker. —◇— RF ; —■— AbF; ⊤standard error bar.

Rumen FOR calculated from abomasal Cr concentration, were similar to those calculated from rumen Cr concentration, but as only 3 deer were involved the data are not shown. They do, however support the results obtained from rumen samples from larger numbers of deer (n=6).

Rumen volume appeared to be increased by administering a large dose of AA, but in unadjusted data the difference was not significantly different ($P>0.05$). When the liveweight of deer was added as a co-variate, the rumen volume was significantly different ($P<0.05$) between the fasted and fasted plus AA treated animals. The rumen volume increased from 13.3 litres to 17.1 litres after administration of AA (Table 3.12).

Table 3.12: Experiment 6; Trial 1 and 2. Mean rumen Cr-EDTA fractional outflow rate (FOR; %/h) and rumen volume (litres) for 6 deer given single intraruminal doses of 180 ml Cr-EDTA (2.77 mg Cr/ml water), either alone or mixed with 2.7 g ascorbic acid/kg liveweight. The deer were fasted for 8 hours before Cr-EDTA administration

Mean	Fasted	Fasted+AA	SEM
Rumen volume (litres)	13.30	17.10	1.85
Rumen volume (litres)[1]	13.30	17.10	1.13
FOR (%h)	5.06	3.52	0.24

[1] Corrected for liveweight by analysis of co-variance

There were no significant changes of pH values both in rumen fluid and abomasal fluid in Trial 1 when Cr-EDTA only was given to deer (Figure 3.13).

Mean concentrations of AA in rumen fluid, abomasal fluid and in blood plasma in 6 deer after a large single dose of AA at 2.7 g/kg liveweight mixed with Cr-EDTA are given in Figure 3.14 A and B (Trial 2). Concentration of AA in abomasal fluid was again much lower than found in rumen fluid during the experimental period of 0 to 22 h. Peak concentrations were found in rumen fluid, abomasal fluid and blood plasma, 1 h after dosing with AA, as also found in Experiment 5,Trial 1 (Figure 3.14A and B and Figure 3.9A and B). The pattern of AA concentration changes with time and the values were also very similar to the results obtained in Experiment 5 Trial 1 (Figure 3.8A and B), showing good repeatability.

