EFFECTS OF FERMENTATION PRODUCTS OF SILAGE ON ITS INTAKE BY CATTLE

by

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SUMMARY

The end-products of silage fermentation have been implicated as factors limiting its intake by ruminant animals, although the contribution of individual chemical components is not clearly understood. A series of experiments were conducted to investigate the effect of short-term intra-ruminal infusions of silage fermentation end-products on roughage intake by cattle.

Infusions of lactic acid, the predominant organic acid found in well preserved silages were found to reduce the short-term intake of roughage by both steers and dairy cows. The short-term intake of hay- and silage- fed steers was reduced by infusions of 32 g lactic acid/kgDMI. The reduction in intake was greater when the acid was infused over two hours as opposed to one hour. The same amount of lactic acid (32 g/kgDMI) reduced the intake of silage fed dairy cows. These results were substantiated by a further experiment in which intra-ruminal infusions of 16, 32 or 48 g lactic acid/kgDMI over a two hour period reduced the short-term intake of silage-fed steers in a dose related manner.

The mechanism by which lactic acid reduces intake was not clear from these trials. Infusions of acetic and propionic acids in the molar proportions to which lactic acid is metabolised in the rumen depressed silage intake by dairy cows, but this was attributed to a fall in rumen pH.

Urea, used to mimic silage ammonia had no effect on voluntary intake of hay- and silage- fed cattle. This supports the theory that rather than ammonia per se limiting intake it is other fermentation end-products, such as silage amines, produced in similar conditions to ammonia that are responsible for poor intakes.

Amines were detected in noticeable amounts in silages made at Hurley. The

predominant one being gamma amino-n-butyric acid (Gaba). Infusion of Gaba intraruminally, reduced the intake of silage-fed steers by up to 22%. Given in combination with putrescine the depressing effect of intake depression was doubled (44%), although these results were not significant. Physical gut fill was found to have little effect in the limitation of short-term silage intake. Rumen emptying studies showed that the maximum amount of digesta present within the rumen, in terms of DM, OM and NDF occurred after the cessation of the first meal.

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ABBREVIATIONS

Aaba	**Alpha amino butyric acid**
Ac	**Acetic acid**
ADF	**Acid detergent fibre**
AP	**Acetic/propionic acid mix**
Bu	**Butyric acid**
CP	**Crude protein**
DM	**Dry matter**
DMI	**Dry matter intake**
DOMD (D-value)	**Digestible organic matter in the dry matter**
EE	**Ether extract**
Gaba (G)	**Gamma-amino-n-butyric acid**
LC	**Lactic acid**
LW	**Liveweight**
NDF	**Neutral detergent fibre**
Pr	**Propionic acid**
Putr. (P)	**Putrescine**
S	**Silage**
S.E.M.	**Standard error of the mean**
Sig	**Significance**
VMH	**Ventromedial hypothalamus**
VFA	**Volatile fatty acid**
W	**Water**

SECTION A

LITERATURE REVIEW

I. THE PRODUCTION OF SILAGE

In the UK, as in many other countries around the world, there are periods of the year when crop growth is restricted, such as in the winter months or during periods of drought. Methods of conservation have evolved to preserve various crops during stages of optimum growth to enable them to be fed to livestock in these periods of shortage.

Traditionally hay making has been the method used for conservation of grass in the U.K. This relies on the harvesting of a high dry matter mature crop which tends to produce a material of low digestibility, often of variable composition and nutritional value, owing to the reliance on good weather during the field drying stages.

An alternative to conserving grass as hay is to ensile it. The process of ensiling relies on the storage of a high moisture crop, which in the case of grass is at an early stage of growth and with a high leaf-to-stem ratio, in anaerobic conditions. During storage organic acids, particularly lactic acid, are produced by the fermentation of water soluble carbohydrates (WSC) in the crop. The lowering of the pH caused by production of these acids, coupled with the exclusion of air from the silo, inhibits the growth of putrefactive microorganisms such as clostridia and bacilli. These organisms would otherwise utilise lactic acid and degrade the plant proteins and amino acids, to produce such end-products as butyric acid, ammonia and amines which have little nutritive value.

Over the last 20 years the amount of grass silage produced in the U.K. has gradually increased until in 1981, for the first time the amount of silage produced in the U.K. exceeded the amount of hay produced (Henderson, 1987). In the EEC as a whole the total amount of silage produced in 1985 was far greater than the amount of hay produced, which contrasts sharply with the amount of each conserved in 1975 (Table 1).

Table 1

The amount of silage and hay produced (million tonnes DM) in the EEC in 1975 and 1985.

	million tonnes DM			
	1975		1985	
	silage	hay	silage	hay
EEC	44.6	58.3	70.9	45.8

Ensiled grass is generally consumed to a lesser extent than the corresponding fresh (Demarquilly, 1973) and dried herbage (Campling, 1966). The reasons for this are not completely understood, although the end-products of silage fermentation have been implicated in the regulation of short-term intake (see Gill et al, 1986), whilst poor microbial protein synthesis in the rumen may limit voluntary silage consumption in the long-term (Austin and Gill, 1981).

This review of literature discusses the silage fermentation process leading to production of compounds implicated in intake limitation, and the mechanisms by which the voluntary intake of ruminants is controlled, with particular reference to the feeding of silage to cattle.

A. The microbiology of ensiling grass

The initial changes to a crop during ensilage are caused by the action of plant enzymes (Sterling, 1963) and in later stages and more importantly by microbial activity.

1. Plant enzymes

Immediately post harvesting and during the initial stages of ensiling, the biochemical changes occurring in herbage arise due to the action of plant enzymes, involved in the processes of plant respiration and proteolysis.

Plant respiration occurs as long as conditions are favourable i.e. oxygen and substrate are readily available (McDonald, 1981) resulting in oxidation of sugars and organic acids. However, the main action of plant enzymes is proteolytic (Lamb, 1917; Gouet et al, 1970; Clark, 1974) releasing peptides, free amino acids and amides (Kemble and MacPherson, 1954). During a three day wilt as much as 20% of the herbage protein N can be degraded to non-protein nitrogen (NPN) (Kemble, 1956) and it has been shown that protein stability is not achieved until the pH of the clamp has fallen to 4.3, when proteolytic enzymes are inhibited.

2. Microorganisms

The four main groups of microorganisms significant in silage fermentation are:

a) lactic acid bacteria

b) endospore forming bacteria (Clostridia and Bacilli)

c) coliform bacteria

d) fungi (yeasts and filamentous fungi)

Lactic acid bacteria enter silage from natural inoculation from farm equipment, since lactic acid bacteria are only present on the plant material in relatively scarce numbers (Stirling and Whittenbury, 1963). Indeed, almost all the microorganisms present on the growing plant are strict aerobes and play virtually no role in silage preservation (Kroulik et al, 1953).

After a 24 hour wilt the number of lactic acid bacteria present per gram of fresh material has been shown to have a 4 fold increase to approximately 1000, this increases to a peak of $8.5x10^8$ approximately four days later at which time they dominate the microorganism population (Henderson, 1987). The two types of clostridial bacteria enter silages from contamination with soil (Rushman and Koch, 1931), as do yeasts and fungi (Henderson et al, 1972) or from contamination with slurry which contains significant levels of the spores (Rodhe et al, 1988).

i. Lactic acid bacteria

In well preserved silages, lactic acid producing bacteria are the dominant microorganisms. These were first categorised into two broad groups by Orla-Jenson (1919).

a) Homofermentative (homolactics) that ferment hexoses to predominantly lactic acid.

b) Heterofermentative (heterolactics) that ferment hexoses to lactic acid and other products such as ethanol and acetic acid.

The dominant microorganisms in most silages are Lactobacillus plantarium and Pediococcus spp. which are both homofermentative and Lactobacillus brevis which is heterofermentative.

The principle substrate for lactic acid producing bacteria are hexoses, glucose and fructose, and the pentoses, arabinose and xylose, previously liberated from plant hemicelluloses by the action of plant enzymes (Dewar et al, 1963).

Lactic acid bacteria have virtually no proteolytic activity with only two amino acids being fermented significantly, arginine and serine (Brady, 1966). There is debate as to whether lactic acid bacteria have the ability to decarboxylate certain amino acids. Cadaverine and putrescine have been found in low pH good quality silages (MacPherson and Violante, 1966) however whether these were produced by lactic acid bacteria or by clostridial bacteria in the early stages of ensilage prior to development of acid conditions is unclear (Woolford, 1984). The production of other amines tyramine (Gale, 1940) and histamine (Rescei and

Snell, 1972) have also been associated with microorganisms active within the silo when lactic acid bacteria dominate the fermentation. These are summarised in Table 2.

Table 2

The role of lactic acid producing bacteria in the deamination of amino acids in silage.

Original amino acid	Amine produced	Bacteria	Reference
Tyrosine	Tyramine	S. faecalis	Gale (1940)
Histidine	Histamine	Lactobacillus spp.	Rescei and Snell (1972)
Lysine	Cadaverine	"	Rodwell (1953)
Ornithine	Putrescine	"	Rodwell (1953)

ii. Endospore forming bacteria

Endospore forming bacteria include the two groups of bacteria Clostridia and Bacilli.

a. Clostridia

Clostridia spp involvement in the silage fermentation is associated with spoilage. The two main types of Clostridia found in silages are:

i Saccharclytictypes

These are often termed lactic fermenting since they utilise both lactic acid and sugars producing butyric acid, carbon dioxide and hydrogen as the main end products. They include Clostridium butyricum and paraputrefactive spheroids. If these bacteria dominate the fermentation, the pH of the silage rises as butyric acid is a weaker acid than lactate and only one mole of butyric acid is produced for each mole of lactic acid utilised. This rise in pH then favours other putrefactive microorganisms including the proteolytic Clostridia.

ii. Proteolytic (putrefactive) types

This group of bacteria attack proteins and amino acids. The three main types of reaction they are involved with are:

- Deamination where ammonia is liberated from an amino acid to leave an organic acid

residue.

- Decarboxylation leading to formation of amines, which have not only been shown to have a role in the limitation of silage intake by ruminants but in addition are potentially toxic to animals (Dain et al, 1955).
- Oxidation/Reduction (Stickland type) reactions where one amino acid is oxidised whilst a second is reduced forming organic acid residues.

The amount of ammonia (NH_3) present in the silo is a good indicator of the amount of proteolytic clostridial activity as it is only produced by other silage microorganisms in very small amounts. Initially these clostridia have the ability to multiply as rapidly as other species of bacteria during the exponential growth stage of bacteria at the onset of ensilage (Gibson et al,1958). However, a rapid development of low pH will inhibit clostridial growth since the optimum pH of clostridial bacteria is 7.0-7.4 and they are unable to tolerate acid conditions (Pelczar and Reid, 1972). This is achieved by either harvesting the crop at a high sugar content or with the use of artificial silage additives (see Section I. B.).

Clostridia favour wet conditions and their growth is stimulated both by high temperatures, a low WSC content and a high buffering capacity of the crop. Wilting the crop prior to ensiling to increase the DM content above 300 kg/DM restricts their growth.

b. Bacilli

Unlike Clostridia, Bacilli can grow under aerobic conditions and hence they are associated with spoilage once the silo has been opened. The end-products of Bacilli fermentation of sugars appear to be acids, including lactate, however they are not as efficient in their production of these acids and hence their growth tends to be discouraged (Woolford et al, 1977).

iii. Coliform bacteria

Coliform bacteria are from the family Enterobacteriaceae and although are thought to be pathenogenic these are not thought to play a role in the silage fermentation process. Enterobacteriaceae ferment sugars to predominantly acetic and formic acids. In addition there is evidence that they are capable of deamination and decarboxylation of amino acids (Beck, 1978).

The optimum pH for their survival is 7.0 and they are generally only active in the early stages of fermentation when the pH favours their growth or clostridial bacteria dominate

the fermentation. (Whittenbury, 1968).

iv. Fungi

The majority of fungi are strict aerobes and are associated with spoilage of a clamp, as they survive on decaying plant material. They are classed as Eukaryotic micro-organisms being either single cells, yeasts, or multicellular filamentous colonies, or moulds.

v. Yeasts

These are not inhibited by acid conditions and can occur in silages at levels up to 10^5 organisms per gram (Ohyama et al, 1979). They are undesirable in silage since they compete with lactic acid bacteria for sugars which they ferment to ethanol. This has little preservative value in silage.

vi. Moulds

The low pH and anaerobis of silage makes mould growth difficult. They are associated with the shoulders and surface of the silo which become exposed to air. Not only do they utilise sugars and lactic acid but metabolise cellulose and other cell wall components and produce mycotoxins which are potentially toxic to animals.

B. Silage additives

To aid the silage fermentation process grass is commonly harvested and ensiled with the use of an appropriate silage additive. The additive may stimulate lactic acid bacterial proliferation within the silo, restrict the growth of putrefactive microorganisms or provide a source of nutrients for the bacteria. Silage additives are classed in five main categories listed below.

1. Fermentation stimulants

These include bacterial cultures to establish lactic acid bacterial dominance. Whilst being successful in improving the fermentation in small scale laboratory silos, the effectiveness of inoculants in farm silos remains unproven (Done, 1986). Intake of inoculant-treated silages improved compared with a control silage although no apparent difference in fermentation quality was observed (Steen et al, 1989).

Carbohydrate sources such as molasses which provide a fermentable substrate and

cell wall enzymes, such as cellulases and hemicellulases, to release otherwise non-fermentable material can be used as silage additives, although their effect on animal performance is not widely known (Gordon, 1989).

2. Fermentation inhibitors

Mineral (sulphuric and hydrochloric) and organic (formic and lactic) acids give rise to an immediate low pH in the silo and create conditions that putrefactive microorganisms cannot tolerate. The original use of mineral acids in silage preservation dates back to 1885 (Watson and Nash, 1960), but problems of handling corrosive acids and poor animal intakes of the resultant silages have limited their use. More recently in Ireland the use of sulphuric acid (at a concentration 45% w/w) as a silage additive resulted in intakes equivalent to those of formic acid treated silages (O'Kiely et al, 1989).

Formic acid was first used in silage production in 1926, but did not become widely used until the introduction of forage harvesters. In the U.K. it is applied at a rate of 2 litres of pure formic/tonne of grass, and appears in many silage additives either as the sole ingredient or in combination with other chemicals (Woolford, 1984). It reduces fermentation in the silo, and lowers the amount of acetic and butyric acids and the degree of protein breakdown in the clamp (Waldo, 1978). Increased DM intakes of growing cattle (20%) and dairy cows (12%) (Waldo, 1978), coupled with improvements in animal performance have been associated with formic acid treated silages (Thomas and Thomas, 1985; Parker and Crawshaw, 1982; Waldo, 1978), the improvements being greatest when the control silage is poorly preserved.

Sterilants such as formaldehyde that inhibit the growth of microflora in general also restrict the amount of plant protein breakdown both in the silo and the rumen.

3. Aerobic deterioration inhibitors

Antibiotics and other antimicrobial agents such as ammonia can be added to silages to prevent the growth of spoilage microorganisms.

4. Absorbents

Absorbents can be added to silages, particularly those that are not wilted or where acid additives are added to reduce the problems of pollution from effluent associated with these silages (Wilkinson, 1988). They include barley, straw and sugar beet pulp.

5. Nutrients

Nutrient additives contribute to the nutritional needs of the animals consuming the silage (Owen, 1971). They can be either energy or protein yielding nutrients and include starches, cereals and nitrogen containing minerals such as urea. Many of the additives classed as absorbents (barley) or fermentation stimulants such (molasses) could also be described as nutrients.

II. CONTROL OF FOOD INTAKE

Voluntary food intake (VFI) is the total amount of food eaten during a given period of time by an animal offered feed ad libitum (Forbes, 1986). Ruminants and monogastric animals are able to regulate their intake by both physical and physiological control mechanisms. Ruminants eat to fulfil the energy and protein requirements imposed by their physiological status, such as pregnancy, lactation and growth. This is often termed the long-term control of voluntary intake. The factors involved in the initiation and termination of each meal are referred to as the short-term controls of voluntary intake. It should be noted that these definitions are only for convenience and do not imply an absolute biological difference (Forbes and Barrio, 1992 (in press)).

The short-term control of food intake by ruminants is achieved by both physical and metabolic control mechanisms, the activation of which is dependent on the characteristics of the ration fed (Conrad et al, 1964). The control of intake of energetically dense feeds is primarily under metabolic control whereas the intake of forages and other energetically less dense materials are limited by physical factors such as gut fill (the space available in the rumen), rather than body energy content (Campling, 1970). However, over a long period of time energy intake matches the energy expenditure in the adult ruminant which results in a constant body weight (Baumgardt, 1970).

VFI is centrally controlled by the hypothalamus, which is situated just above the pituitary gland in the brain. It receives information about the animals' nutritional status from metabolic, hormonal, neurogenic, thermal and cortical factors which it integrates before taking appropriate action by either activating or deactivating the animal's food seeking behaviour. A satiety centre, the ventral medial hypothalamus has been identified as being responsible for production of the sensation of fullness, whilst the lateral hypothalamus, the

feeding centre appears to initiate feeding.

III. THEORIES OF INTAKE CONTROL

Various theories have been put forward as to what controls voluntary food intake. Many theories have concentrated on the role of single factors such as physical gut fill, the amount of fat stored in the body or blood glucose levels. Although these are discussed below it is unlikely that any single factor is acting alone in regulating intake. It is more probable that control of intake is multifactorial involving input of signals from various receptors in the body.

A. Glucostatic theory

Circulating levels of glucose have been implicated in the control of voluntary intake in non-ruminant animals. Infusions of glucose into the portal vein of chickens (Shurlock and Forbes, 1981), dogs (Russek, 1970) and rats (Campbell and Davies, 1974) have been shown to reduce food intake, whilst the same effect was seen with infusions into the duodenum of fasted pigs (Houpt et al, 1970). Receptors sensitive to blood glucose have been found in the ventral medial hypothalamus of the brain (Mayer, 1955), these were inactivated by injections of gold thioglucose causing obesity and hyperphagia in mice (Brecher and Waxler, 1949). In addition further glucose receptors have been found in the liver (Russek, 1970) and the duodenum (Stephens, 1980) of non-ruminant animals.

It is believed that glucose does not play a role in the regulation of intake by ruminant animals since no effect on VFI was seen when intravenous injections of glucose were given to cattle (Dowden and Jacobson, 1960) or sheep (Manning et al, 1959) either during or after a meal. In addition administration of gold thioglucose had no effect on intake (Baile et al, 1969).

B. Lipostatic control

In the case of most adult animals a constant body weight is maintained despite changes in food quality (Kennedy, 1953) due to regulation of intake by the ventral medial hypothalamus preventing deposition of excess fat. Mayer (1955) suggested that food intake must therefore be adapted to caloric expenditure. Indeed, removal of inguinal fat from rats

resulted in an increase in intake and of fat deposition (Liebelt et al, 1965). It has been reported that undomesticated and wild ruminant animals do not become obese, although annual fluctuations in body weight are seen (Forbes 1982). Thin non-pregnant cows ate more DM than fat cows fed either a hay or a hay concentrate diet (Bines et al, 1969), whilst a decline in intake of fresh pasture has been reported with dry cows approaching maximum fatness (Hutton, 1963).

C. Thermostatic control

Mammals maintain their body temperature relatively constantly. The thermostatic control of intake suggests that intake is controlled to regulate heat production within the body. This was first suggested by Brobeck (1948) who proposed that 'animals eat to keep warm and stop eating to prevent hyperthermia'. Animals tend to have a greater appetite in cold weather than they do in warm weather, with the appetite of sheep increasing after shearing (Ternouth and Beattie, 1970).

Whilst heating and cooling of the hypothalamus decreased and increased the intake of goats (Andersson and Larsson, 1961), it had no effect on the intake of pigs (Carlisle and Ingram, 1973). It would be expected that for the thermostatic control of intake to hold true an increase in the temperature of the hypothalamus would occur during eating. This has been found to be the case in sheep (Dinius et al, 1970), but not goats (Baile, 1968), and similar increases were seen when sheep were force-fed or sham-fed. Forbes (1986) concluded that temperature changes in the hypothalamus and the periphery are likely to be due to excitement rather than ingestion and that thermostasis was not the main controller of VFI.

D. Physical control of intake

Ruminants in their natural habitat and in many farming systems, obtain most of their energy requirements from low digestible bulky roughage feeds, the control of whose intake is predominantly by physical means. The principal limitation of intake of fibrous feeds is the capacity of the rumen, this being determined by the body size, shape and other contents of the abdomen such as fat or a foetus (Baile and Forbes 1974).

Tayler (1959) found a negative correlation between the weight of abdominal fat and the intake of herbage by cattle. The herbage intake of ewes (Reid and Hinks, 1962; Owen,1963) and cattle (Campling, 1966) has been shown to decrease in later stages of pregnancy as the competition between the foetus and the rumen for abdominal space

increases. This is despite the fact that the rate of passage of particles out of the rumen increases during pregnancy (Baile and Forbes, 1974).

Evidence for the role of physical distension in the regulation of VFI comes from studies of the amount of digesta in the rumen termed 'the rumen fill' and both the addition and removal of material from the rumen. These points are discussed below.

1. Rumen fill

It has been hypothesised that ruminants eat to similar levels of fill in the reticulorumen at the end of a meal when offered different roughages ad libitum (Kruger and Muller, 1959).

Blaxter (1962) found similar amounts of DM in the digestive tract of sheep at the end of a meal when fed three different roughages. Freer and Campling (1963), comparing hay and dried grass found similar amounts of digesta in the reticulorumen immediately after feeding and concluded that it was the amount of material in the reticulorumen that signals the end of a meal. Both of these findings are in contrast to earlier work of Campling et al (1961), where different amounts of digesta were present in the reticulorumen at the end of the first meal of cows fed various poor quality roughages, although the amounts reduced to similar levels by the next meal.

Thiago and Gill (1986) found that the weight of digesta in the rumen of steers fed hay ad libitum reached a maximum 8 to 14 hours after the feed was first offered. This suggests that the weight of digesta in the reticulorumen was not the sole factor limiting voluntary hay intake in the first eight hours after feeding, although its importance may increase later in the day. In addition the weight of digesta in the rumen fell between 14 to 24 hours post feeding but a marked increase in feeding was not seen until fresh feed was offered.

When forages are fed to ruminants in the form of silage the evidence that short-term intake is controlled by factors other than the weight of digesta in the reticulorumen is even more marked. In a comparative study silage and hay of similar digestibility were fed for 5 hours to non-lactating dairy cows. The weight of digesta in the rumen of the silage fed animals was lower than that in the rumen of hay fed animals when they ceased eating (Campling, 1966). Steers fed restricted (20 gDM/KgLW) amounts of either hay or silage once daily, finished eating silage at a lower weight of rumen digesta DM than the hay fed animals. The level of rumen digesta DM of the silage fed steers never reached that of the hay fed steers (Thiago and Gill, 1986). It was postulated that at least with the first meal of silage,

factors other than the weight of digesta in the reticulorumen were making a contribution to intake limitation.

With ground and pelleted rations the degree of physical fill in the reticulorumen is unlikely to be limiting VFI. There is evidence though, that intake might be limited by the amount of digesta in the intestines and the abomasum (Campling and Freer, 1966) although this could be related to the rate of disappearance of digesta from the reticulorumen.

2. Ruminal additions and removal of materials

Insertion of water filled balloons into the rumen has been used as a method of demonstrating the role of physical fill in the regulation of VFI. A 2lb water filled balloon placed in the rumen of hay fed sheep decreased intake by 0.27 (Davies, 1962). Insertions of 50-100 lbs of water filled balloons into the rumen of mature cows for 14 days decreased the intake of hay fed ad libitum by 0.54 lb for every 10 lb of water added (Campling and Balch, 1961). In contrast increases of up to 68% of rumen fill in the reticulorumen (digesta and distending material) failed to disrupt intake of hay fed cattle (Mowatt, 1963). Work by Carr and Jacobson (1967) found no significant decrease in intake when 4.5 kg or 8.7 kg of ballast was inserted into either the dorsal or ventral sacs of the rumen of hay fed steers. They concluded that the reductions of intake (caused by distention) noted by Campling and Balch were due to the unphysiological amounts of ballast they used.

Additions of up to 45 litres of water directly into the rumen had no effect on the intake of hay fed cattle (Campling and Balch, 1961). Similarly when 8 l of water was added to the rumen of sheep there was no effect on roughage intake (Davies, 1962). Dilutions of the diet by additions of water directly to hay (Hillman et al, 1958) or silage (Thomas et al, 1961) before feeding did not result in a reduction in voluntary intake. It is likely that water added directly into the rumen or extracellular water in the feed is rapidly absorbed, and does not limit VFI by having a physical presence in the rumen. However, intracellular water found in low DM silages (Thomas et al, 1961) and grasses (Halley and Dougal, 1962) may account for the low intakes of these roughages, since it acts in a similar fashion to the water in the balloons to depress intake by physical fill.

3. Mechanoreceptors

The ruminant animal senses the degree of physical fill via mechanoreceptors in the gut wall. Mechanoreceptors sensitive to distention are found in the rumen forestomach, with

stretch receptors being reported in the wall of the rumen and the reticulum by Leek and Harding (1975). Leek (1986) concluded that the degree of physical fill in the rumen is sensed by tension receptors in the epithelium which respond to increased distension.

Grovum and Philips (1978) found that the major role of tension receptors was in the reticulum since administration of inert material into the abomasum of sheep had no effect on intake. Neither did insertion of balloons filled with 800 ml of water into the rumen, whilst insertion into the reticulum markedly depressed intake (Grovum, 1979).

Distension of the abomasum of adult sheep fed a ground or pelleted alfalfa diet decreased intake (Grovum 1979) which indicated the presence of tension receptors in this part of the intestinal tract. It was not evident from these results whether or not the depression in intake was caused by a reduction in the rate of passage of digesta out of the rumen due to the presence of balloons in the duodenum. In addition, the author pointed out that there was no evidence that these receptors are stimulated during meals in adult ruminants. It is possible that these may be active in pre-ruminant calves.

E. The role of metabolites in intake control

In the early 1960's it was becoming apparent that factors apart from physical fill were important in regulating the voluntary intake of roughages. Work by Blaxter et al (1961) and Campling and Murdoch (1966) showed that the ability of concentrates to depress ad libitum roughage intake was greater for high quality roughages than for poorer roughages. It was evident that the intake of high quality roughages was not purely limited by physical fill and led to the hypothesis that the end-products of digestion acting via chemoreceptors in the intestinal tract were involved in the regulation of VFI of ruminants.

1. Volatile fatty acids

Work to investigate the role of the three primary organic acids produced as end-products of digestion as signals of satiety began in the early 1960's.

Thomas (1960) found that additions to the rumen of either 126 g acetic acid or 100 g of propionic acid (both neutralised to pH 5.0) had no effect on the ad-libitum hay fed dairy heifers when administered in four doses throughout the day.

Short-term (4 hour) infusions of 870 g of acetic acid in 8 litres of water, or 260 g of butyric acid in 4 litres of water, both in free acid form, reduced the ad-libitum intake of hay fed lactating cows by 35% and 16.5% respectively (Montgomery et al, 1963). When the

acids were infused in the salt form, partially neutralised to pH 5.0 with NaOH, no significant reduction in food intake was seen as was the case with propionic acid in either form.

These results are in partial contradiction to those of Simkins et al (1965) who found that the intake of dairy heifers fed an alfalfa hay ration was reduced when propionic acid (partially neutralised to pH 6.0) was infused at levels between 439 to 893g over a five hour period. Partially neutralised butyric acid at levels between 365 to 744g was also seen to depress intake of long hay, but no effect on intake was seen with infusions of up to 1269g of acetate in the salt form. In the same experiment when a pelleted ration of alfalfa (75%) and corn (25%) was fed, acetate and propionate infusions both restricted intake as did isocaloric mixtures of all three acids Ac, Pr and Bu (60,20,20) when partially neutralised to pH 6.0.

The higher volatile acids valeric and caproic are only produced and absorbed in very small amounts in the rumen and their role in VFI is believed to be non existent. The mechanisms via which the principle rumen VFA's may signal satiety through chemoreceptors are discussed below.

i. Acetic acid

Intraruminal infusions of acetic acid are known to depress voluntary food intake. Injections of acetate into selective areas of the goats rumen have shown that the greatest depressing effect on intake occurs in dorsal rumen as opposed to the ventral rumen, the reticulum or the abomasum (Baile and McLaughlin, 1970). Later work showed that exposure of as little as 5% of the rumen, the 'Parlov pouches', to high concentrations of acetic acid depressed intake significantly (Baile and Martin, 1972). Since intrajugular injections of sodium acetate did not depress intake of goats compared to intraruminal injections, Baile and Mayer (1969) concluded that receptors sensitive to acetate were present in the lumen side of the rumen, and were not activated by blood acetate levels. It should be noted however that Baile (1971a,b) also achieved a 20% depression in intake when acetate was infused into the ruminal vein of sheep, although De Jong (1986) points to the unphysiological changes in blood metabolite concentrations that would result during much of Bailes infusion experiments which could limit VFI. Receptors sensitive to acetate concentrations in the rumen are thought to be neural since any effect of ruminal infusions are blocked if a local anaesthetic is included in the infusate (Martin and Baile 1972).

ii. Propionic acid

Intraruminal infusions of propionate have also been shown to have a depressing effect on the intake of cattle, sheep and goats. The mode of action of propionate in satiety was thought to be different from that of acetate since selective injections into the dorsal and ventral parts of the rumen, the reticulum and the abomasum depressed intake equally (Baile and Mclaughlin 1970). It was initially thought that propionate receptors were present in the ruminal vein as injections at this site were more potent at reducing intake than when given intraruminally (Baile, 1971b). However, it is now believed that propionate receptors are infact in the liver as sheep continued to eat during 3 hour portal vein infusions of propionate following denervation of the liver (Anil and Forbes 1984).

iii. Butyric acid

In comparison to acetate and propionate the intake depressing effects of both intraruminal and intravenous infusions of butyrate have been extremely variable, and it is generally regarded as being much less important as a signal of satiety. Butyrate is produced at half the rate of Ac and Pr in the rumen and is of little physiological significance after absorption since 90% is converted to 3OH-Butyrate in the rumen wall resulting in very low plasma levels.

iv. Problems associated with the theory of VFA's as signals of satiety

It should be pointed out that over the last ten years the concept of VFA's as signals of satiety has been challenged.

In the light of previous work and from his own findings, De Jong (1986) concluded that VFA's make only a minor contribution to the control of meal size, duration and frequency under normal feeding conditions. He was critical of the infusion work of Baile and others where infusions were used to investigate role of VFA's in VFI regulation, citing that;

- infusions were only given during meals
- the infusion rate was greatly above the rumen production rate of these VFA's and was therefore unphysiological
- no account was taken of the ruminal changes in acidity, VFA's or tonicity
- the slow mixing of ruminal contents meant that infusates may have been acting locally and not representing the physiological changes that occur in the rumen as a whole.
- no author monitored whether depressions of food intake by either intraruminal or

intravenous infusions of VFA's was a specific satiety effect or whether it was due to sickness or malaise, although most trials did use sodium salts and not free acids.

Whilst many experiments investigating the role of VFA's in intake regulation have used levels of infusions above physiological limits, this is deemed necessary to induce a response. The work of Grovum (1986) is in general agreement with De Jong's interpretation of earlier experiments to elucidate chemoreceptors involved in VFI regulation. He concludes that there is little evidence for receptors in the rumen specific for acetate or in the liver specific for propionate and states that many of the earlier depressing effects of VFA's acting via chemoreceptors can be attributed to the tonicity of the infusate acting via osmorecptors and not chemoreceptors.

2. Lactic acid

Lactic acid is an intermediate in the metabolism of carbohydrates and whilst on most diets the half life of lactic acid is relatively short, about 25 minutes, (Chamberlain et al, 1983) large quantities can accumulate in the rumen of animals fed large quantities of readily fermentable carbohydrates. This can result in a metabolic disorder referred to as lactic acidosis (Dunlop and Hammond, 1965). The role of lactic acid in the control of VFI is unclear. Intraruminal infusions of lactic acid depressed the intake of cattle (Montgomery et al, 1963), sheep (Baile and Pfander, 1966) and goats (Baile and Mayer, 1969) whilst other workers have found no effect of infusion on intake of sheep (Morgan and L'Estrange, 1977). Infusions of lactic acid into the jugular veins of cows (Dowden and Jacobson, 1960) and portal vein of goats (De Jong, 1981) had no effect on VFI.

It is unclear whether there are ruminal receptors specific to concentrations of lactic acid in the rumen. It is believed that the epithelial receptors sensitive to acetic acid may respond to lactic acid (Gregory, 1987). A reduction in intake would therefore occur due to a slowing of reticulorumen motility.

On diets that contain fairly small amounts of lactic acid, such as silage based rations, the predominant end-products of its metabolism by microorganisms in the rumen are acetate and propionate (Gill et al, 1986), both of which have been implicated in the limitation of VFI. Where the level of lactic acid is much higher, such as when concentrate feeds are offered the main end products of digestion are propionate and butyrate (Chamberlain et al, 1983).

3. Amino acids and ammonia.

The role of amino acids as feedback signals in the control of intake of non-ruminant animals has been implied, since injections of L-lysine into the portal vein of chickens (Rusby et al, 1987) depressed voluntary intake. Intravenous injection of glycine, alanine and lysine at unphysiological levels depressed the intake of sheep fed a concentrate ration (Baile and Martin, 1971). Baile and Forbes (1974) however, concluded that absorbed amino acids were unlikely to be involved in the regulation of short-term intake as they are absorbed from the small intestine several hours after ingestion (Purser et al, 1966). In addition on most silage the amount of protein absorbed from the small intestine is low compared with protein intake (Beever, 1980).

Due to protein degradation in the rumen coupled with extensive protein breakdown in the silo, substantial quantities of ammonia appear in the rumen following ingestion of silage, which is quickly absorbed. Ammonia is potentially toxic to animal cells in large quantities (Visek, 1968) and has been implicated in the limitation of silage intake, although the mode of action is unclear.

4. Rumen fluid pH

The pH of the rumen fluid per se may be responsible for the cessation of a meal rather than the increased presence of any one particular acid in the rumen fluid. Bhattacharya and Warner (1968) found that the intake of hay fed steers was depressed ($p < 0.001$) by up to 50% following infusions of phosphoric, lactic or citric acids to lower and maintain rumen pH at pH 6.0 and concluded that pH per se was responsible for the reduction in intake. A similar conclusion was drawn from comparing intraruminal infusions of acetic acid or potassium acetate, where the acid depressed intake and the salt did not.