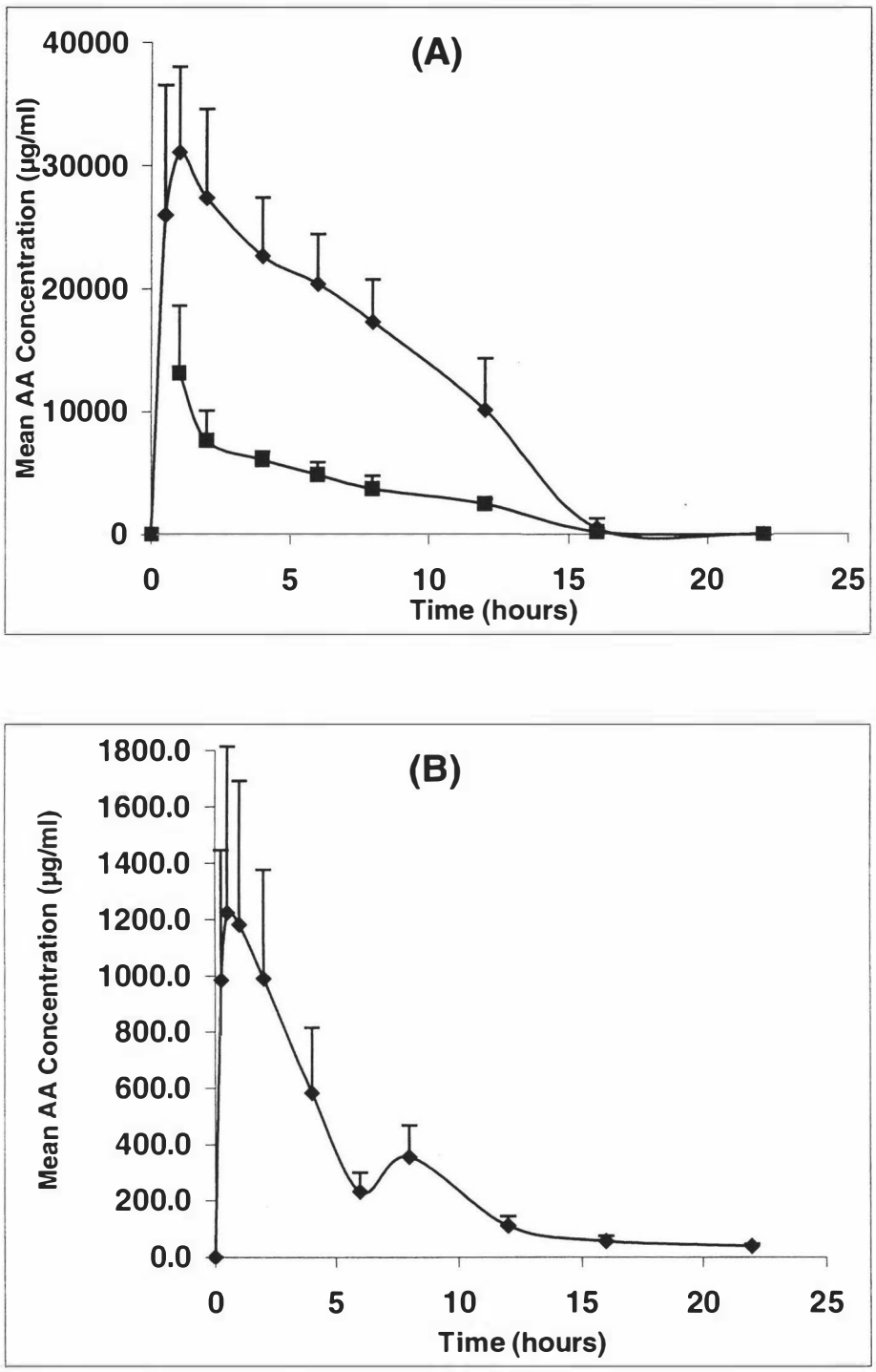


Figure 3.14 Experiment 6; Trial 2. Mean rumen and abomasal fluid (A) and blood plasma (B) ascorbic acid (AA) concentrations during 0 to 22 h in 6 deer given single intraruminal doses of AA at 2.7g/kg mixed with Cr-EDTA 180ml (concentration of Cr-EDTA, 2.77mg/ml water). —◆— RF ; —■— AbF (A) ; —◆— blood plasma (B) and ⊤ - standard error bar.

Good linear relationships were obtained between $\log_e$ rumen AA concentration and time, with r^2 values of 0.83- 0.98. Fractional disappearance rate (FDPA) of AA for a typical deer is shown in Figure 3.15. Mean values for FDPR, FOR, their $T_{1/2}$ values and rumen outflow of AA in g/d are given in Tables 3.13 and 3.14.

AA disappeared from the rumen with a half-life about 6 h.. This means that half of the AA has disappeared in this time and the remainder disappeared between 6 h. and infinity (as the process is exponential). Ascorbic acid disappears from the rumen in 3 different ways i.e. Rumen degradation, rumen absorption and rumen outflow, of which only the latter was measured in this study. Rumen outflow (RO) represents 29% of the AA dose given (FOR/FDPR) and half of the rumen AA outflow occurred in about 20 hours i.e. $T_{1/2}$ for liquid FOR (Table 3.13). For the deer in this experiment, rumen outflow was calculated to be 100g (Table 3.14), with half of this occurring in 20 hr; this is a small proportion of the dose administered (361g).

Figure 3.15 Fractional disappearance rate of ascorbic acid from the rumen of a typical deer given ascorbic acid (2.7 g AA/kg liveweight) mixed with 180 ml of Cr-EDTA (2.77 mg Cr/ml of water) via rumen fistula