Leek and Harding (1975) showed that chemoreceptors are present in the anterior rumen and reticulum, the activity of which is changed by the pH of rumen fluid whatever acid is applied (Chritchlow and Leek, 1981).

5. Osmolality of rumen contents

Ternouth and Beattie (1970) found that infusions of increasing concentrations of the sodium salts of acetate, propionate and butyrate caused a successive decrease in VFI of lucerne chaff fed sheep. The authors concluded that the precise molecule infused into the rumen was less critical than the resulting rumen osmolality. In 1971 the same authors found

that infusions of water into the rumen, lowering the osmolality of rumen fluid, caused an increase in intake and implied osmorecptors were involved in the normal control of intake. This contrasts with the work of Campling and Balch (1961) which found that intra-ruminal infusion of 45 litres of water had no effect on the intake of hay fed cattle. Bergen (1972) found that when rumen osmolality reached 400 mOsm/kg feed intake was markedly reduced in sheep, but this level was seldom reached on high roughage or alfalfa silage rations. Bergen concluded that rumen osmolality is not an important factor in VFI regulation.

The mode of action of osmolality in limiting intake is not clearly understood. Gregory (1987) found that raising and maintaining ruminal osmolality at 420mOsm had no effect on reticulorumen motility. In contrast, comparing the effects of NaCl loading of the rumen and the abomasum on the tonicity of the rumen contents and jugular blood, Carter and Grovum (1988) localised the osmotic effects of salts to the wall of the reticulorumen. Epithelial receptors sensitive to hyper- and hypo- osmotic solutions were isolated by Leek and Harding (1975).

IV. THE EFFECT OF PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE DIET ON VOLUNTARY FOOD INTAKE

The physical and chemical characteristics of the diet offered have an influence on VFI. They are discussed here with specific reference to silage, although studies involving other forages are included for comparison. Included in this section is the palatability of the feed. This is the animal's overall impression of the feed and is often underestimated in the regulation of voluntary intake, especially silages (Forbes, 1986)

A. Palatability

The palatability of a feed includes its colour, form, smell and taste.

Sheep are thought to be colour blind which in the case of ruminant animals would imply that colour is not an important factor in palatability (Tribe and Gordon, 1949). The form in which a feed is presented to the animal is important, for example pelleted roughages are eaten in larger quantities than unpelleted roughages (Heaney et al, 1963). It is difficult to assess the relative importance of how the form in which grass within silages effects palatability. It is known that cows prefer short- as opposed to long-chopped silages (Castle

et al, 1979) but generally short chopped silages are better preserved than those that are long-chopped, therefore making it difficult to attribute increased intake to physical form as opposed to some factor of fermentation quality.

The smell of the feed is important in feed selection in sheep (Arnold, 1970), although olfactory bulbectomy of sheep in another trial did not affect daily intake of a complete pelleted diet (McLaughlin et al, 1974). Additions of silage extracts from silages deemed poor quality by their chemical analysis did not affect the intake of sham fed sheep (Buchanan-Smith, 1990). Whilst the sensation of taste would have been involved in the selection of these silages, it may have been anticipated that the presence of butyric acid and possibly amines such as putrescine and cadaverine in these extracts would have inhibited the sheeps intake.

The taste of a feed is probably the most important palatability factor when silages are fed to ruminant animals. Ruminant animals are known to be sensitive to bitter, salty, sour and sweet flavours (Jacobs et al, 1978). A fifth taste (umami) has been proposed as existing, at least in sheep (Grovum and Chapman, 1988). Cattle are able to detect tastes with a greater sensitivity than sheep, with goats intermediate.

Poorly preserved silages are eaten to a lesser extent than well preserved silages. Sheep fed one of 3 silages ate a similar amount of each. When given free access to choose between the three, they ate considerably less of the poor quality silage (Forbes, 1988). Two silages were made from the same sward of grass, one was ensiled using good silage techniques and an additive, whilst the second was made in a deliberate attempt to create a poor quality silage. When these were fed to dairy cattle, considerably more of the well produced silage was eaten (Baker et al, 1991), the main difference between the two being their amine content (see Experiment 1).

In a comprehensive study into the effect of the fermentation end-products on palatability, Buchanan-Smith (1990) found that when acetic acid was added to silage, the intake of sham-fed sheep was depressed. However, when acetic and lactic acids were added to the silage in combination, or when butyric acid was added alone, no effect on intake was seen. The difference in response to these acids was attributed to the fact that the pH of all silages was adjusted to pH 4.5 prior to feeding. At this pH lactic acid would have been in the salt form as opposed to the free form and less likely to affect palatability, acids in the free form having a sour taste. This does not however account for why no effect of butyric acid was found.

In the same study no effect of additions of ammonia were seen. The amines

cadaverine and putrescine and GABA depressed intake up to the highest level they were applied and the author points out that the animals may have become accustomed to these additions and this was not taken into account in the experimental design.

B. Physical characteristics

The physical form of the diet can affect its voluntary intake by ruminant animals. Forages tend to be bulky fibrous foods that impose a physical limit on voluntary intake. Van Soest (1965) attributed this limitation of forage intake to the cell wall constituents, the structural carbohydrates, as measured by the acid detergent fibre method. In this study and in the work of Osbourn et al (1974) a negative correlation was obtained between DM intake and cell wall constituents. Increasing the crude fibre content of the diet of sheep not only decreased the daily intake of sheep but altered meal patterns, reducing both their size and length (Dulphy et al, 1980).

1. Reduction of forage particle size

Grinding (Wilkins et al, 1972) and pelleting (Heaney et al, 1963) of dried roughages increases their intake by ruminants, the effect being greater with low quality as opposed to high quality forages (Campling and Freer, 1966). The mincing of silage has also been shown to increase its voluntary intake (Thomas et al, 1976). This has been associated with a decrease in the particle size and a faster rate of passage through the rumen which enables a greater quantity of food to be eaten.

Silages, with the exception of those made as 'big bale silage' are generally chopped prior to ensiling. A reduction of forage particle length aids the compaction of the material so excluding oxygen and liberates the intracellular contents therefore assisting a rapid fermentation.

Silage intake is improved by chopping of the grass prior to ensiling. Deswysen et al (1978) found that sheep fed a short- as opposed to a long-chopped silage had a higher intake of the short-chopped material. Cattle consumed a greater amount of flail cut material than silage made without cutting or laceration (Murdoch, 1965), whilst precision chopping of forage before ensiling increased the voluntary intake by sheep (Dulphy and Demarquilly, 1973). Precision chopping of silage increased the intake by sheep in comparison to flail chopping in a comparison of 9 silages (Dulphy, 1980). These increases in intake could have been attributed to an improvement in silage quality seen when grass is lacerated prior to

ensiling as opposed to a reduction in the particle sizes.

2. Digestibility

The digestibility of the dry matter of a feed has been shown to be positively correlated to intake below 0.67 and negatively correlated above 0.67 for dairy cows of moderate lactation (Conrad et al, 1964) suggesting that with poor quality feeds physical rather than metabolic controls of intake were likely to operate. The actual value where control of intake changes from physical to metabolic control will vary between diets and animals and the two mechanisms will not be exclusive of each other.

The intake of fresh and dried forages is positively related to intake (Balch and Campling, 1962). More specifically the increase in hay intake seen with increasing digestibility was a result of reducing the effect on rumen fill (Blaxter, Wainman and Wilson, 1961). In early studies of the effects of silage fermentation upon intake (Wilkins et al, 1971; Demarquilly, 1973) the digestibility of the silage was not thought to be closely related to intake. Wilkins (1981) concluded that the poor relationship between intake and digestibility of silages was due to silages being cut at an early stage of growth resulting in poor fermentation quality.

In later studies, discussed in more detail in Section IV.C.2., statistical correlations of digestibility and intake of silage have been found to be positively related (Lewis, 1981; Gill et al, 1988). The discrepancy between these results may be attributable to the advances made in ensiling techniques over the last 20 years, with a greater use of additives used today to regulate and improve the fermentation.

3. Dry matter

The voluntary intake of silage increases with increasing DM. The wetting of silage before feeding did not affect the DM intake of silage (Thomas et al, 1961), whilst additions of water 12 hours prior to feeding to sheep lowered their intake (Dodsworth and Campbell, 1953). Extracellular water is not likely to limit VFI since it is rapidly absorbed across the rumen wall and Clancey et al, (1977) concluded that water content per se is not involved in intake regulation.

Wilting of grass has for many years been used as a means of improving fermentation quality. Wilting generally results in an increase in silage intake of between 5% (Rohr and Thomas, 1984) and 18% (Steen and Gordon, 1984), although generally a depression in

liveweight gain is seen when wilted as opposed to unwilted silage is fed. This improved intake is a consequence of improved fermentation (Wilkins, 1988) and the reduction in animal performance the consequence of a reduced digestibility of the diet (Gordon, 1989). Wilting of silage resulted in a 4% higher intake by lactating dairy cattle, but again animal performance in terms of milk yield was reduced by 2% (Rohr and Thomas, 1984). Whilst wilted silages may enhance DM intake, Gordon (1989) calculated that the loss in animal product per hectare caused by wilting of grass prior to ensiling in dairy and beef enterprises could be as high as 13%.

C. Chemical characteristics

The reduced intake seen when silages are fed as opposed to other forms of herbage have been attributed to their chemical composition.

1. Protein

Low protein diets are associated with poor intakes by both monogastric and ruminant animals. Ruminants needs for dietary protein are lower than those of monogastric animals since the microorganisms of the gut are able to utilise NPN in the diet and urea in the saliva for protein synthesis. A fall in intake of mature sheep and cattle occurs when the dietary protein level falls below 8 - 10% (Blaxter and Wilson, 1963; Elliot and Topps, 1963). In the case of the lactating dairy cow where protein requirements are high, levels of dietary protein below 12% reduce intake (Bines, 1979). Protein deficiencies may be due to a reduction in bacterial and protozoal cellulytic efficiency in the rumen (Campling, Freer and Balch, 1962). Egan (1965) suggested that a protein deficiency would lead to depressed intake as key enzymes needed in the metabolic pathways to utilise digestion end-products would not be able to function without these limiting amino acids. A build up of digestion end-products would result, stimulating the chemoreceptors involved in intake regulation.

Silage diets are characterised by the rapid degradation of soluble protein and NPN in the rumen, resulting in a pronounced peak in rumen ammonia and a reduction in available amino acids and peptides. The fixation of ammonia by rumen bacteria requires energy. On many silage diets there is a lack of energy substrates in the rumen due to the poor ATP yield from silage fermentation end-products. This coupled with the decrease in the availability of amino acids and peptides results in a poor rate of microbial protein synthesis (Thomas and Thomas, 1985). The Agricultural Research Council (ARC, 1984) adopted a value of 23 g

microbial N/kg organic matter digested in the rumen (DOMR) for silages and 32 g N/kg DOMR for hays and grasses, whilst a value of 50 gN/kg DOMR has been reported for fresh grass in a more recent study (Beever et al, 1986). A poor rate of microbial protein synthesis in the rumen could lead to a slowing of the rate of digestion and hence reduce voluntary silage intake (Chamberlain et al, 1989).

The reduced microbial synthesis on silage diets leads to a lower duodenal concentration of methionine and lysine, two of the limiting amino acids for growth and lactation (Thomas and Chamberlain, 1982). Increasing the amount of protein (fishmeal, blood meal, meat and bone meal mix) reaching the abomasum either by direct infusion, or increasing the dietary supply of protein increased food intake of dairy cows by up to 12% (Chamberlain et al, 1989).

The intake of silage diets appears to be enhanced by the inclusion of a rumen undegradable protein supplement (Gill and Bursted, 1985), the intake response to supplementation is less pronounced with good quality silages (Gill et al., 1987) rather than when poor quality silages are fed (Gill and England, 1984).

2. End-products of silage fermentation and intake

The influence of the chemical composition of the diet on VFI increases when silage is fed, due to the presence of fermentation end-products. It is well documented that the voluntary intake of forage is reduced by ensiling compared to both fresh forage or conservation in the form of hay. Ensiling appears to have a greater effect on the reduction of intake by sheep than cattle (Greenhalgh and McDonald, 1978). Demarquilly (1973) showed the intake of sheep to be 33% higher for fresh forages than silages made from the same crop, whilst Campling (1966b) found non-lactating dairy cows ate 28% more hay than silage made from the same sward. This is supported by work in which silage extracts have been infused directly into the rumen and depressions in intake have occurred (Clancey, Wangsness and Baumgardt, 1977; Buchanan-Smith and Phillip, 1986). These results imply that there is some factor, possibly an end-product of fermentation, present within silage that is limiting its voluntary consumption by ruminants.

Investigations into the end-products of the silage fermentation that play a role in VFI generally take one of two forms:

a) derivation of statistical correlations between the effects of concentrations of different components of silage on intake

b) measurement of adding silage extracts or individual silage constituents to either the feed or the rumen and VFI (Gill et al, 1986).

i. Statistical correlations between silage composition and VFI

Statistical studies have been carried out with each of the three principle forms of livestock, sheep, dairy cows and beef steers, to correlate fermentation parameters with VFI. The results of analysis of data of physical characteristics of silage such as digestibility and DM are also included in this section. Multiple regression analysis using data accumulated from a number of sources has shown that only ammonia-N as a proportion of total-N has a significant negative effect on the intake of dairy cows, whilst DM content and DM digestibility are both positively related to intake (Lewis, 1981). However, Vadiveloo and Holmes (1979) found that 0.27 of the variation in intake could be attributed to the effects of different source.

Gill, Rook and Thiago (1988) analysed data collected over a period of 7 years at Hurley that included 206 lactations and the feeding of 13 different silages. A one way analysis of variance found that 0.24 of the total variation of intake could be attributed to differences among silages. Linear regression analysis of various silage characteristics showed significant ($p < 0.001$) positive correlations between DM, DOMD, pH, lactic acid as a proportion of total acids and total-N, while significant negative correlations were found with lactic acid, acetic acid, butyric acid and total acids.

Wilkins et al (1971) used regression analysis to correlate concentrations of silage constituents of 70 grass and legume silages and the intake of mature and wether sheep. The results indicated that total-N, DM and lactic acid as a proportion of total acids were all positively correlated with silage DM intake while ammonia-N as a proportion of total-N, acetic acid and total acid concentration were all negatively correlated with DM intake. In a later study (Wilkins et al, 1978) that included 142 silages made from grass, legumes, maize or sorghum fed to wether sheep, similar negative correlations with ammonia-N as a proportion of total-N, and with butyric acid ($p < 0.05$) and intake were found. No other correlation was found with individual organic acids and silage intake. However, when silages made with a formaldehyde additive were excluded (these type of silages have a low acid content) from the regression analysis a negative correlation ($p < 0.05$) was obtained between total acid content of the silage and VFI. These results show that the same factors limiting silage intake in dairy cows may also be operating in sheep.

In a series of studies Rook and Gill (1990) and Rook, Dhanoa and Gill (1990) have studied the relationship between silage composition and intake of beef steers from data accumulated at three different sites. In the initial study, simple and multiple linear regression were used. It was concluded that silage intake was positively related to the toluene dry matter (TDM), but as the result was curvilinear there was unlikely to be any improved intake from wilting to above 250 g/kgTDM. The concentrations of the butyric and acetic acids were found to be more important than their level in relation to the other acids, both being negatively correlated with intake. Ammonia-N was negatively related to intake, although it ranked below other measures of fermentation quality, and was highly correlated with silage VFA's. This implied that ammonia-N per se was not responsible for intake limitation.

In the second study the effect of collinearity between the independent variables was removed by use of ridge regression techniques to improve the predictions. Butyric acid was found to be the most important of the silage organic acids in relation to VFI, being strongly negatively related to intake. The effect of ammonia-N was small once collinearity was removed and in both studies the lactic acid content of silage was deemed to be of little importance in intake regulation.

These types of statistical analysis relating silage composition to intake provide useful information about the type of silage, recognisable by the profile of the end-products of fermentation, that may or may not be readily eaten by livestock.

To identify which silage fermentation end-products are directly involved in the limitation of silage intake, experiments have to be conducted where the nutrient supply is in some way manipulated either by additions to the diet or by infusions into the rumen.

ii. The effect of manipulating nutrient supply

The two groups of silage fermentation end products implicated as being involved in regulation of VFI are the nitrogenous compounds or the organic acids.

a. Nitrogenous compounds

As stated earlier high levels of ammonia in a silage are associated with spoilage and low voluntary intakes. Intra-ruminal infusions of ammonium salts, especially NH_3Cl, have been shown to depress the voluntary intake of silage by heifers (Thomas et al, 1961) and to reduce the frequency and size of meals of animals fed high concentrate diets (Conrad, Baile and Mayer, 1977). The exception to this is ammonium lactate which had no effect on

voluntary intake, which may be due to its buffering activity that unlike ammonium chloride upsets the animals acid-base balance. In these experiments no account was taken of the effect that intra-ruminally infusing salts has on the osmolality of the rumen fluid and it may have been the tonicity of NH_3Cl as opposed to the presence of NH_3 ions that restricted VFI.