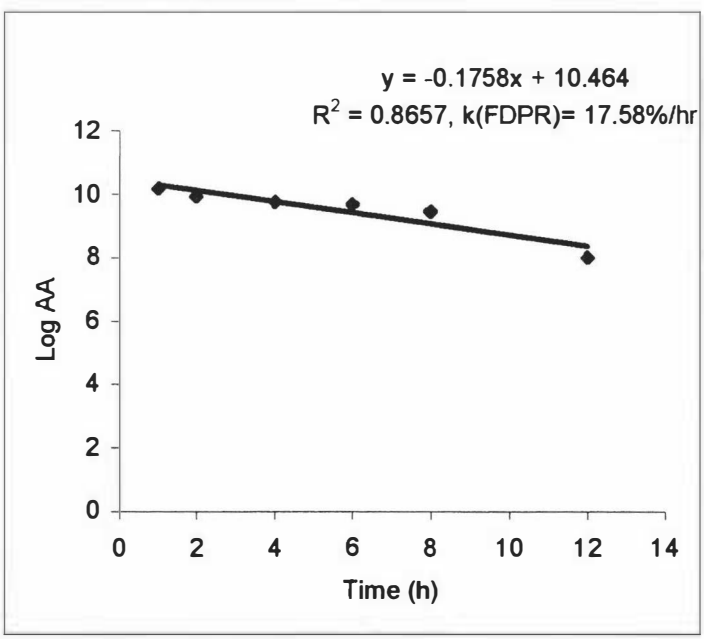


Table 3.13 Mean and standard error values of Rumen Fractional Disappearance Rate (FDPR), Fractional Outflow Rate (FOR), Fractional Degradation Rate (FDR)+ Fractional Absorption Rate (FAR), FOR/FDPR ratio, half life values for FDPR and FOR for six deer given a single intraruminal dose of ascorbic acid in Experiment 6, trial 2

	Mean	SEM
FDPR (%/h)	13.33	2.08
FOR (%/h)	3.52	0.28
FDR+FAR (%/h)	9.81	1.91
FOR/FDPR	0.29	0.04
$T_{1/2}$ FDPR (h)	5.95	0.97
$T_{1/2}$ FOR (h)	20.33	1.59

SEM- Standard error of mean
FDPR- Rumen Fractional Disappearance Rate
FOR- Fractional outflow Rate
FDR- Fractional Degradation Rate
FAR- Fractional Absorption Rate
$T_{1/2}$ Half life

Table 3.14 Mean and standard error values of rumen outflow of ascorbic acid in six deer in Experiment 6, trial 2

	Mean	SEM
Dose AA (g)	361.13	32.52
RO (g)	100.06	8.47
RO (g/kg liveweight)	0.38	0.12

SEM- Standard Error of Mean
RO- Rumen Outflow

The mean pH values of 6 deer in Trial 2 are given in Figure 3.16. Rumen pH declined rapidly after intraruminal AA administration and this was followed by a rise in abomasal fluid pH as found in Experiment 5 (Figure 3.9A and B). Rumen pH was significantly lower than pre-dosing pH values ($p<0.001$) up to 16 h after dosing, after which the difference became non-significant at this time. ($p>0.05$). Abomasal pH values were higher than pre-dosing values at 1 h ($p<0.05$), 2 h ($p=0.088$), 4 h ($p<0.01$) and 6 h ($p<0.05$) after dosing; thereafter (at 8 h, 12 h, 16 h and 22 h) all values were not significantly different from pre-dosing values ($p>0.05$). In both Experiment 5 (Figure 9A and B) and Experiment 6 (Figure 3.16) the normal rumen pH values were reached 22 hours after intraruminal AA administration and normal abomasal fluid pH was attained 8 h after AA administration.

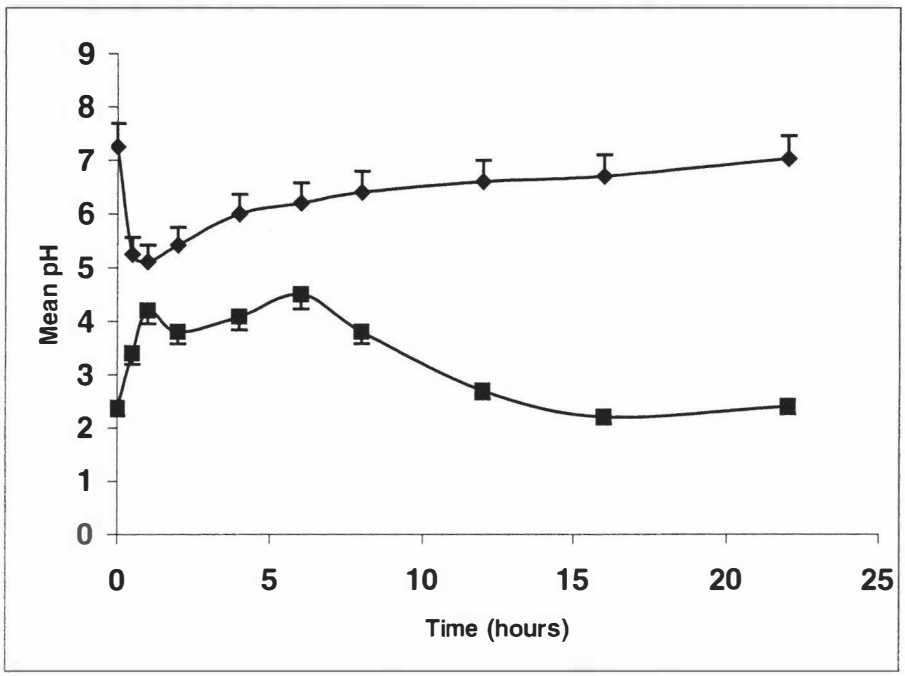


Figure 3.16. Experiment 6; Trial 2. Mean rumen and abomasal fluid pH during 0 to 22 h in 6 deer given single intraruminal doses of AA 2.7g/kg mixed with Cr-EDTA 180 ml (concentration of Cr-EDTA, 2.77 mg/ml water). —◆— RF ; —■— AbF and ⊤⊥standard error bar