Urea is rapidly metabolised within the rumen to form ammonia and is used in production systems as a supplementary nitrogen source. Chalupa et al (1979) demonstrated that even on diets containing urea below toxic levels intake was reduced. This effect was attributed to a development of malaise as a result of ammonia production. Urea is not as osmotically active as ammonium salts and is sometimes used as an infusate to mimic additions of ammonia. Recently it has been shown that intra-ruminal infusions of urea have markedly depressed the silage intake of both lactating and non-lactating dairy cows when the crude protein content of the total diet exceeded 220g CP/kgDM (Choung, Chamberlain and Thomas, 1989) again suggesting a role for ammonia in limiting silage intake.

In contrast to this when alfalfa silage fed heifers were given intra-ruminal infusions of silage juice only 40% of the intake depression could be attributed to ammonia (Clancey et al, 1977) and the presence of some other factor(s) reducing VFI was suggested.

This is supported by Barry et al (1978) who from a study of lucerne silages concluded that it was probably products of decarboxylation rather than products of deamination within the silo that were associated with depressions of intake. Experiments have shown that individual intraruminal infusions or dietary additions of decarboxylation products have varying results. No depressions of intake have been seen with work using tyramine, tryptamine (Neumark, Bondi and Violante 1964; Thomas et al, 1961), histamine (Mcdonald, Macpherson and Watt, 1963) or gamma amino-n-butyric acid (Clapperton and Smith 1983, cited by Thomas 1990). Contrary to these findings histamine has been shown to decrease intake (Neumark et al, 1968) as have intra-ruminal infusions of gamma amino-n-butyric acid (Buchanan-Smith, 1982) which decreased VFI of wether sheep fed alfalfa pellets, although not significantly. Gaba is of particular interest in regards silage intake as it has been shown to be involved in the control of both satiety and hunger in the brain (Morley, 1980).

b. Organic acids

The organic acids acetic, propionic, butyric, lactic and formic acids are found within silages either as end products of fermentation or, in the case of formic acid, as an additive to aid preservation.

Whilst increasing the amount of formic acid applied to silage may well result in an increase intake due to improved fermentation quality (Wilson and Wilkins, 1973), Wilkins and Valdemoro (1973) demonstrated that formic acid per se can inhibit intake since intra-ruminal infusions lowered intake.

Partial neutralisation of silages with sodium bicarbonate to restrict the total amount of free acids that are present, prior to feeding, have resulted in increased intakes of well preserved grass and maize silages (McLeod, Wilkins and Raymond, 1970; Thomas and Wilkinson, 1973). Partial neutralisation of maize silage resulting in increased intake was attributed to an increase in blood pH and a reduction in acid-base stress.

The role of acetic and propionic acids in VFI have been discussed in detail earlier (Section III.E.1).

Lactic acid is the predominant acid found in well preserved silages and can occur at levels of up to 180 g/kg DM (Chamberlain et al, 1983). It is also found in the rumen as an intermediate of carbohydrate metabolism.

Little work has been conducted to investigate the effect of lactic acid on the intake of silage fed animals although mixed results as to its effect on the intake of dried forage fed ruminants have been obtained.

Infusions of 360g lactic acid into the rumen of lactating holstein cows lasting for four hours depressed the daily consumption of hay by 5% (Montgomery et al, 1963) and caused an increase in the molar proportions of acetic acid and a decrease in propionic acid within the rumen. Greater reductions of intake were seen when sheep fed dried grass were given infusions of lactic acid at levels up to 2.10 moles/day. (Wilkins and Valdemoro, 1973). Intake was not depressed until the level of lactic acid infused reached 0.9 moles/day, at levels of 1.2 moles/day intake was depressed by 9.2% and above this level intake dropped by up to 85%, the critical level being between 1.35 and 2.10 moles/day. It was calculated that 1.2 moles of lactic acid represented 7% of the total DMI, a level not excessively high in silages.

Mixing of lactic acid to the diet before feeding has also been shown to limit VFI. Additions at levels of 26.9 g/kg DM, 60.5 g/kgDM, and 171.5 g/kgDM of 80% lactic acid to a mixed sward of grass silage reduced the intake of 5 month old sheep by 53.5%, 57.7% and 78% respectively in relation to the control (McLeod et al, 1970). Additions of lactic acid at a level of 50 g/kg DM depressed the intake of silage, made without additive, fed to growing calves by 12% (23.9 verses 21.2 g/kgLW ($P<0.001$)) (Thomas et al, 1980). This depression of intake was alleviated when fishmeal was added to the diet at a level of

50g/kgDM. The authors suggested that this was caused by an increase in the protein to energy ratio in the rumen.

In 1977, Morgan and L'Estrange using sheep fed grass pellets compared intra-ruminally infusing lactic acid at levels of 600, 800 and 1000 mmol/kgDM fed with additions to the diet of the same levels upon VFI, measured at 09:00 and 21:00 hours. Infusions caused a decrease of VFI of up to 67.7% ($P<0.001$) whilst no effect was seen when lactic acid was added to the diet, although in both cases a decrease in the molar proportion of acetic acid ($P<0.05$) and an increase in the molar proportion of propionic acid ($P<0.001$) was seen. It was concluded that lactic acid was unlikely to limit VFI. The depressions in intake caused by ruminal infusion were attributed to a decreased motility of the rumen wall, caused by a problem of administration of the acid, and not due to increased ruminal lactic acid concentrations or an increase in the ruminal concentrations of acetic or propionic acids.

D. The effects of supplementation on silage intake

Phipps (1986) calculated that silage diets barely met the maintenance requirements of the cow, or 50% of the total energy needs over the winter period. This is despite the ability to produce silages with high concentrations of ME and CP and illustrates the limitations to the nutritional value of silages for feeding to animals with high metabolic requirements such as the lactating dairy cow. The conventional means of overcoming the deficiencies of silage is to supplement the diet with increasing amounts of concentrates.

The effect of concentrates on silage intake is influenced by 3 components of concentrate feeding;

- the amount of concentrate given
- the number of meals per day in which the concentrate allocation is given
- the nutritional composition of the concentrate feed

As the level of concentrate in the total diet increases, energy intake will increase, whilst the forage intake will decline as the substitution rate expands (Leaver, 1983). An increase in the total amount of concentrate given will generate a more extreme rumen environment that will not favour cellulolysis and will inhibit forage intake (Sutton, 1981).

Barley based concentrate rations have depressed silage intake of dairy cows by 0.51 kg/kg barley DM, when up to 6.0 kg of barley was offered to cows (Castle and Watson, 1976). Cereals such as barley have a high content of starch in them that are readily fermented in the rumen with a subsequent reduction in rumen pH. Cellulytic bacteria in the rumen are

inactivated at pH 6.0 - 6.1 (Mould and Orskov, 1983), it is believed that the fall in pH, depresses their activity and reduces intake (Tayler and Wilkins, 1976). Successful attempts to reduce the rate of ruminal degradation of starch in order to reduce the substitution rate have involved the treatment of barley with acid-formaldehyde (Kassem et al, 1987). The treatment of barley grain with sodium hydroxide prior to feeding reduced the substitution rate of hay (Orskov and Fraser, 1975), probably because of a neutralising effect on the acids produced during digestion.

To alleviate the detrimental effects of starch based concentrate feeds on cellulolysis recent studies have investigated replacing starchy concentrates with digestible fibre sources such as sugar beet pulp or rice bran. When fibrous concentrates were included in the concentrate feeds of dairy cattle, as opposed to barley, the intakes of silage (Thomas et al, 1986) and hay (Sutton et al, 1987) increased, although these results have not always been consistent (Castle et al, 1981) and in some cases higher intakes of silages have occurred when barley is fed (Mayne and Gordon, 1984).

The inclusion of protein supplements in a cereal concentrate feed reduces the substitution rate of silage. Castle (1982) reported this reduction in the substitution rate to be greater than would be expected simply from the reduction in the starch content and implied that supplementary protein has a stimulatory effect on silage intake. When the CP content of a concentrate feed was increased from 214 to 403 g/kgDM the substitution rate of silage declines from 0.59 to 0.37, although the mode of action of protein supplementation is unclear (Reeve, Baker and Hodson, 1986). The addition of fishmeal at levels of 50, 75 and 100 g/kg silage DM offered increased the silage DM intake of calves fed ad libitum (England and Gill, 1985).

Increasing the number of times that a concentrate feed is offered to animals within the day is likely to result in less fluctuations in fermentation parameters and a more stable rumen pH (Sutton, 1986). In practice concentrate feeds may only be available to cows in the milking parlour and so limit their feeding to twice daily. In modern dairy production systems, the use of on farm computers to recognise individual cows and allocate the appropriate amount of ration, make frequent feeding of concentrates outside of the parlour a possibility. However, Gordon (1984) reported little evidence of the use of these machines increasing forage intake.

V. PHYSIOLOGICAL CONTROL OF VOLUNTARY INTAKE

The physiological state of the animal will impose some control or influence upon it's voluntary intake, this includes growth and fattening in the case of beef steers and lactation, pregnancy and oestrus in the dairy cow.

A. Lactation

In general the intake of lactating cows is higher than the intake of non-lactating cows (Hutton, 1963; Hunter and Siebert, 1986) and a positive relationship is found between milk yield and intake (Vadiveloo and Holmes, 1979).

After partuition milk yield rises to a maximum level between 35 to 50 days post partum, after which point it steadily declines (Wood, 1969). This rise in yield is accompanied by a growth in energy demand and is reflected by an increase in appetite. The rise in intake is slow, peak intake occurring at 16 weeks but ranging from 5 to 35 weeks post partum (Bines, 1976). This lag is extended on roughage diets (Owen et al, 1968; Forbes, 1970a) and is larger with heifers compared to cows (Bines, 1976). In the early stages of lactation the intake of cows generally does not meet it's energy demands and results in mobilisation of body tissues.

Several hypotheses exist as to the cause of this depression in intake immediately post partum and during early lactation.

The abdominal fat deposited during pregnancy is believed to impose a restraint on the capacity of the gut and thus limits intake by means of physical fill. Mobilisation of this adipose tissue has to occur before intake can increase to a maximum (Bines, 1976; Garnsworthy and Topps, 1982). In general fat cows have been found to consume less food than thin cows (Land and Leaver, 1980).

Rather than the amount of abdominal fat present, the slow metabolic response to the increased nutrient demands of the cow (Forbes, 1986) and hypertrophy of the digestive tract and the liver (Campbell and Fell, 1970) has been suggested as reasons for the low intakes in early lactation.

The effects of endocrine changes in late pregnancy may reduce intake. Following injection with 10 mg of 17 beta-oestradiol ewes took 2 weeks to recover to the same level of intake recorded prior to treatment (Forbes, 1986).

B. Pregnancy and oestrus

The intake of monogastric animals increases during pregnancy on account of the increase in nutrient demand. The intake of both heifers (Penzhorn and Meintjes, 1972) and ewes (Forbes, 1970) increases in mid lactation. Whilst in the last months of gestation cows generally eat less food than in the earlier months of pregnancy (Owen et al, 1968). This has been attributed to a reduction in abdominal space caused by the growing foetus. Cross-sectioning of the abdomen of pregnant ewes has shown that the rumen is compressed by the uterus up to five weeks before partuition (Forbes, 1968) so impeding VFI.

It would appear that this is not the sole reason, since the intake of concentrates which is not primarily controlled by physical means has been reduced when fed as the main feed to heifers in late pregnancy (Aitken and Preston, 1964). The increased production of oestrogens by the placenta in late pregnancy is one possible metabolic factor that may be responsible for this reduction in intake (Forbes, 1971).

The elevated levels of circulating oestrogens have been suggested as causing the decline in food intake (Hurnik et al, 1975) and the reduction in the time spent eating (Putman and Bond, 1971) by cows at oestrus.

C. Growth and fattening

The intake of a growing animal increases as it grows, although there is a decline in the amount of food eaten in relation to metabolic liveweight as the animal gets larger. Blaxter (1962) suggested that intake was related to a metabolic liveweight raised to the power 0.75. This may be true for the adult animal but for the growing animal it is probably somewhat lower, values to the power 0.6 (Forbes, 1971b) and 0.61 (Hodgson and Wilkinson, 1967) having been determined.

Fat cattle tend to eat less than thin cattle (Mather, 1959). Tayler (1959) found an inverse relationship between the weight of internal fat and the intake of herbage, probably due to a reduction in rumen capacity, which would appear to support the finding of Mather.

The plasma insulin levels of goats rose as they became fatter (De Jong, 1981), as did the levels detected in sheep which rose from 10 to 300 iu/ml over a 35 week period as the animals reached maturity (Vandermeerschen-Doize et al, 1982). These findings imply that fatness does not simply inhibit intake by physical limitation of rumen capacity as the levels of insulin detected in the plasma would be expected to fall as the intakes decreased (Forbes, 1986).

VI. THE USE OF NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) IN ANIMAL AGRICULTURE

Near infrared reflectance spectroscopy (NIRS) has become a widely used method in both the human food industry and the animal feeds industry as a rapid method for feedstuff analysis. The theory of NIRS, its history, present usage and future applications as regards its role in agriculture are discussed below.

A. The theory of NIRS

Near infra-red spectroscopy covers the spectral range from 1100 to 2500 nm. Energy in this range is directed via a monochromator grating onto a cell containing a sample. The monochromatic radiation interacts with the sample and may be absorbed, transmitted or reflected. The energy, either reflected or transmitted, from the sample cell is collected by lead sulphide detectors. Two types of reflectance occur.

i) Specular (or regular) reflectance which occurs at the sample-air interface and carries information about the physical nature of the sample particle surfaces.

ii) Diffuse reflectance occurs when the energy passes the air to surface interface and is randomly reflected and scattered at further interfaces within the sample. Diffuse reflectance carries information about the chemical nature of the sample, since the bonds within the molecules of the sample (-CH, -OH, -NH, -SH) absorb energy at specific wavelengths and modify the intensity of the radiation emerging from the sample.

Reflected energy is collected on the detectors which are set to maximise the amount of diffuse reflectance collected. The packing density of the sample and the sample particle size affect the path length through the sample cell, to keep these as uniform as possible the samples are usually ground before analysis. However, spectra of the same sample tend to have the same shape but due to differences in the path length are displaced from each other (Baker and Barnes, 1990). This problem of variable path length is overcome by first and second order derivative spectroscopy.

Calibration of the machine is carried out by relating wet chemistry analysis values, or in vivo data, to log 1/R absorbence of samples using multi linear regression. Approximately 100 samples are needed to test and calibrate an NIRS instrument successfully (Shenk et al, 1976), which means that the accuracy of NIRS analysis is limited by the accuracy of the chemical analysis in the first place.

B. The history of NIRS

NIRS dates back to 1800, when Herschel observed radiation beyond the red portion of the visible light spectrum, that was detectable with a photographic plate. The first NIRS spectrum, from 700 to 1200 nm, was measured in 1881 (Abney and Festing).

NIRS as it is today, did not evolve until the 1950's, with the work of Norris. Norris working for The United States Department of Agriculture (USDA), and using technology developed for communications during the second world war, embarked on a programme to develop a technique for the rapid analysis of the moisture content of grain. This involved various spectroscopic methods including visible and near infrared transmittance and reflectance.

Initially samples were prepared in carbon tetrachloride or methanol extracts (Hart et al, 1962; Norris and Hart, 1965). However, problems of safety in the use of solvents prevented these methods being widely used. Transmission directly through thin layers of dried sample was first used by (Ben-Gera and Norris, 1968), but it is only recently that this has been used in a commercial machine (Rosenthal and Rosenthal, 1981).

Difficulties were also encountered in the early analyses due to the overlapping of absorbence wavelengths that occurs from the protein, oil, and starch components of the feed. However, this problem is eliminated today, as the incorporation of a microcomputer which permitted elimination of these wavelengths or determination of their constituents (Osbourne and Fearn, 1988).

The use and increasing widespread availability of computers in the 1970's made the use of NIRS a more practical proposition in many situations. Computers enabled the vast amount of multiple linear regression involved in NIRS to be calculated easily and these were starting to become a fixture in many research institutions. Over the next 15 years the advances in microcomputer technology continued to be applied to NIRS, which eased the problems of tedious calibration. This enabled its use in laboratories by relatively unskilled staff and hence the overall appeal of the technique.

C. Present usage of NIRS

Today NIRS is used extensively in both the animal and human food industries. It has advantages over chemical methods of proximate analysis of feedstuffs in that they are often time consuming, expensive and hazardous. NIRS, once a machine has been successfully calibrated, is rapid, clean and repeatable.

The Canadian Grain Commission were one of the first groups to use NIRS commercially as a standard analytical tool. In 1975 they adopted NIRS as the means by which the protein content of wheat was measured when grain was bought and sold.

Norris et al (1976) demonstrated that NIRS could be used in the analysis of forages, including fresh grasses and those conserved as hay and silage. Using 9 wavelengths for the prediction curves the standard errors of prediction were $\pm$ 0.95% for CP, $\pm$ 3.1% for NDF and $\pm$ 5.1% for dry matter digestibility by sheep.

The samples used for analysis in this study were dried and ground before being analysed. As regards silage, drying will dramatically alter the chemical composition. Volatiles such as ammonia and the short chain organic acids are lost by drying, so any subsequent analysis of dried silage samples will not detect these compounds.

Recent studies have attempted to analyse undried silage samples (Reeves et al, 1989; Reeves and Blosser, 1991). In the first of these experiments strong correlations were obtained between the DM and CP content of the silage and NIR spectra. Poorer correlations were obtained with silage acids, although the best of these was with the two major silage acids, lactic and acetic (Reeves et al, 1989).