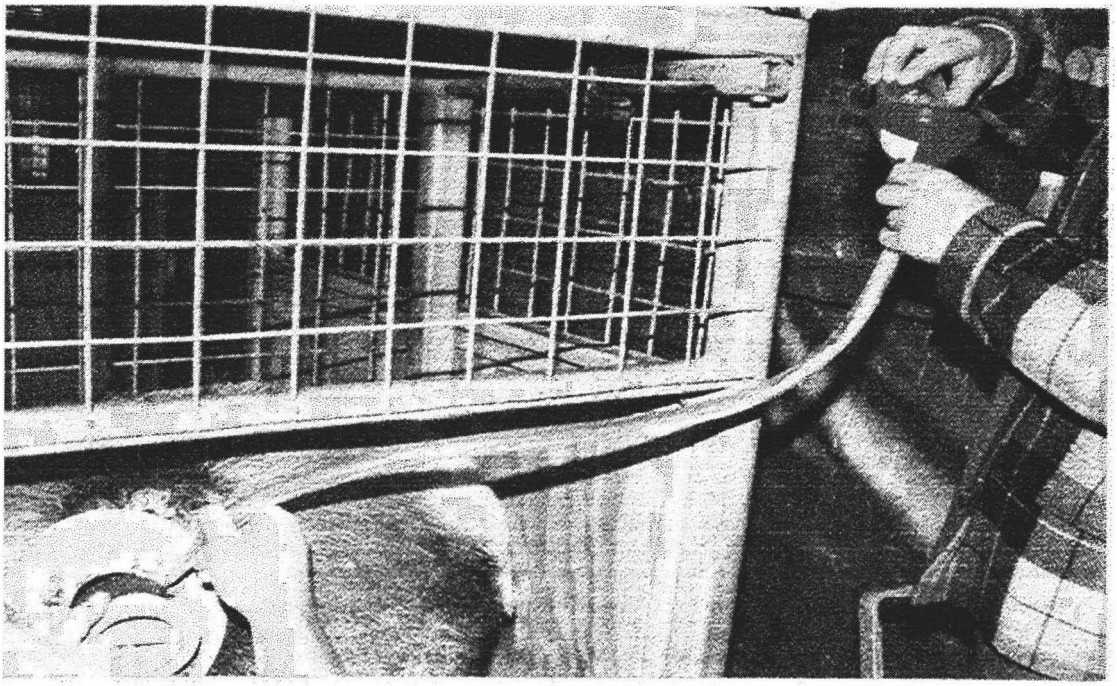


Plate 3.1 A ruminally fistulated deer was given ascorbic acid via rumen fistula

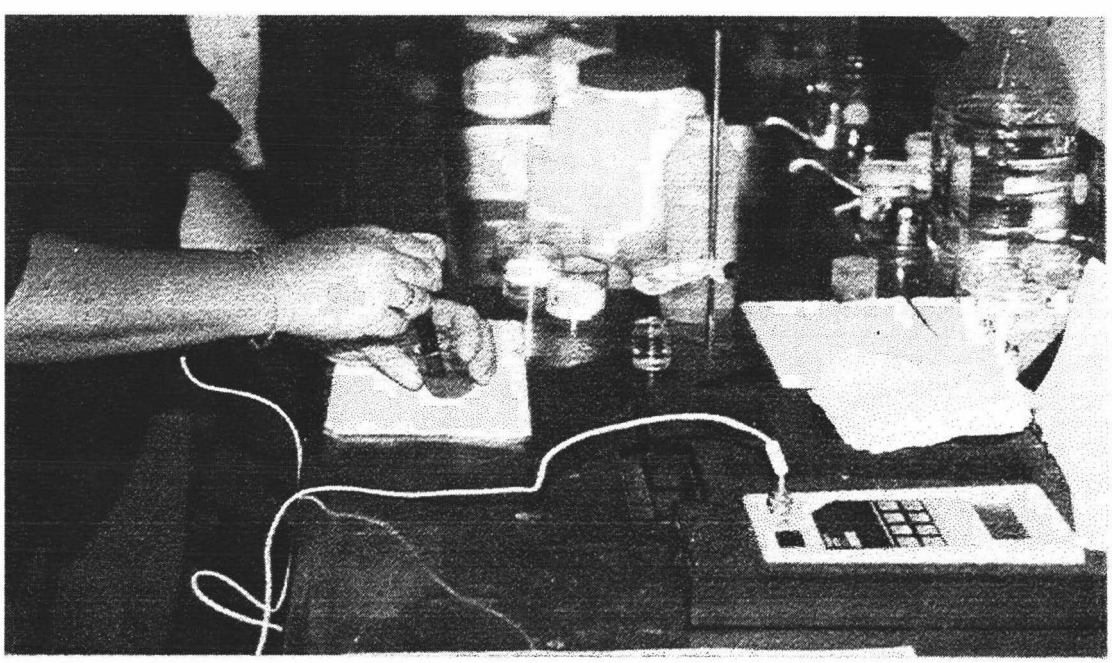


Plate 3.2 Rumen and abomasal pH values were determined at the Deer Unit soon after taking the samples

3.4. DISCUSSION AND CONCLUSION

This study developed methodology for increasing plasma AA concentration in farmed red deer, using a single oral or intraruminal administration of AA prior to a simulated slaughter situation, such that similar values were found to those produced by Stevenson-Barry (personal communication). The study then went on to evaluate different formulations of AA for increasing plasma AA, concentration and studied ruminal disappearance and outflow of AA. Although Invermay research has shown that a single high oral dose of ascorbic acid to deer (100 g or 200 g in 200 or 500 ml liquid to 70 kg deer) can elevate plasma concentration for up to 3 hours (J Stevenson- Barry personal communication), no other work has been carried out in this area in deer. Therefore the results of this study will mainly be compared with the administration of AA to other ruminants. As the present work was a part of a series of studies on "Defining Vitamin C Supplementations Techniques in Farmed Deer to Improve Venison Shelf life" conducted by AgResearch, the results obtained in this study have considerable relevance to the New Zealand deer farming industry.

Criteria involved in the development of suitable methodology include defining an appropriate dose, route and interval between repeat single doses of AA in deer. It was concluded at the end of Experiment 1 that single intraruminal doses of 2.7- 2.9g AA /kg liveweight should be used for the remaining experiments to produce a high concentration of AA in rumen fluid and in blood plasma. The dose rate involved was considered to be too high to give safely by the oral route. At the end of Experiment 2, it was also deduced that giving repeat intraruminal single doses (2.7g AA/kg live weight) a minimum of 2-week periods apart would be appropriate in future work. A two-week period between doses of AA produced