The second of these studies investigated the effect of grinding and presentation cell size on NIRS analysis of wet silages. The best correlations with DM, CP and ADF content were obtained when the samples were ground in the presence of dry ice and analysed in a non-rotating rectangular cell, approximately 15 cm in length. The grinding of samples before NIRS analysis has been advocated by other workers in order to achieve a sufficiently fine subdivision of sample material to obtain accurate analysis (Martin et al, 1985).

Whilst the results of these trials demonstrate that the major non-volatile components of wet silage can be analysed by NIRS, the determination of organic acids is not satisfactory for analytical purposes. It was questioned by Reeves and Blosser (1991) whether it was better to determine accurately an altered composition (dried samples) or to determine less accurately and more conveniently an unaltered composition (wet samples).

As the knowledge of interpretation of NIRS spectra increases it is likely that wet silages will be routinely analysed by NIRS in the future.

VII. SUMMARY

It is apparent that many factors affect the voluntary intake of silage. For example, the physiological state of the animal to which the silage is fed, the stage of growth of the material at harvest, the conditions in which the silage is stored and the choice of additive used (if any), will all influence the intake of the ensiled feed. The work reported in this thesis focuses on the end-products of fermentation and the role these play in the regulation of silage intake in cattle.

Statistical correlations between silage characteristics and voluntary intake by cattle have shown that various fermentation end-products are negatively associated with silage intakes. These include compounds such as ammonia (Wilkins et al, 1971, 1978; Lewis, 1981: Rook, Dhanoa and Gill, 1990), commonly associated with spoilage organisms, and lactic acid (Wilkins et al, 1971; Gill, Rook and Thiago, 1988), the predominant organic acid in well preserved silages. However, when the intake of these nutrients has been manipulated in order to identify the components of silage that depress voluntary intake, either by additions to the diet or intra-ruminal infusions, the results have not been consistent.

Intra-ruminally infused lactic acid has been shown to depress the intake of hay fed cows (Montgomery et al, 1963) and sheep fed a dried grass diet (Wilkins and Valdemoro, 1973), whilst additions of lactic acid to silage prior to feeding depressed the intake of both growing cattle and sheep (Thomas et al, 1980; McLeod et al, 1970). In contrast, Morgan and L'Estrange (1977) found no effect on voluntary intake of adding lactic acid to the diet prior to feeding.

Ammonium salts have been shown to depress the intake of silage fed heifers (Thomas et al, 1961) and alter the feeding pattern of goats (Conrad, Baile and Mayer, 1977). Recent debate has questioned the role of silage ammonia in the limitation of intake with the amino acid decarboxylation end-products, the amines, now being considered more important in intake limitation (Barry et al, 1978).

This thesis investigates the effect of short-term intra-ruminal infusions of specific silage fermentation end-products, lactic acid, ammonia-N, Gaba and putrescine on roughage intake by cattle in order to try to identify the role they play in intake regulation.

SECTION B

GENERAL MATERIALS AND METHODS

I. MEASUREMENTS AND SAMPLING PROCEDURES

A. Rate of eating

All animals were individually housed in cow standings, modified for the experiments with steers by the insertion of a slatted plastic flooring to allow free flow of urine away from the beds. At the front of each standing a feed bin was mounted upon an electronic balance (August Sauter GmBH, Model E1200, Germany), which allowed for both the rate and pattern of the animals roughage intake to be monitored. The balances were connected through a serial port box to a BBC B micro computer which ran a programme in OS9, enabling the weight of feed in front of the animal to be recorded every two minutes. 'Daily' patterns of intake were monitored over a 23.5 hour period commencing at the time that fresh roughage was offered to the animals each morning. Data were transferred to a Vax/VMS mainframe computer and analysed using Genstat 5.0.

In all trials roughage was offered ad libitum. However, due to the bulk of the feeds and to keep the feeding regimen in line with other animals housed in the buildings, fresh feed was given in two batches, a.m. and p.m., the times varying with experiment.

In all experiments the animals had free access to water from drinking bowls and to mineral licks. No attempt was made to monitor water intake.

B. Feed sampling

During all experimental digestibility periods and at other regular intervals, feed samples were collected daily (^1000 g hay and silage and 200 g concentrate) and stored as bulk samples, the silage at -4°C and the hay and concentrates at room temperature, until the end of each period when they were sub-sampled for analysis. For each of the feeds three 400 g samples were dried at 105°C for 24 hours to obtain an oven-dried value; in the case of silages this was corrected at a later date for volatile losses. A second sample of 500 g was freeze dried, ground to pass through a 1 mm screen and used for chemical analysis. A third sample of 500 g of silage was taken and used for analysis requiring fresh material.

In addition, every time silages were removed from the clamp three 400 g samples were oven dried to obtain a dry weight which was later corrected for volatile losses.

C. In vivo digestibility

Apparent whole tract digestibility was measured in experiments 2, 3 and 4 by

collection of faeces over an 8-10 day collection period. The digestibility coefficient was calculated as the difference between the amount of feed consumed and the amount excreted in the faeces, expressed as a percentage of the amount of feed consumed. The amount of feed offered was recorded daily as was the amount of feed refused and the amount of faeces excreted.

1. Feed refusals

During the digestibility periods daily refusals of the roughage offered were collected 23.5 hours after the previous a.m. feed, and 15% was taken for a bulk sample. Three 400 g samples were taken and oven dried to obtain an oven-dried weight which was later corrected for volatile losses. Silage refusals were stored frozen at -4°C and hay refusals at room temperature until the end of the digestibility period when, after thawing and mixing a sub-sample of 500 g was freeze dried and ground to pass through a 1 mm screen for chemical analysis. Feed refusals were analysed for NDF, ADF, total-N, ash and organic matter.

2. Faeces

Total faeces excreted by the animals was collected over either an 8 or 10 day digestibility period depending on the experiment.

Faeces were collected from the steers by means of a piece of polythene tubing held up to the steers' anus by a body harness. This extended into a plastic bag residing in a bin at the rear of the animals' standing which was removed and changed daily.

Cows faeces were collected in a large three-sided bin placed at the rear of the cow standing which was emptied into a pre-weighed tub at regular intervals throughout the day using a dustpan. A piece of funnelled rubber was attached to the skin around the cow's vagina using evo-stick adhesive three days before the first collection of faeces was made. A flexible piece of washing machine tubing attached to this funnel allowed the urine to drain away from the faeces and prevent contamination.

Daily faecal output was recorded and a 5% sub-sample of mixed wet faeces taken, bulked and stored frozen until the collection period was complete. The bulk sample was then thawed and mixed and three 400 g samples were oven dried at 105°C for 48 hours to obtain a dry matter value. A 500 g sample was freeze dried and ground to pass through a 1 mm screen and analysed for organic matter.

D. Infusions

Infusion solutions were made up on the day that they were to be infused, the metabolite to be infused being thoroughly mixed with the appropriate amount of water (see individual experiment for volumes). They were infused into the dorsal rumen at the rate of 900 ml/hour (except experiment 1 where the rate was 1000 ml/hour) using a peristaltic pump (Watson-Marlow Ltd, England). The infusates entered the rumen through a piece of plastic tubing, 30 cm long, that had a small perforated bottle filled with polythene beads connected to the end in the rumen so that the infusate was dispensed into the rumen. All infusions were short-term and were set up so that the infusate first entered the rumen at the same time as the a.m. feed was offered.

E. Rumen fluid sampling

In all experiments rumen samples were taken on each day of infusion and in experiments 2 and 3 on one day prior to any infusion period. The samples were taken at regular times throughout the day, the exact times depending on the individual experiment.

Samples were collected through a piece of stiff plastic tubing 40 cm long which passed securely through a bung in the rumen cannula into the ventral rumen. The sampling tube was held in place by a weight attached to the bottom which also served to seal the tube. Small holes drilled in the bottom 20 centimeters of the sampling tube to allow for the collection of rumen fluid. These were prevented from becoming blocked by a piece of muslin secured tightly above and below them that served to strain the fluid upon collection.

Samples were collected using a 50 cm disposable syringe. The pH of the fluid was measured immediately upon removal from the rumen. In experiments 1 and 2 an aliquot was immediately analysed to determine osmolality using a freezing point depression osmometer (Knauer, Germany). In experiments 3 and 4 an aliquot was taken and stored frozen at -20°C and subsequently thawed, centrifuged at 3000rpm for 15 minutes and 50 ul analysed to determine osmolality using an Advanced Micro Osmometer (Model 3MO, Advanced Instruments, Inc, Masschusettes 02194).

The remaining rumen fluid was acidified to below pH 2 using 3-4 drops of concentrated H_2SO_4 to prevent loss of ammonia and then stored frozen before being analysed for ammonia, lactic acid and VFA concentrations. In addition samples taken in experiment 1 were analysed for their amine content.

F. Milk sampling

During experiment 3, milk samples were taken at three different times during the digestibility trial, a sample was taken at both the afternoon and the following mornings milking. It was then analysed for fat, lactose and protein content.

II. ANALYTICAL PROCEDURES

A. Rumen fluid analysis

Rumen samples were thawed at room temperature, thoroughly mixed and centrifuged at 3000 rpm for 15 minutes, the supernatant was decanted off and used for chemical analysis.

B. Ammonia-N

In experiments 1, 2 and 3 ammonia-N concentration in rumen fluid was determined by a colorimetric method using a flow injection analyser (Chemlab Instruments Ltd, Essex). The sample was made alkaline with dilute NaOH to release ammonia which then reacts with salicylate and dichlorosicyanurate; the later decomposes in the alkaline solution to release hypochlorite ions which bond the salicylate to form an indophenol, that can be measured at 650 nm. In experiment 4, 2.5 ml of rumen fluid was deproteinised using 2.5 ml 0.2 M HCL made up to 50 ml and then filtered. The diluted samples optical density was then measured at 600 nm by the method of Fawcett and Scott (1960) using a Cobas Mira auto-colourimeter.

C. Lactic acid

Rumen fluid samples were analysed for total lactic acid content using a spectrophotometric method. Both the D(+) and L(-) isomers of lactic acid are oxidised to pyruvate by NAD in the enzymatic reactions catalysed by D(+) and L(-) lactate dehydrogenases with the subsequent production of NADH, the production of which is measured at 340nm in this colorimetric reaction.

Lactic acid + NAD+ ----------> Pyruvate + NADH + H+

1. Reagents

Glycine buffer: 1.14g glycine + 0.6ml hydrazine hydrate in 29.4 ml distilled water

NAD reagent: 3.75 mg NAD (Sigma N8881)/ml buffer

Enzyme reagent: 10 ul of L(-) lactate dehydrogenase (Sigma L0672) and 18 mg D(+) lactate dehydrogenase per ml buffer.

2. Method

The samples were analysed on a 9 place discrete analyser (Labsysytems Oy, Analyser Model FP-90L, Finland).

10 ul of sample, standard and blank (distilled water) were pipetted (Labsystems Oy, Chemistry Sampler, Model FP-801, Finland) into a 9 place sample vessel to which 190 ul of buffer and 0.4 ml of NAD reagent were added mixed and incubated (Labsystems Oy, Incubator, model FP- 401, Finland) for 10 minutes at 37°C. The optical density was read at 340 nm and 0.1 ml of enzyme reagent added, the samples mixed and incubated for 30 minutes at 37°C, before the optical density was again read at 340 nm.

D. Volatile fatty acids

The previously centrifuged rumen samples were analysed for volatile fatty acid content using a gas chromatogram. The same column and packing material, and general conditions were used to analyse all samples. However, analysis for samples from experiments 1,2 and 3 were carried out at Hurley and for experiment 4 at Leeds. These specific operating conditions refer to the analysis at Leeds.

1. Operating conditions and equipment

Column : 1.5 m long spiral glass tube with 4 mm internal diameter.

Packing material : 5% FFAP on 80/100 Chromosorb W HP (Alltech UK).

Carrier gas : Nitrogen, flow rate 35 ml/min

Column temperature : 120°C

Injector temperature : 240°C

Detector temperature: 260°C

Column conditioning temperature : 200°C

2. Analysis of samples

5 ul of sample was loaded onto the chromatograph column using an automatic sample injector (Pye-Unicam, England) and the peak area of each acid in the sample recorded on an integrator. Every 12th sample a standard solution of VFA (10 ml acetic, 3 ml propionic, 3 ml iso-butyric, 2 ml n-butyric, and 1 ml of both iso-valeric and n-valeric acids in 1l of 0.4

M H_2SO_4) was run.

E. Amine determination

The content of amines in silages and the rumen fluid samples taken during experiment 1 were determined using a conventional ion exchange chromatograph (Biotronik LC 2000 Amino Acid Analyser, Frankfurt, Germany) by a method developed at Hurley. 10 g of wet rumen liquor was mixed with 30 ml of lithium citrate buffer (pH 2.2, 15.02 g trilithium citrate $4H_20$, 13.5 ml HCl (37%), 20 ml thiodiglycol, 1 ml phenol made up to 1 l with deionised double distilled water) and left to stand in a fridge for 12 hours. For silage 100 g of wet silage was taken and soaked in 100 ml of buffer overnight, the procedure for two types of samples was the same. The sample was filtered (Whatman 541 paper), made up to 50 ml with lithium citrate buffer and then deproteinised by the addition of 3 g of 5-sulphosalicylic acid. The mixture was left to stand at 4°C overnight before being filtered through a 0.45 µm membrane filter.

The prepared samples were then analysed on the ion exchange chromatograph through a 35 x 0.6 cm column under the following conditions.

1. Operating conditions

Resin type	Durrum DC6a (Li^+)
Resin bed height	18 cm
Buffer flow rate	35 ml/hour
Ninhydrin flow rate	20 ml/hour
Ninhydrin reaction time	4 min
Reaction temperature	100°C
Column temperature	50°C for 10 mins, 68°C to end of run.

Each sample took 310 minutes to analyse, and was brought off the column by this series of buffers:

	BUFFER					
	A	B	C	D	E	F
pH	5.20	6.60	8.70	10.5	12.3	*
Lithium hydroxide (g)	*	*	*	*	*	33.8
Trilithium citrate $4H_20$ (g)	33.8	33.8	33.8	33.8	33.8	*
Lithium chloride (g)	31.2	35.4	60.6	60.6	74.4	*
Final Volume (l)	2.0	2.0	2.0	2.0	2.0	2.0

F. Analysis of feeds, faeces and digesta

Fresh silage was analysed for toluene and alcohol-corrected dry matter, ammonia, pH and fermentation acids content. Freeze dried silage, hay and concentrate feeds were analysed for dry matter (DM), ash, organic matter (OM), NDF, ADF and total-N content. Rumen digesta was analysed for NDF, ADF and OM. Fishmeal samples were analysed for DM, OM, total-N and ether extract (EE). Faeces samples were analysed for DM and OM. The methods used for these analyses are those described in MAFF/ADAS Reference Book 427 unless stated otherwise.

1. Neutral detergent fibre (NDF)

NDF was determined by the method of Van Soest and Wine (1967) except that declain and sodium sulphite were omitted, sand was used as a filtering aid, and the samples were refluxed in a 200 ml tubes on a heating block digesta. The NDF content of the compound feeds of experiment 3 were analysed in the same way as for the forages except that the enzyme alpha-amylase was added during refluxing to solubilise any starches present and to allow for easy filtration (Robertson and Van Soest, 1981).

2. Acid detergent fibre (ADF)

ADF determinations were made by the same methods of Van Soest, (1963 and 1973) except that the samples were refluxed in the same way as for NDF (above).

3. Starch

In experiment 3 the compound feeds were analysed using the method of Wainman et

al (1981). The sample is first treated with hot ethanol to remove glucose and other sugars before being heated to gel the starch. The enzyme amyloglucosidase is then used to degrade the starch to glucose which after suitable dilution is measured colorimetrically using the specific enzyme glucose oxidase. The amount of starch present is calculated as follows:
% Starch = % Glucose x 0.9.

4. Toluene dry matter

Silages were distilled in toluene to determine a correction factor that was then corrected for alcohols and used to amend the 'on farm' oven dry weights of the silage samples. Portions of the volatile residue were subsequently analysed for the titrimetric estimation of dissolved acids (Dewar and McDonald, 1961) and the alcohol content.

5. Alcohols

Ethanol and propanol present in silage extract were quantified after gas chromatographic separation on a packed column of SP1200 on Chromsorb W-AW (Jouany, 1982). Silage DM was corrected for the presence of these.

6. Milk analysis

Fresh milk samples were analysed for fat, protein and lactose using a 203B Milko-Scan infrared milk analyser (Foss Electric (UK) Ltd.)

SECTION C

EXPERIMENTAL

Experiment 1

I. The effect of short-term intra-ruminal infusions of silage decarboxylation end-products on the voluntary intake of silage-fed steers.

A. Introduction

When referring to silages the word amine is commonly used to describe the end-products of amino acid decarboxylation even though they include some compounds that are not true amines such as gamma amino-n-butyric acid (Gaba). These decarboxylation products have been found both in silages deemed to be well preserved and in those that have undergone a clostridial fermentation and their production pathways are open to debate. Two such decarboxylation products are Gaba and putrescine. Production of Gaba is associated with spoilage organisms whilst putrescine has been associated with both Clostridia spp and lactic acid bacteria. Levels of Gaba and putrescine within silages range from 30 mg and 2 mg/kg DM (MacPherson and Violante, 1966) up to levels of 12900mg and 2750 mg/kg DM (Hughes, 1970) respectively. Four silages made at Hurley in the summer of 1988 and fed over the following winter were found to contain varying quantities of amines (Table 3). Amines are of interest when studying the voluntary intake of silage since Gaba has been found to be a neurotransmitter involved in intake regulation. It is believed that Gaba plays a dual role in intake regulation by both stimulating the ventromedial satiety centre and inhibiting the lateral hypothalamic hunger centre of the brain (Morley, 1980).