repeatable results (Figure 3.4) and gave sufficient time for VFI to increase following the depression after dosing. The validity of this approach is illustrated by the non-significant period effects for all criteria measured in Experiment 3, the longest experiment in this series, confirming that the measurements were repeatable with time. A shorter time interval between repeat doses of AA via abomasal administration may be

possible, given the similar results in plasma AA concentration in doses given 2 days apart in Experiment 1 (Figure 3.1), explained by the absence of a microbial population in the abomasum.

The reason for few data being available on the concentration of AA in blood plasma and rumen fluid following an oral / ruminal administration of ascorbic acid in ruminants is due to rapid and pronounced destruction of AA by rumen micro organisms, reported as early as 1940 (Knight et al. 1941). They observed that ruminal fluid had a strong degradation effect on L-ascorbic acid but the exact type of microorganisms involved is not known.

The idea to use different formulations of AA in this study such as ethyl cellulose coated ascorbic acid (EC) and silicone coated ascorbic acid (SC) as well as pure ascorbic acid (AA) was to identify the best bioavailability type for a single high dose technique. In this study, it was observed that administration of all 3 types of AA significantly increased AA concentration in both rumen fluid and blood plasma in a similar manner, with maximum concentrations occurring about one hour after administration (Figure 3.5). Area under the concentration vs. time curve (AUC) values of AA, was determined as AUC is considered to be the best way of assessing the bioavailability of AA in the animal body (Hidiroglou *et al.* 1997). It was observed that the AUC, area under the curve corrected for baseline concentration (AUCB) and maximum concentration (MAX) of AA in both rumen fluid and blood plasma in this study were not significantly different between the three formulations of AA (Table 3.6 and 3.7), indicating that their rates of rumen degradation and their bioavailability must have been similar.

The work conducted by Hidiroglou *et al.* (1997) in sheep studied the effect of single oral administration of five different formulations of AA such as pure AA, EC, SC, sodium ascorbate (SA) and Rovimix C coated with silicone (RC). They administered a single dose of each formulation equivalent to 4 g of AA orally in capsule form to each of five sheep (45-50 kg BW; 1 year old). Blood samples were taken at 0, 10 and 30 min, once every hour up to 7 h and 24 h after administration of AA. Unlike in our studies, they did not obtain high concentrations of blood plasma AA following dosing with any of the five

formulations of AA and observed that the AUC of AA in blood plasma were not statistically significantly different from zero. The difference between this study and the present study can be explained by the low dose rate used by Hidiroglou *et al.* (1997) of approximately 0.08 g AA/kg liveweight.

In other work, the absorption of 4 different formulations of AA namely ascorbic acid crystalline (AA), ascorbyl palmitate (AP), ascorbyl stearate (AS) and formulated ascorbic (AF) was investigated in six mature Thoroughbred horses (463kg liveweight) following a single oral administration of 20g equivalent of AA (Snow and Frigg 1989). Although there were no high concentrations of AA observed in blood plasma, they found that crystalline ascorbic acid (AA) had poor absorption compared to the other preparations. The mean AUC were 18.2, 57.1, 42.6, 32.2 for AA, AP, AS and AF respectively. Based on these results AP showed a 3.1 (significant) times higher, 2.3 for AS (not significant) and AF had 1.8 (not significant) higher AUC compared to AA. The reason for the differences between this study and the present study may also be due to the low dose rate used by Snow and Frigg (1989) of approximately 0.04g AA/kg liveweight, which nevertheless showed that low oral doses of AA can increase plasma AA concentration in monogastric animals with hindgut microbial fermentation.

Based on the results of the present study, it is believed that the main route of AA absorption may have been from the rumen, despite degradation by rumen microorganisms in the single ascorbic acid technique. The relatively high FDPR of 13.3%/hour in Experiment 6 showed that AA disappeared relatively quickly from the rumen, with half of it having disappeared by approximately 6 hours (i.e. $T_{1/2}$). The ratio FOR/FDPR showed that approximately 29% of the AA dosed followed out of the rumen and into the abomasum. However, as the half life of solute outflow from the rumen (including AA) was approximately 20 hours, half this quantity or about 14.5% of the dose administered would flow out in this time; the remaining 14.5% of the dose would flow out between 20 hours and infinity, as outflow is an exponential process. Therefore, under the conditions of these experiments, where approximately 350 g of AA were administered as single doses, approximately 50 g (0.38 g AA/kg liveweight) would flow

out of the rumen in the first 20 hours after dosing. This is approximately half the dose administered into the abomasum in Experiment 1 and 5 and is unlikely to have been a major contributor to increase plasma AA concentration immediately following intraruminal dosing.

Support for rumen absorption is shown is shown by maximum concentrations of AA in both rumen fluid and blood plasma occurring at the same time, approximately 1 h after a single intraruminal administration of AA (Figures 3.3, 3.4, 3.7 and 3.14). A low pH value of 5.0 (Figure 3.5) during the initial period (one hour), after dosing was observed which may have reduced the rumen AA degradation by microorganisms. This is supported by the addition of sodium bicarbonate ($NaHCO_3$) to AA, which increased rumen pH by 0.5-1.0 units (Figure 3.6) but also increased ruminal degradation of AA, as shown by the reduced area under the rumen AA concentration vs. time curve (Table 3.9). Low pH values (5.0) in the rumen fluid of deer in the initial period after intraruminal administration of AA (Figure 3.5) may also have caused a reduction in the contraction frequency of the rumen wall. This may explain the reduced rumen liquid FOR in deer dosed with AA, allowing more time for absorption from the rumen (Figure 3.12 and Table 3.12).

A comparison of rumen liquid FOR measured in the present study (Experiment 6) with fasted deer with that found by Dominique *et al* (1991) with fully fed deer is shown in Table 3.15. These results show that compared with deer fed the same diet *ad libitum*, fasting dramatically reduced rumen liquid FOR and this was further reduced by giving a single large dose of AA into the rumen. The later comparison is however confounded by time, but as the values for the fasted deer were so much lower than those of fully fed deer, this is unlikely to effect the conclusions drawn. This comparison illustrates the long residence time of solutes in the rumen of fasted deer given a large intraruminal dose of AA, allowing more time for AA to be absorbed from the rumen.