Barry et al (1978) postulated that it was probably products of decarboxylation rather than products of deamination in the silo that are associated with intake limitation, although intra-ruminal infusions of amines have had varying effects on voluntary food intake. Intra-ruminal infusions of Gaba have been shown to decrease the intake of sheep fed alfalfa pellets (Buchanan-Smith 1982), whilst in other trials tyramine, tryptamine (Neumark et al, 1964) and histamine (McDonald et al, 1963; Koers et al, 1976) had no effect on VFI.

B. Materials and methods

1. Animals

Four beef steers which had previously been cannulated in the rumen (80 mm internal diameter, University of Sydney, Australia) had an average liveweight over the experimental period of 444 kg $\pm$ 21 kg. The steers were kept in individual modified cow standings during the measurement periods.

2. Feeds and feeding

Silage, cut on the 24 May 1988 from a sward of perennial ryegrass (Lolium perenne, cv Francis), wilted for 24 hours before being ensiled with 4.75 l/tonne ADD-F (BP Chemicals, UK, Ltd.), was offered as the sole feed. Fresh feed was offered ad libitum (intake + 15%) at 08:30 h and at 15:00 h and was withdrawn from the animals at 08:00 h, 23.5 hours after first having been offered. One hundred grams of mineral supplement (Dairy Super, FSL Bell Ltd., Corsham, Witshire.) was added to the forage at feeding.

Fresh silage was taken from the clamp every second day and if not offered immediately was stored at -4°C for 12 hours and then thawed for 12 hours before feeding.

3. Experimental design.

The four steers were used in a 4x4 Latin Square design consisting of three short-term treatment infusions, Gaba, putrescine and Gaba and putrescine in combination and a control infusion of water. The steers were adapted to the silage diet over a period of 12 days. The infusions were carried out over days 13 - 25 of the trial with each infusion day being followed by a two day recovery period.

4. Infusions

The infusions commenced at 08:30 h, when feed was first offered, and proceeded for one hour in which time 1 l of infusate was administered. The treatment infusates contained either 6 g/kgDMI (equivalent to 0.46 g/kg$LW^{0.75}$) putrescine (P) (Aldrich D1,320-8), 24 g/kgDMI (equivalent to 1.89 g/kg$LW^{0.75}$) Gaba (G) (Aldrich A4,44-1), a combination of the two (G/P) based on the average intake over the previous three days to the first infusion. A control of 1 l of water comprised the fourth treatment. These levels of amines are higher than 'normal' values usually found in grass silages.

5. Measurements

Meal patterns and the rate of silage intake were recorded on each day of infusion. Rumen fluid samples were taken at 08:15, 08:25, 08:45, 09:00, 09:15, 09:30, 09:45, 10:00, 10:30, 11:30, 12:30, 13:30, 14:30 and 15:30h on days of infusion.

C. Results

The levels of amines detected in various silages made at Hurley are summarised in Tables 3.

Amines were detected in all of the silages that were analysed, although the levels present within the silages varied considerably. However, Gaba was the predominant one in all but one of the silages and tryptamine was not detected at all. The total amine content of the silage used in this trial was the lowest of all those analysed and was generally well preserved (Table 4).

Table 3

The content of amines (mg/kgDM) in various silages made at Hurley.

	SILAGE.			
	Amine content (mg/kgDM)			
	1	2	3	4
Gaba	191.9	130.5	>200.0	154.3
Histamine	13.8	122.0	142.7	163.5
Putrescine	31.9	18.2	71.4	26.4
Cadaverine	21.7	35.8	147.8	41.4
Tyramine	36.8	81.0	154.5	107.3
Tryptamine	NOT DETECTED			

Silage 1 : The silage used in this trial

Silage 2 : The silage used in experiment 2

Silage 3 : A high digestibility 'bad' silage (Baker et al, 1991)

Silage 4 : Unknown source, made at Hurley in 1988

Table 4

The chemical composition of the silage.

pH		3.80
DM	(g/kg)	266.20
Total-N	(g/kgDM)	11.16
Insol-N	"	6.84
Ammonia-N	"	1.30
NDF	"	439.70
ADF	"	288.00
Lactic acid	"	76.61
Acetic acid	"	22.68
Propionic acid	"	0.00
Butyric acid	"	0.35

Problems with the feed monitoring equipment meant that a full analysis of the daily pattern of voluntary intake was not possible such as the daily number of meals or the size and length of the first meal. Table 5 summarises the intake of the steers at various time intervals past the a.m. feed expressed in terms of gDM/KgLW during the infusion period, these are illustrated in Figure 1.

Intake (gDM/kgLW) over the 24 hours during and after the infusion was unaffected by infusion of either Gaba or putrescine when infused alone in comparison to the control infusion of water, whilst a combination of the two depressed total daily intake by 3.7 gDM/kgLW although this reduction was non significant ($P > 0.05$).

Table 5

The effect of intra-ruminal infusions of decarboxylation end-products on the voluntary silage intake of steers at various times past initial feeding.

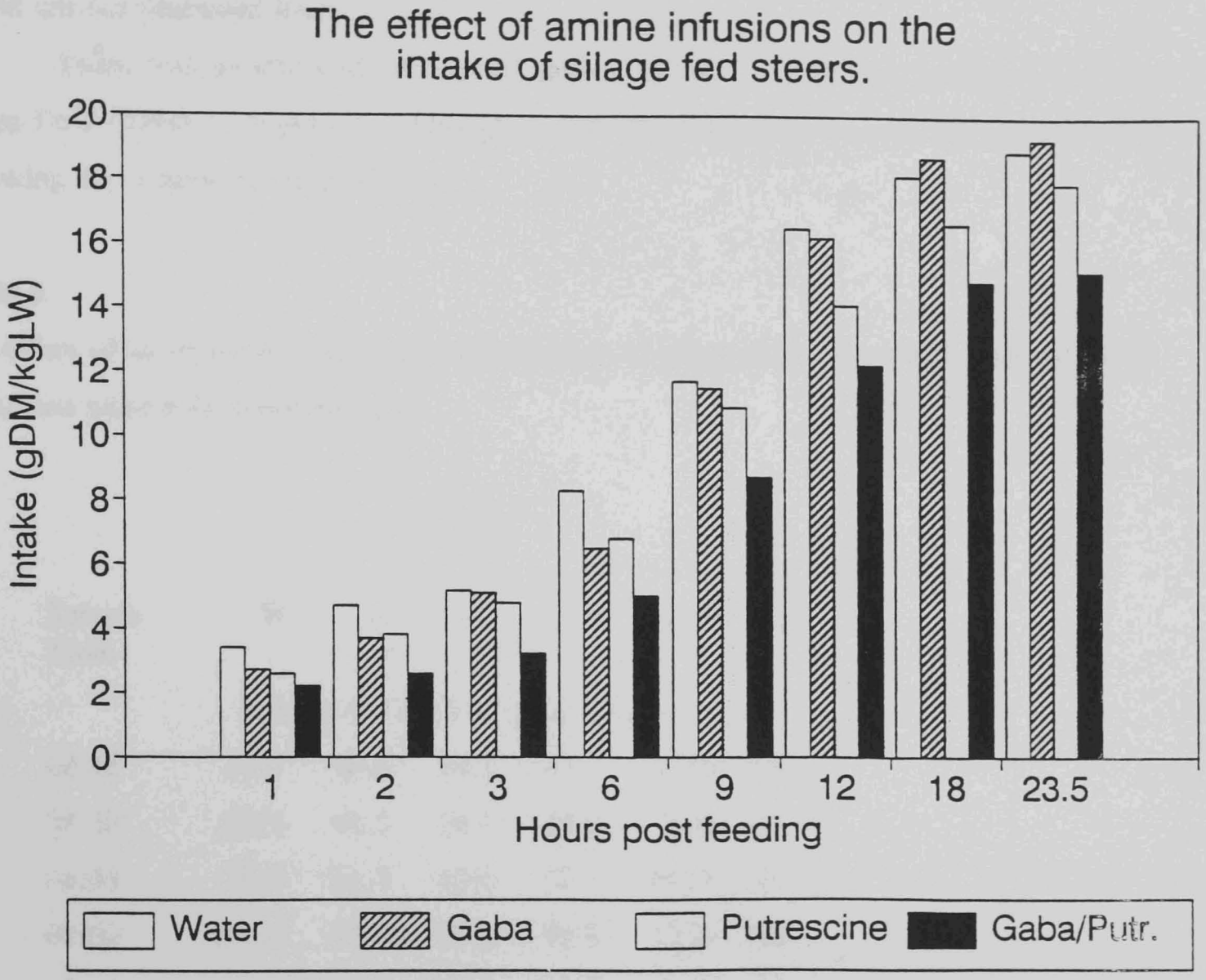
Time post feeding	Infusion W	G	P	G/P	S.E.M.	Sig
	I N T A K E (gDM/kgLW)					
1	3.4	2.7	2.6	2.2	0.39	NS
2	4.7	3.7[a]	3.8	2.6[c]	0.30	0.022
3	5.2	5.1	4.8	3.2	0.43	0.052
6	8.3	6.5	6.8	5.0	0.72	NS
9	11.7	11.5	10.9	8.7	0.67	0.072
12	16.5	16.2	14.1	12.2	1.12	NS
18	18.1	18.7	16.6	14.8	1.22	NS
23.5	18.9	19.3	17.9	15.1	1.26	NS

Across rows superscripts indicate a significant difference from the control (a=$P<0.05$ and c=$P<0.001$)

At the end of the infusion period, one hour after feed was first offered there was no significant reduction in intake caused by any of the treatments. However, two hours after feeding Gaba reduced silage intake by 1.00 gDM/kgLW ($P<0.05$) in comparison to the control whilst the combination of Gaba/Putr. depressed intake by 2.1 gDM/kgLW ($P<0.001$), and neither of these were significantly different from the intake of animals given putrescine alone. Three hours after feeding there was no significant difference in intake between the treatments and the control, although the intake of silage with the combination of decarboxylation end-products was depressed by 2.18 gDM/kgLW.

The intake of the steers given the combination treatment remained lower than that of the control group, albeit non significantly ($P>0.05$), throughout the remainder of the day, whilst the intakes following the other two treatments were similar to that of the control.

Figure 1


The effect of amine infusions on the intake of silage fed steers.
Intake (gDM/kgLW)
0
2
4
6
8
10
12
14
16
18
20
1
2
3
6
9
12
18
23.5
Hours post feeding
Water
Gaba
Putrescine
Gaba/Putr.

No amines were detected in the rumen fluid samples apart from Gaba and putrescine during their infusion. The maximum concentration of Gaba detected in the rumen fluid following intra-ruminal infusion was 250 nanomoles/ml, and that of putrescine 170 nanomoles/ml. As amines were not commonly detected in the samples, and the length of time each assay took to complete (5 hours) not all rumen fluid samples were analysed, and the results are not presented here.

There was no effect of infusion of amines on the concentration of ammonia in the rumen fluid (Table 6, Figure 2), although a more pronounced peak was seen post feeding following the control infusion of water.

Table 6

The effect of short-term intra-ruminal infusions of silage decarboxylation end-products on rumen ammonia concentration.

	Infusion					
Sample Time	W	G	P	G/P	S.E.M.	Sig
	A M M O N I A (mmol/ml)					
08:15	60.1	46.5	34.1	44.7	10.03	NS
08:30	53.4	44.5	36.1	34.9	7.86	NS
08:45	76.9	61.8	52.0	72.7	80.74	NS
09:00	111.6	91.7	75.8	98.9	12.41	NS
09:15	142.5	114.7	101.5	112.5	16.96	NS
09:30	158.5	130.4	116.5	126.6	18.07	NS
09:45	115.6	130.5	131.8	124.3	19.36	NS
10:00	171.7	127.5	128.7	125.0	18.40	NS
10.30	174.4	115.9	128.9	118.0	16.68	NS
11:30	118.8	136.1	122.7	122.5	16.64	NS
12:30	19.3	125.2	96.5	129.2	9.49	NS
13:30	90.8	95.3	83.4	109.9	8.57	NS
14:30	86.1	82.9	96.8	123.5	11.03	NS
15:30	89.8	113.6	95.0	125.8	11.17	NS

The pH of rumen fluid was also unaffected by any of the treatment infusions (Table 7, Figure 3), although a slight non-significant rise of 0.25 pH units was seen between 1 and 2 hours post feeding on the combination treatment.

Table 7

The effect of intra-ruminal infusions of decarboxylation end-products on the pH of rumen fluid of silage fed steers.

	Infusion					
Sample	W	G	P	G/P	S.E.M.	sig
	R U M E N pH					
08:15	6.86	6.86	6.78	6.89	0.109	NS
08:30	6.91	6.85	6.78	6.88	0.102	NS
08:45	6.87	6.92	6.88	6.93	0.071	NS
09:00	6.81	6.81	6.86	6.83	0.083	NS
09:15	6.78	6.80	6.95	6.92	0.083	NS
09:30	6.48	6.80	6.89	7.06	0.087	NS
09:45	6.74	6.78	6.85	7.13	0.159	NS
10:00	6.71	6.81	7.01	7.13	0.181	NS
10:30	6.63	6.75	6.85	7.04	0.098	NS
11:30	6.59	6.64	6.68	6.73	0.040	NS
12:30	6.63	6.64	6.68	6.64	0.040	NS
13:30	6.54	6.56	6.64	6.62	0.029	NS
14:30	6.57	6.54	6.66	6.61	0.044	NS
15:30	6.67	6.45	6.67	6.62	0.054	NS

Figure 2

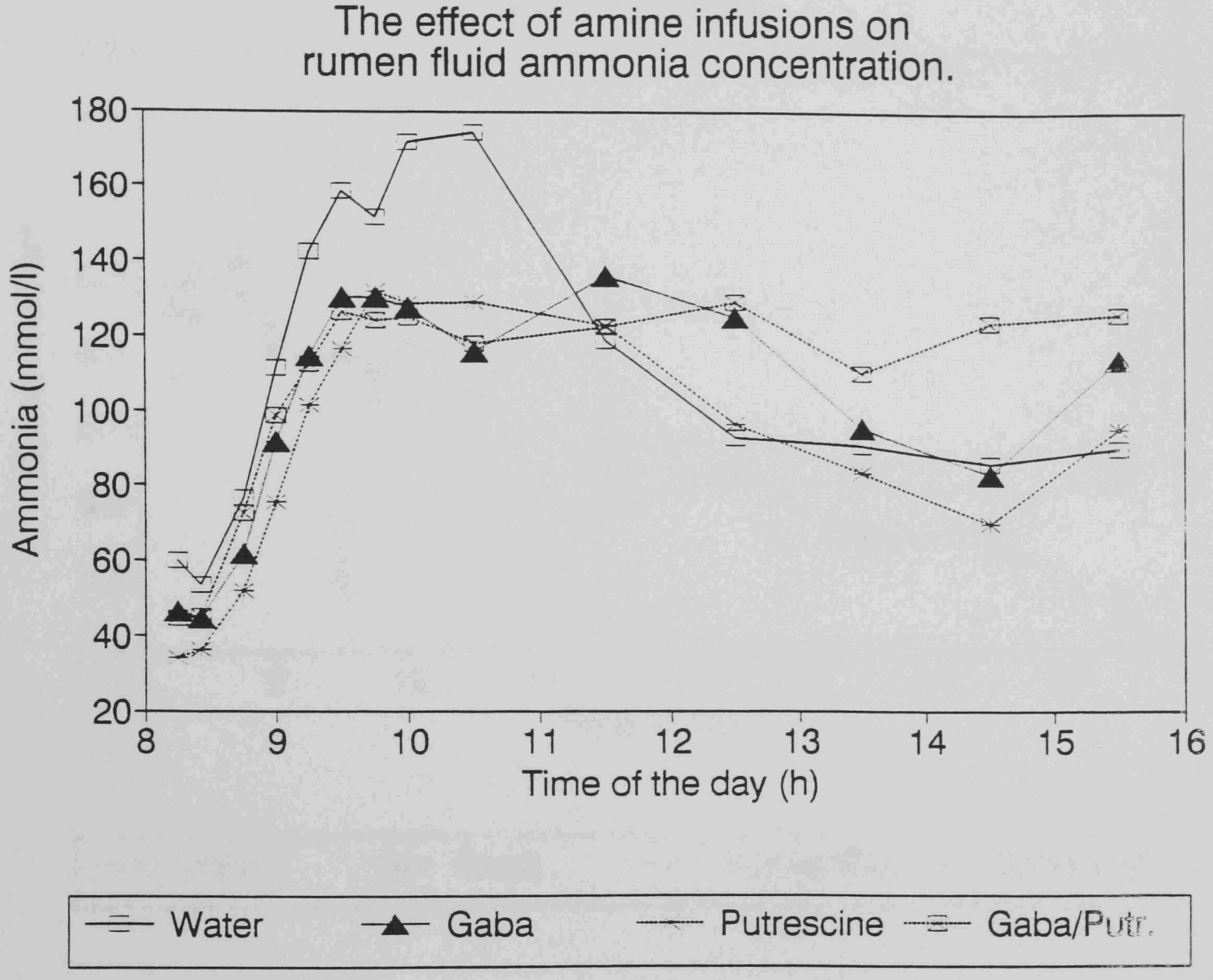
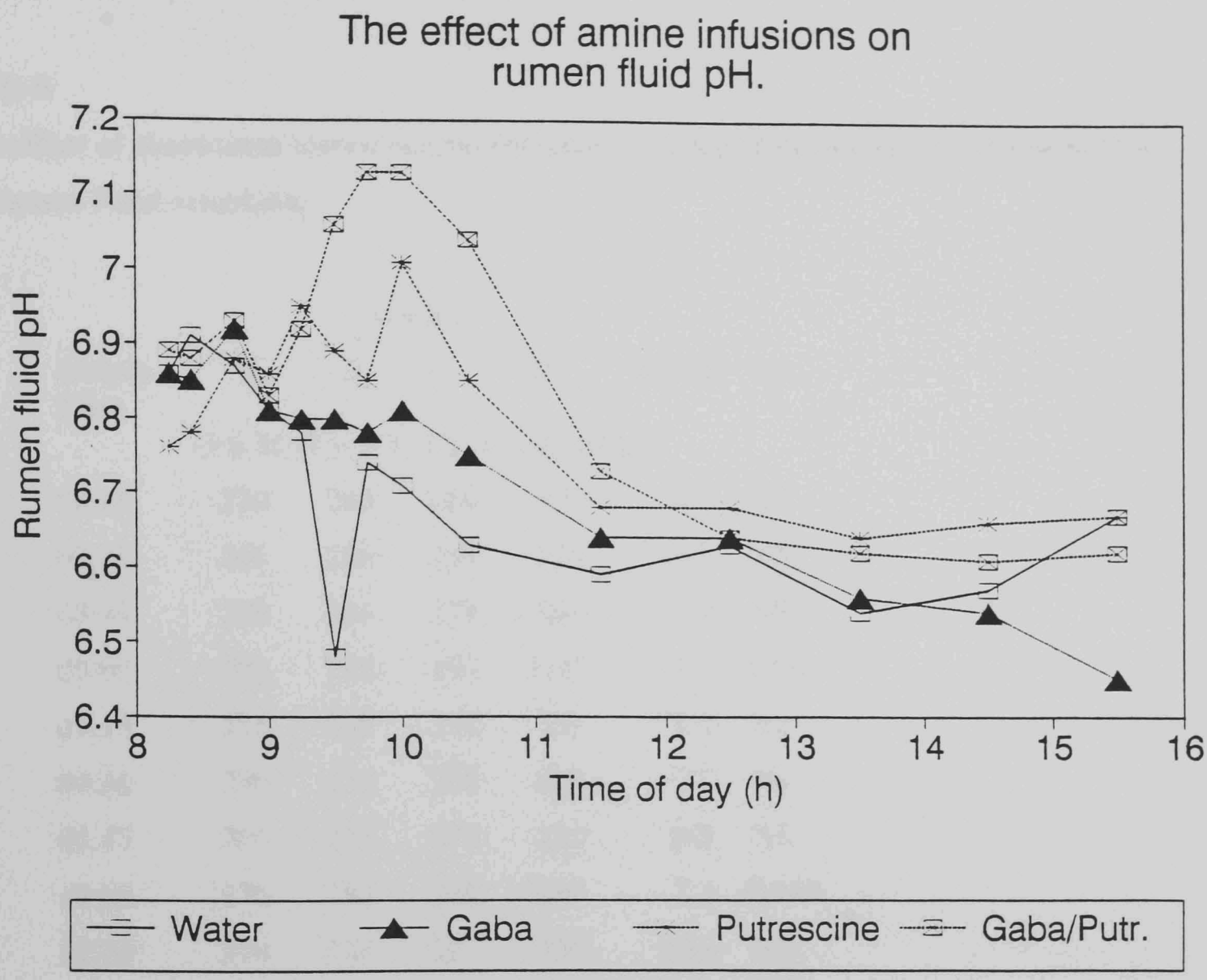

The effect of amine infusions on
rumen fluid ammonia concentration.
Ammonia (mmol/l)
180
160
140
120
100
80
60
40
20
8
9
10
11
12
13
14
15
16
Time of the day (h)
Water
Gaba
Putrescine
Gaba/Putr.

Figure 3


The effect of amine infusions on rumen fluid pH.
Rumen fluid pH
7.2
7.1
7
6.9
6.8
6.7
6.6
6.5
6.4
8
9
10
11
12
13
14
15
16
Time of day (h)
Water
Gaba
Putrescine
Gaba/Putr.

Rumen fluid osmolality was not affected by intra-ruminal infusions of putrescine or Gaba alone, the osmolalities of the rumen fluid following a similar pattern to the control (Table 8, Figure 4), peaking at approximately 300 mOsm one hour after feeding. The combination infusion produced a significantly higher ($P<0.001$) rumen fluid osmolality of 314 mOsm 30 minutes after feeding. This gradually declined to similar levels to the control and other treatments three hours post feeding.

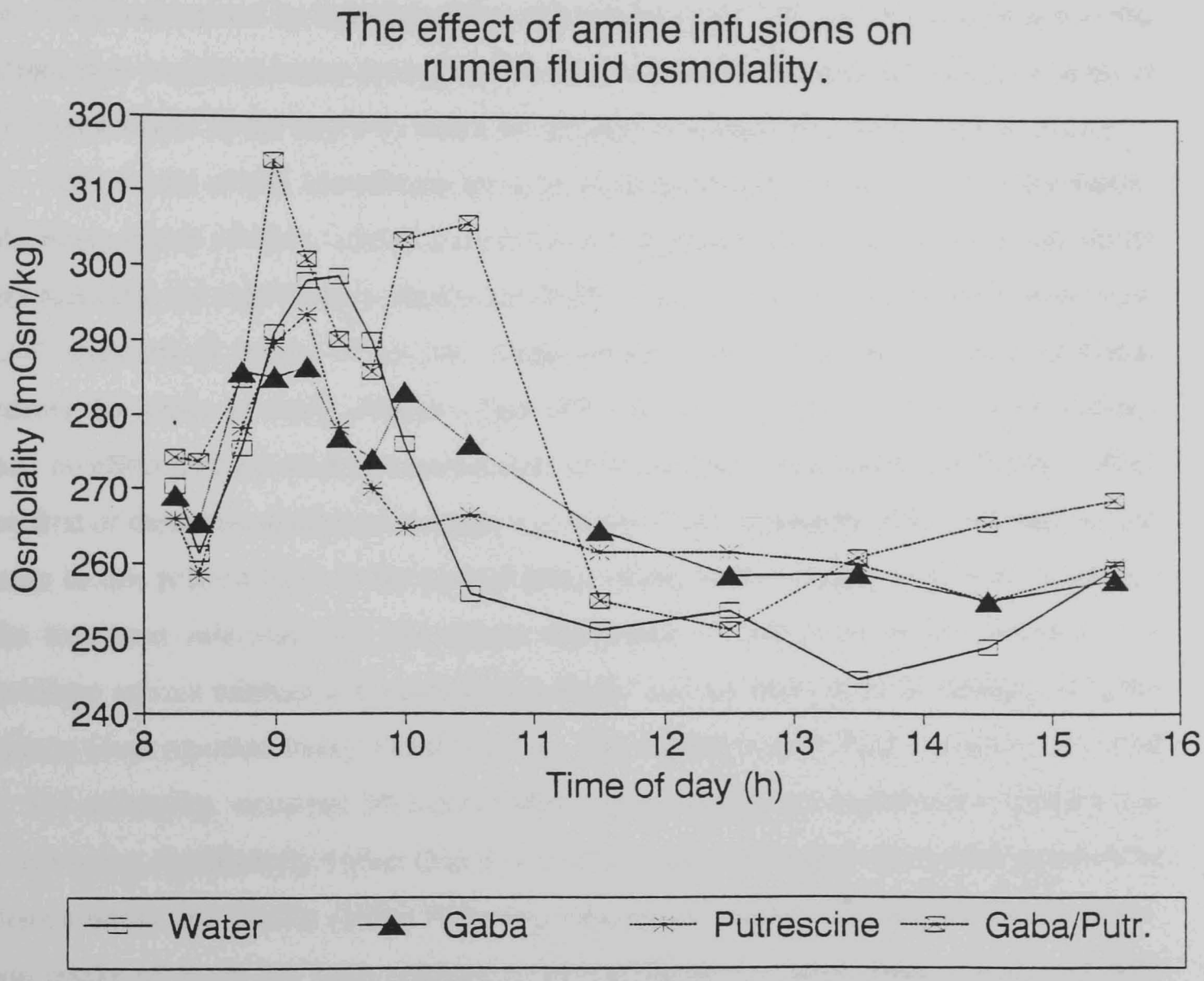
Table 8

The effect of short-term intra-ruminal infusions of silage decarboxylation end-products on rumen fluid osmolality.

	Infusion					
Sample Time	W	G	P	G/P	S.E.M.	sig
	O S M O L A L I T Y (mOsm/kg)					
08:15	270	269	266	274	4.4	NS
08:30	261	266	259	274	4.7	NS
08:45	276	286	279	295	9.3	NS
09:00	291	295	290	314[b]	5.1	0.046
09:15	298	287	294	302	8.3	NS
09:30	299	277	278	290	7.7	NS
09:45	290	274	270	286	8.8	NS
10:00	276	283	265	304[a]	7.2	0.044
10:30	256	276	267	306	14.4	NS
11:30	251	265	262	255	14.6	NS
12:30	254	259	262	251	10.5	NS
13:30	245	259	260	261	6.3	NS
14:30	249	255	255	266	4.3	NS
15:30	260	258	260	269	6.1	NS

Across rows superscripts indicate a significant difference from the control (a=$P<0.05$ and b=$P<0.01$)

Figure 4


The effect of amine infusions on rumen fluid osmolality.
Osmolality (mOsm/kg)
320
310
300
290
280
270
260
250
240
8
9
10
11
12
13
14
15
16
Time of day (h)
Water
Gaba
Putrescine
Gaba/Putr.

D. Discussion

Intra-ruminal infusions of Gaba and putrescine had no significant effect on the intake of silage-fed steers over the 24 hours during and following infusions. Depressions in intake of 21.3% and 19.2% below the level of the control group, were seen at two hours post feeding following infusions of Gaba and putrescine alone. When the amines were administered in combination, intake was depressed by 44.6% in relation to the control, close to the sum of the reductions caused by infusion of the individual amines. The cummulative intake of this treatment then remained lower throughout the rest of the day although this was as a result of the reduced intake in the first two hours as opposed to a limitation effect later in the day.

The results of this experiment are in general agreement with the work of Buchanan-Smith. Forty grams of Gaba infused intra-ruminally depressed the intake of sheep fed alfalfa pellets although not significantly (Buchanan-Smith, 1982). In a later study short-term intra-ruminal infusions (4 hours) of lucerne silage extract, with an addition of 40 g of Gaba, depressed the intake of sheep offered a high DM lucerne silage up to 4 hours post feeding, but had no effect on total intake measured over 20 hours (Buchanan-Smith and Phillip, 1986). In the first of these two studies no account was taken of the osmolality of the infusate, as was the case in this present trial. In the second study saline, with a similar or greater osmolality to the treatment infusates, did not reduce the intake of the sheep to the extent of the Gaba/silage extract mixture and ruminal osmolality was not thought to be limiting VFI, the maximum level reported being 375 mOsm/kg. The highest rumen fluid osmolality reported here, 314 mOsm/kg, occurred 30 minutes after feeding during the combination infusion and although being significantly higher than that of the control was below the values reported by Buchanan-Smith and Phillip (1986) following infusions of amines and the feeding of silage. Indeed intake of sheep has been reported to be unaffected by osmolalities within the rumen below 400 mOsm/kg (Bergen, 1972).

The mechanism by which silage amines limit feed intake is not clear. Gaba is of particular interest as regards the control of silage intake since it has been found to be a neurotransmitter involved in the control of feed intake in the hypothalamus. It is believed to have a dual role, having an inhibitory effect on the lateral hypothalamus, the hunger centre, and increasing appetite by inhibiting the ventro medial hypothalamus, the satiety centre (Morley, 1980).

The exceptionally low recovery rate of amines in the rumen was unexpected, since in the case of Gaba nearly 180 g was infused into each animal, and no amines besides those

that were infused were detected. This would imply that either Gaba and putrescine have exceptionally short half lives in the rumen due to metabolism by microorganisms, rapid absorption across the rumen wall or the amines were lost during storage and preparation of the samples.

It is possible that amines are degraded further by rumen microorganisms, although in this trial there was no significant increase in the ruminal ammonia concentration following infusion of the amines. Indeed the largest peak in rumen ammonia concentration was seen 2 hours after the onset of feeding following the control infusion of water. However, both of the infusates have low nitrogen contents (Gaba 13 % and putrescine 26 %) and it would be difficult to conclude from these results that these amines are not broken down further in the rumen to release ammonia.

The pH of the rumen fluid was unaffected by infusions of amines. At all times it remained above 6.5 and above the value of 5.0 below which feed intake is severely reduced (Baile and Forbes, 1974).

There are no data apparently available on the effect of intra-ruminal infusions of putrescine alone on voluntary intake. When infused in combination with Gaba, alpha amino butyric acid (Aaba), cadaverine, histamine and tyramine the intake of lucerne silage by sheep was depressed between 4 and 8 hours post feeding (Phillip et al, 1981). Other amines have been found to have varying effects on VFI. Histamine reduced the intake of hay fed sheep when 214 mg was given intra-ruminally in the presence of formic acid, the predominant acid in many silage additives such as ADD-F used in this trial (Neumark, 1967). Histamine has been shown to be potentially toxic to animals following intravenous injection causing complete rumen stasis and preventing eructation (Dain et al, 1965).

The amines, methylamine, ethylamine and dimethylamine although not commonly reported as being found in silages have been found to stimulate gastrin secretion in the stomach of rats (Lichtenberger et al, 1892), and gastrin has been reported to limit the intake of sheep (Grovum, 1982).

The amounts of amines found in silages made at Hurley in 1988 were within the wide range detected reported by other workers. Gaba has been reported as occurring at levels up to 16 g/KgDM in lucerne silages (Oshima et al, 1979), well in excess of the highest value reported here <200 mg/KgDM. The levels of putrescine and cadaverine found in this study range from 18.2 and 21.7 mg/Kg DM in a well preserved silage up to 71.4 to 147.8 mg/Kg respectively in a notoriously poorly preserved silage (silage number 3). These compare to

values of 49 to 86 mg/KgDM of putrescine and 27 to 180 mg/KgDM of cadaverine found by MacPherson and Violante (1966). Putrescine has also been recorded in grass silage at extreme levels of up 2750 mg/KgDM (Hughes, 1970). This experiment has shown that amines can still occur at similar levels to those detected in silages in the 1960's. This is despite the advances made in the ensiling process over the last twenty years. It would also confirm the widely-held belief that amines present within silage are involved, albeit partially, in the limitation of silage intake.

It is acknowledged that the levels of amines used in this trial to reduce intake are greatly in excess of levels normally found in grass silages. However, it is unlikely that an animal fed silage would encounter these two alone, as at least four other amines were detected in grass silages. At two hours post feeding the effect on intake of intra-ruminally infusing the two amines together was more than twice the effect when they were administered alone. It may therefore be postulated that if a cocktail of amines were to be infused which comprised all of those compounds that are commonly found in silages a much larger reduction in intake would occur. Indeed the reasons for using putrescine in preference to cadaverine, tyramine, histamine or other amines were purely financial.

II. A study of a badly fermented silage

Following completion of this amine trial, the silage referred to as number 3, in Table 3, the summary of silage amines detected at Hurley, was fed to two of the steers. This high digestibility 'bad' (HDB) silage was made from a crop of primary growth perennial ryegrass (Lolium perrenne cvs Melle and Endura) cut on May 19 and 20 1987. It was purposely ensiled using extremely poor ensiling techniques, no additive was used , the clamp was left open overnight between the two days of filling, and very little consolidation of the material in the silo occurred (Baker et al, 1991). Using the same sward of grass as for this poor quality silage, a high digestibility 'good' silage (HDG) was made in an identical silo. Alternate loads of harvested material was put into either clamp and good silage practices were applied to the HDG silage, including application of formic acid at 3.2 l/t.

The analysis of the silages upon opening the silos showed the main difference in fermentation parameters to be the higher content of acids in the HDB silage. Despite these relatively similar fermentation characteristics, dairy cattle in weeks 8 - 22 of lactation, fed no concentrates, ate 44% less of the HDB compared to the HDG. Approximately half a tonne of HDB silage remained after being used in the above trial, which was subsequently cold

stored in 25 kg sacks for 12 months. The silage was thawed over 72-96 hours and fed to two of the beef steers over a 9 day period.

The analysis of the silage before and after cold storage is shown in Table 9. Cold storage of the silage had resulted in a decrease in the amount of acetic acid and ammonia-N in the silage, and a slight increase in the amount of lactic acid, although only three samples were analysed post cold storage and the number used originally is not known.

Table 9

The effect of cold storage for 12 months on the composition of silage.

		Original	After storage
DM	(g/kg)	226.0	237.0
pH		3.9	3.8
total-N	(g/kgDM)	31.4	30.16
NH_3N	(g/kg total-N)	117.9	90.3
lactic acid	(g/kgDM)	111.8	117.2
acetic acid	"	40.4	24.9
propionic acid	"	3.0	1.4
butyric acid	"	2.3	1.6

Due to the shortage of the amount of silage available the steers were abruptly changed onto the HDB silage. Their intake immediately fell and remained low for the remainder of the week (Table 10).