Table 3.15 A comparison of nutritional treatments on rumen fractional outflow rate (FOR) measured with chromium EDTA and rumen mean retention time 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 Domingue *et al* (1991) for fully fed deer.

	FOR (%/h)	MRT (h)	$T_{1/2}$ FOR (h)
Domingue *et al* (1991)			
Summer- *ad libitum*	15.8	6.3	4.39
Winter- *ad libitum*	16.3	6.1	4.25
Present investigation			
Fasted (30 hr)	5.1	19.6	13.6
Fasted + ascorbic acid (30 hr)	3.5	28.6	19.8

[1] Calculated as 1/ FOR

FOR- Rumen Fractional out flow rate

MRT- Rumen Mean retention time, $T_{1/2}$ (half life)

Based on the results of this study it seems that the absorption of AA from the small intestine of deer may not be a major route following a single large dose of AA into the rumen. Evidence for this includes a much lower AA concentration in abomasal fluid than in the rumen fluid (Figure 3.8A), perhaps due to the reduced rumen liquid FOR following a single large dose of ascorbic acid into the rumen. Nevertheless, absorption from the small intestine will augment the larger amount of AA that must be absorbed from the rumen and can be considered as a secondary site of absorption. Its contribution will increase with increased time from dosing.

During Experiment 3, low pH levels (5.0) were found in the rumen fluid during the first 60 minutes after dosing with each type of the 3 formulations of AA (Figure 3.5). As we cared for the animals according to the instructions provided by the Director Animal

Welfare Science and Bioethics, Massey University, it was important to overcome low rumen pH values due to introduction of AA, especially the values of less than 5.5, which is the bottom end of the normal physiological range for rumen pH in deer (Kusmartono 1996). Therefore it was deduced that to use an alkaline, sodium bicarbonate ($NaHCO_3$) along with AA in a single dose method to maintain normal rumen pH values. Although it was possible to maintain the rumen pH values over 5.5 in the ascorbic acid plus $NaHCO_3$ group of deer (Figure 3.6) during the course of the experiment, it was observed that this treatment reduced the AUCB value in rumen fluid but not in blood plasma, relative to the deer dosed with AA only (Tables 3.9 and 3.10), indicating increased rumen AA degradation. Sodium bicarbonate is a constituent of ruminant saliva and rumen infusion of $NaHCO_3$ has been shown to increase rumen liquid FOR in sheep (Harrison *et al.* 1975). Addition of $NaHCO_3$ in the rumen may therefore have increased the flow rate of AA from the rumen of deer and so increased the proportion of AA absorbed from the intestines. Therefore absorption of AA in deer dosed with $NaHCO_3$ plus AA may have been from the small intestines as well as from the rumen. It is required to develop new strategies to keep the rumen pH over 5.5 while maintaining high concentrations of AA in blood plasma in the single dose AA technique in deer, which should be one of the major areas of study in the future.

All three formulations of ascorbic acid (AA, EC and SC) showed similar rumen degradation in this study and resulted in similar bioavailability. Therefore it is important to identify any other formulations of AA in a single dose of administration to increase the blood plasma AA concentration in deer, possibly with a lower dose rate and with rumen pH not falling below 5.5 units.

Methods available for achieving these objectives include reducing rumen AA degradation, so increasing the proportion absorbed from the rumen, and increasing rumen outflow of AA. As rumen solute FOR was so low in the fasted animals, it is difficult to see the absorption from the intestines could ever be a major source of AA absorption in the single large oral administration method. Solubility is a pre-requisite for degradation of all substances by rumen micro-organisms, including AA. Therefore a

logical route to follow is the evaluation of formulations of AA that have reduced solubility, such as calcium ascorbate. Such studies would be easier to interpret if methodology could be developed to quantify the individual processes of rumen degradation and rumen absorption.

The suggested plasma AA concentration vs. time profile of an ideal compound, relative to AA found in the present study is shown in Figure 3.17. It is suggested that instead of AA used in this study, a less soluble form such as calcium ascorbate should be evaluated in future studies. Less soluble forms of AA would probably give a higher minimum pH value than pure ascorbic acid, which gave minimum value of pH 5.0 in this study. Less soluble forms of AA may be degraded more slowly by rumen microorganisms, to produce high AA concentrations in the blood plasma for a longer period of time. Thus, it may be possible to use a lower dose of AA than used in the present study (2.7g AA/kg liveweight) in deer in future studies and to produce a plasma AA concentration vs. time curve closer to the ideal formulation shown in Figure 3.17.

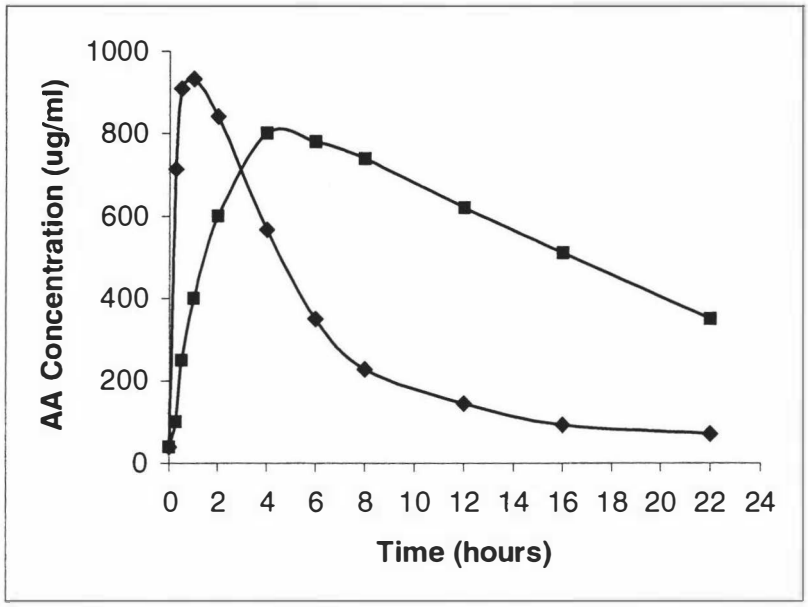


Figure 3.17. Mean AA concentration in blood plasma during 0-22 hr in deer given single doses of AA at 2.7g AA equivalent /kg liveweight through rumen fistula in the present study and suggested blood plasma concentration during the same time of deer given single doses of an "ideal compound" by the same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

On the basis of the new methodology developed and the results obtained in the present study, it is recommended that the following long term research should be considered for future development.

• To identify less soluble forms of AA for the use in the single dose technique to obtain high concentrations of AA in blood plasma without lowering the rumen pH values below 5.5. Once these new formulations are identified, they should be compared with the 3 types of AA used in the present study to see if there are any significant differences in bioavailability of AA in deer. The ideal AA formulation should give longer ruminal fractional disappearance time (i.e.increased $T_{½}$) and increased blood plasma concentration over a longer time period. A key development will be finding compounds that will elevate plasma AA concentration, but at a lower dose rate than used in the present study.

• To develop methodology to quantify rates of rumen degradation and rumen absorption of AA, to complement the methodology developed in this study for FDPR and FOR.

• The identification of the individual species of the rumen bacteria, protozoa and fungi that are involved in the degradation of AA should be carried out, as there are no research findings on the degradation of AA by individual rumen microbial species. These findings will be useful in studying the rumen degradation of AA in more detail.

• Once the "ideal" AA formulations have been identified at the research level as the best bioavailable types, these products should be tested on deer at the commercial Deer Slaughter Plant. Animals should be slaughtered at the time when peak blood plasma concentrations are achieved, which should be determined (the time of peak concentration after dosing with AA) from earlier experiments. This will enable Researchers to study the differences in colour stability in venison treated with AA and without AA at 0° C temperature.

• The "ideal" AA need to be formulated as a stable suspension using colloidal silica instead of slurries used in the present experiments. This would make oral administration easier. This, plus identifying a lower dose rate, may result in oral administration using conventional drenching equipment being a practical proposition.

It can be concluded that the single dose AA technique can be used in farmed deer to successfully increase AUC, AUCB and MAX of AA in rumen fluid and in blood plasma. There were no significant differences between the three formulations of ascorbic acid namely pure AA, EC and SC used, indicating that the rumen degradation and bioavailability of these three different formulations were similar. It was deduced that repeat intraruminal single doses of AA in deer should be a minimum of a 2-weeks period apart in order to avoid any adaptation by rumen micro-organisms, to produce repeatable results in terms of rumen and blood plasma AA concentrations and to allow VFI to recover. Finally, it was concluded that the main site of absorption of AA occurred through the rumen epithelium, followed by much lower flow into the abomasum and

absorption from the small intestines in deer given a single large oral or intraruminal dose of AA.

These research findings suggest further avenues for future research in farmed deer in New Zealand. In the long term, the results of this study and the future research findings will be beneficial to improve the colour stability of New Zealand chilled venison in the international market place.

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