Table 10

The average daily intake (gDM/kgLW) of silage HDB by two steers over a 9 day period

	DAY								
	1	2	3	4	5	6	7	8	9
Intake (gDM/kgLW)	14.8	10.2	13.1	13.1	15.5	12.6	12.7	14.9	12.6

The average intake measured over this period was 13.3 gDM/kgLW, substantially lower than the intakes of the same steers recorded over the digestibility periods of experiment

2 (18.5 gDM/kgLW), when fed a reasonably well preserved silage. Whilst these intakes are not statistically comparable they illustrate the poor ingestibility of this HDB silage.

Buchanan-Smith (1990) found that the amines cadaverine, putrescine and Gaba reduced the intake of sham-fed sheep and implicated them as factors affecting palatability. The levels of all the amines detected here, including these three, were found to be high in comparison to those detected in other silages, which might partially explain the poor intakes recorded.

Experiment 2

The effect of short-term intra-ruminal infusions of urea or lactic acid on the voluntary intake of silage- and hay-fed steers

A. Introduction

Lactic acid is the predominant organic acid found in well preserved silages, occurring at levels of up to 180 g/kgDM (Chamberlain et al, 1983). Whilst lactic acid production within the silo is desirable to reduce the pH and to inhibit growth of putrefactive microorganisms, it has been shown that lactic acid can reduce the intake of both hay- (Montgomery et al, 1963) and silage-fed (Thomas et al, 1980) cattle.

Ammonia present within silage is used as a measure of silage quality with high ammonia levels indicative of a poor fermentation. Correlations of silage intake with concentrations of end-products of fermentation have shown that silages with higher ammonia levels generally have poor intakes. In addition ammonium salts and urea have been shown to reduce the intake of goats fed a high concentrate ration (Conrad et al, 1977), but whether reductions in intake are due to the osmolality of the infusate or to ammonium ions per se is unclear.

The objective of this trial was to investigate whether intra-ruminal infusions of lactic acid or urea (used to mimic the effect of silage ammonia) depressed the voluntary intake of steers adapted to a diet with either high (silage) or low (hay) ammonia and lactic acid concentrations, and how any depressions might be mediated.

B. Materials and Methods

1. Animals

The same steers used in experiment 1 were used in this trial. Their average liveweight at the start of the experiment was 390 kg. They were kept in the individual modified cow standings (see experiment 1) during the measurement periods, but prior to the experimental periods and during the diet changeover period they were housed in individual resting pens.

2. Feeds and feeding

Either hay or silage was offered ad libitum (intake+15%) as the sole feed throughout the experiment. Fresh food was offered twice daily at 09:30h and 16:00h and was withdrawn from the animals at 09:00, 23.5 hours after first having been offered. At all times water, and

minerals (Baby Red Rockies, Tithebarn.) were available from automatic drinking bowls and licking blocks respectively. In addition 100 g of mineral supplement (Dairy Super, FSL Bell Ltd., Corsham, Wiltshire.) was added to the forage at feeding.

The silage came from a sward of late maturing perennial ryegrass (Lolium perenne, cv Endura) cut on June 13 and 14 1988 using a precision chop forage harvester and ensiled in a clamp silo after treatment with formic acid (Add-F at 3.84 l/tonne). Fresh silage was taken from the clamp every second day and if not offered immediately was stored at -4°C for 24 hours and then thawed for 24 hours before being offered. The hay was cut on the 16 June 1988 from a permanent pasture and baled on 20 June. The hay was chopped into 3-5 cm lengths using a hay chopper and stored in hessian sacks prior to the experiment.

3. Experimental design

The four steers were used in a 2x2 crossover designed experiment; in each dietary period two steers were fed hay and two silage. Two 4x4 Latin squares of intra-ruminal infusions, consisting of three amounts of either lactic acid or urea and a control infusion of water were completed in each dietary period.

The steers were randomly allocated to either the hay or the silage diet and adapted to the diets over the first 14 days, with measurements being made on days 14-39 of each period. On days 40, 41 and 42 the animals' diets were gradually changed over and 11 days later the experimental procedure was repeated.

4. Measurements

In vivo whole tract digestibility was recorded over days 14-22 of the trial. The rate and pattern of roughage intake was recorded on three days during this period and also on each day of infusion. Rumen samples were taken from the steers on day 23 of the trial prior to any infusion at 09:15, 09:25, 09:45, 10:00, 10:15, 10:30, 10:45, 11:00, 11:30, 12:30, 13:30, 14:30 15:30, 16:30, 18:30, 20:30, 22:30 and 06:30h and at the same times on infusion days up to 16:30h.

5. Infusions

In each dietary period the latin square of urea infusions preceded infusions of lactic acid. Infusions commenced at 09:30 and lasted for one, two or three hours and proceeded at a rate of 900 ml/hour. The same amount of urea (Biuron, 46% N; Chemie Linz, Germany)

or lactic acid (Analar, variable mix of D(+) and L(-) isomers; BDH Ltd, Poole, England) was administered during each infusion, 0.77 g urea/kgLW$^{0.75}$ (which was calculated as being equivalent to 10.9 g urea/kgDMI, DMI being calculated over the digestibility period) or 2.26 g lactic acid/kgLW$^{0.75}$ (equivalent to 32 g lactic acid/kgDMI) and hence it was length of infusion that varied and not the amount of metabolite given. These values are based on the amounts of urea and lactate found to depress intake of a concentrate ration when infused into the rumen of goats (Conrad et al, 1977).

6. Statistical analysis

The data obtained during the digestibility study comparing the difference between parameters measured on the hay and silage diets were analysed by Anova using Minitab, Statistical Software, release 7 and Genstat 5, release 1.3. The data obtained during the infusion periods were analysed separately for the effect of urea and lactic acid on various parameters by Anova using Genstat 5, release 1.3. The Genstat programme enabled the effect of the infusions on silage and hay intake to be considered both separately and together.

C. Results

1. Pre-infusion

The analysis of the feeds is shown in Table 11. The silage was reasonably well preserved with a pH of 3.82 and a predominantly lactic acid fermentation, although the ammonia-N accounted for 134 g/kg of the total-N, which is towards the upper end of the normal range found in silages. Both the hay and the silage had low in vivo D-values of 0.58 and 0.62 respectively, measured over a seven day faecal collection period. The slightly lower D-value of the hay is reflected in the higher content of both NDF and ADF. These differences and those between the nitrogen content of the two feeds, hay (17.8 gDM/kgLW) and silage (19.1 gDM/kgLW), illustrate the fact that the two feeds were from different sources.

Table 11

The chemical composition of the silage and hay.

		Silage	Hay
DM	(g/kg)	246.5	815.4
pH		3.82	
organic matter	(g/kgDM)	918.9	930.2
total-N	"	19.07	17.83
insn-N	"	7.89	12.13
NH_3N	"	2.56	
NDF	"	423.6	594.4
ADF	"	259.7	365.3
lactic acid	"	86.68	
acetic acid	"	22.84	
propionic acid	"	0.72	
i-butyric acid	"	0.31	
n-butyric acid	"	0.24	
D-value*	"	0.617	0.580

- These results are the mean composition of six bulk samples of each feed.

- The amine content of the silage is reported in Table 3.

* Calculated over a seven day faecal collection for all animals, except for one animal on the hay diet (5 day collection) and one animal on the silage diet (6 day collection).

The total daily intake of the feeds recorded over the seven day digestibility period is shown in Table 12. The intake of silage,18.5 gDM/kgLW,was significantly higher ($P < 0.001$) than the intake of hay,15.9 gDM/kgLW. It was noted that the highest individual daily intake was on the hay diet in period one, a value of 23.5 gDM/kgLW being recorded; however, there was no valid reason for excluding this result from the statistical analysis.

Table 12

The total daily intake of silage and hay (gDM/kgLW) measured over a 7 day digestibility period.

Hay	Silage	S.E.M.	Sig
15.9	18.5	1.335	***

* One animal's silage intake only recorded over six days as the animal became lame and the experiment was delayed for two weeks.

** One animal's hay intake only recorded over five days as the animal escaped from the standing on day six of the digestibility trial.

The pattern of intake over 23.5 hours post a.m. feeding was monitored on three different days during each digestibility period and these results are summarised in Table 13.

Silage intake was consistently higher than hay intake at all times throughout the day. However a significant difference ($P < 0.01$) only occurred between the total daily intakes, 18.5 gDM/kgLW silage compared to 15.5 gDM/KgLW hay, these values being similar to the intakes recorded over the digestibility period.

The overall daily pattern of intake differed between the two diets. Silage was consumed in more frequent ($P < 0.01$) and smaller meals than hay throughout the day, 12.25 silage feeding bouts of an average size of 1.6 gDM/kgLW compared to 8.67 hay feeding bouts of a size of 2.0 gDM/kgLW. Although 88.3% of the total daily intake of the hay diet was consumed in the first twelve hours of feed being offered compared to 80.8% of the silage diet, this was not significantly different.

Table 13

The pattern of intake (gDM/kgLW) of hay and silage at various times throughout the day.

Hours post feeding	Hay	Silage	S.E.M.	sig.
	Intake (gDM/kgLW)			
1	3.6	3.8	0.777	NS
2	4.8	5.3	1.147	NS
3	5.6	6.2	1.273	NS
6	7.2	8.2	1.272	NS
12	13.7	15.0	1.431	NS
23.5	15.5	18.5	1.431	**
No. of meals per day	8.67[+]	12.25[!]	1.431	**
Average meal size	2.0[+]	1.6[!]	0.364	NS

All means based on 12 observations except

[+] 11 observations.

[!] 9 observations.

The size of the first meal after feed is offered tends to be the major bout of eating for the day. The size and duration of this is shown in Table 14. A minimum inter-meal interval of 8 minutes was used in the analysis of all data. This value was obtained by plotting the frequency distribution of intervals between meals and noting where the change in slope occurred (Metz, 1975). This value may not be exactly 8 minutes, but was somewhere between 6 and 10 minutes, the recording equipment was set to log data every two minutes. A pause in eating of 16 and 24 minutes was then used to illustrate the extent to which eating ceased after the first meal.

Table 14

The size and length of the first meal of silage- and hay-fed steers.

	DIET			
End of meal pause.	Hay	Silage	S.E.M	Sig
		Size (gDM/kgLW)		
8 minutes	4.3	5.3	1.44	NS
16 minutes	4.2	5.6	1.47	NS
24 minutes	5.1	5.9	1.47	NS
		Length (mins)		
8 minutes	92.3	83.3	25.87	NS
16 minutes	94.1	89.4	25.77	NS
24 minutes	95.4	107.1	27.34	NS

There was no difference ($P > 0.05$) in the size of this meal between the hay and the silage diets however the end of the meal was defined, although the first meal of silage tended to be the larger. When an 8 or 16 minute pause in eating was used as a definition as to the end of a meal, hay meals were 9 and 5 minutes longer than silage meals. This altered when 24 minutes was used to define the end of a meal with the silage meals lasting for 12 minutes longer than meals of hay. Although this was not significant it could be attributed to the fact that more frequent meals were taken on the silage diet than with hay, and meals which were previously separate are now joined together.

There was no significant effect of diet upon either the total VFA concentration or the amounts of acetate, propionate within the rumen (Figure 5, Appendix Tables A.1, A.2). The level of butyrate (iso- and n-) in the rumen was significantly higher ($P < 0.05$) between 3 and 13 hours after feeding of silage. The higher levels of total VFA's in the rumen was as a result of the increased Pr and Bu, in the rumen of silage-fed steers.

Despite this higher total acid content in the rumen of silage-fed steers, ruminal pH did not differ significantly between the two diets (Figure 6, Appendix A.3).

There was no difference between the ruminal osmolalities recorded on the silage and hay diets and for this reason they have not been included in the results (Appendix Table A.4).

On both diets rumen osmolality peaked at 285-290 mOsm/kg at one hour post feeding, plateaued for 30 minutes and then gradually declined to pre-feed levels between 250-260 mOsm/kg nine hours after feeding.

The ruminal concentrations of ammonia are shown in Table 15 and Figure 7. Pre-feeding, there was a significantly higher concentration of ammonia in the rumen of silage-fed animals. Post feeding there was no significant difference between ruminal ammonia concentrations on either diet.

Table 15

Ruminal concentrations of ammonia (mg/l) of hay- and silage-fed steers.

Sample Time	Hay	Silage	S.E.M	sig
	Ammonia (mg/ml)			
09:15	43.15	24.25	4.059	0.043
09:25	43.35	23.55	2.613	0.017
09:45	53.15	31.85	7.127	NS
10:00	64.38	60.38	13.720	NS
10:15	83.80	103.35	23.076	NS
10:30	101.9	109.1	36.708	NS
10:45	109.2	118.1	46.216	NS
11:00	111.5	120.6	52.995	NS
11:30	117.2	116.9	53.856	NS
12:30	102.3	91.28	32.016	NS
13:30	95.20	42.25	29.721	NS
14:30	85.43	57.67	17.155	NS
15:30	81.05	76.60	19.891	NS
16:30	73.18	91.00	16.486	NS
18:30	63.07	113.15	17.861	NS
20:30	23.65	53.00	9.479	NS
22:30	15.20	31.07	9.471	NS
06:30	19.52	20.02	19.34	NS

Figure 5

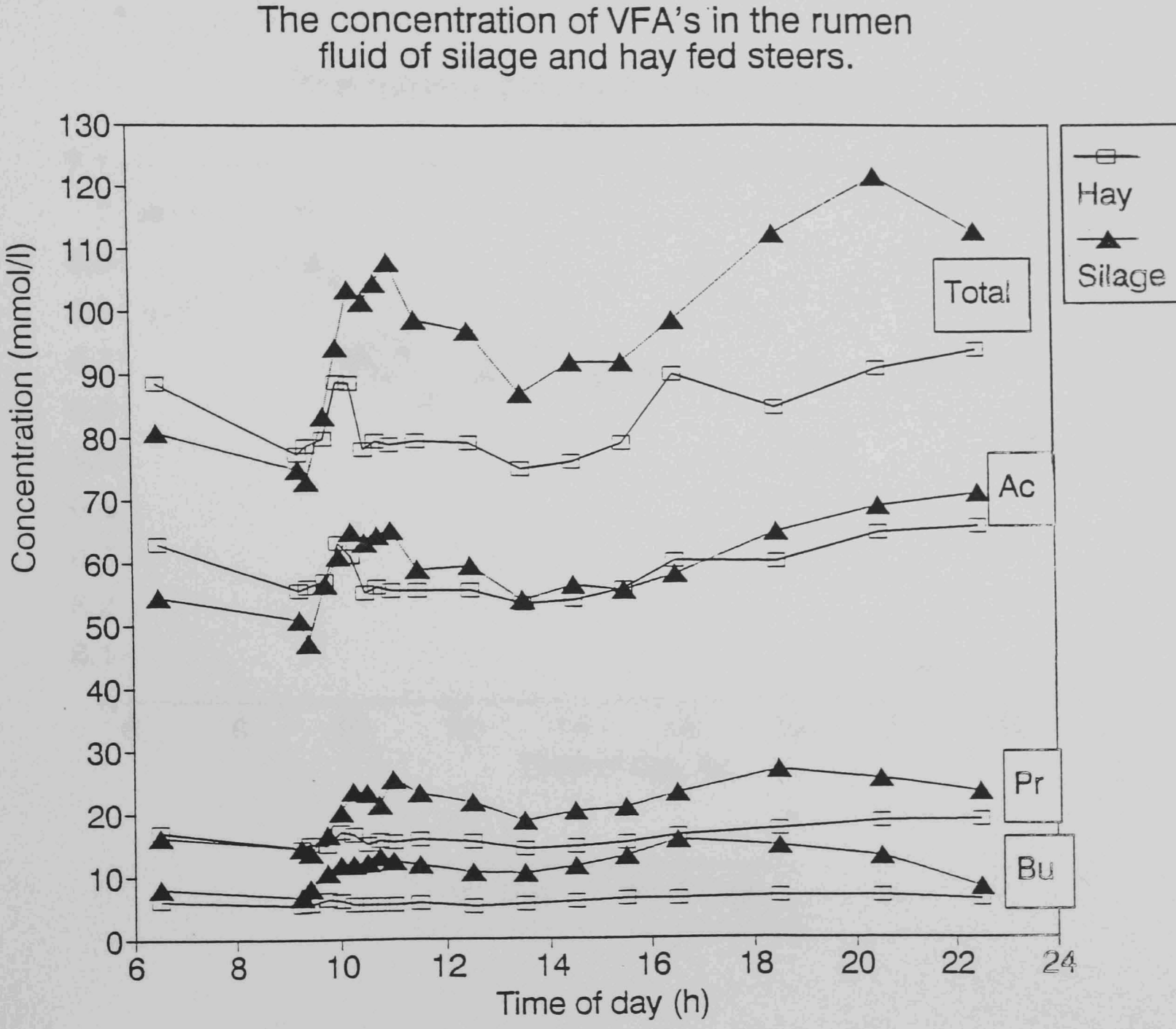

The concentration of VFA's in the rumen fluid of silage and hay fed steers.
Concentration (mmol/l)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
6
8
10
12
14
16
18
20
22
24
Time of day (h)
Hay
Silage
Total
Ac
Pr
Bu

Figure 6

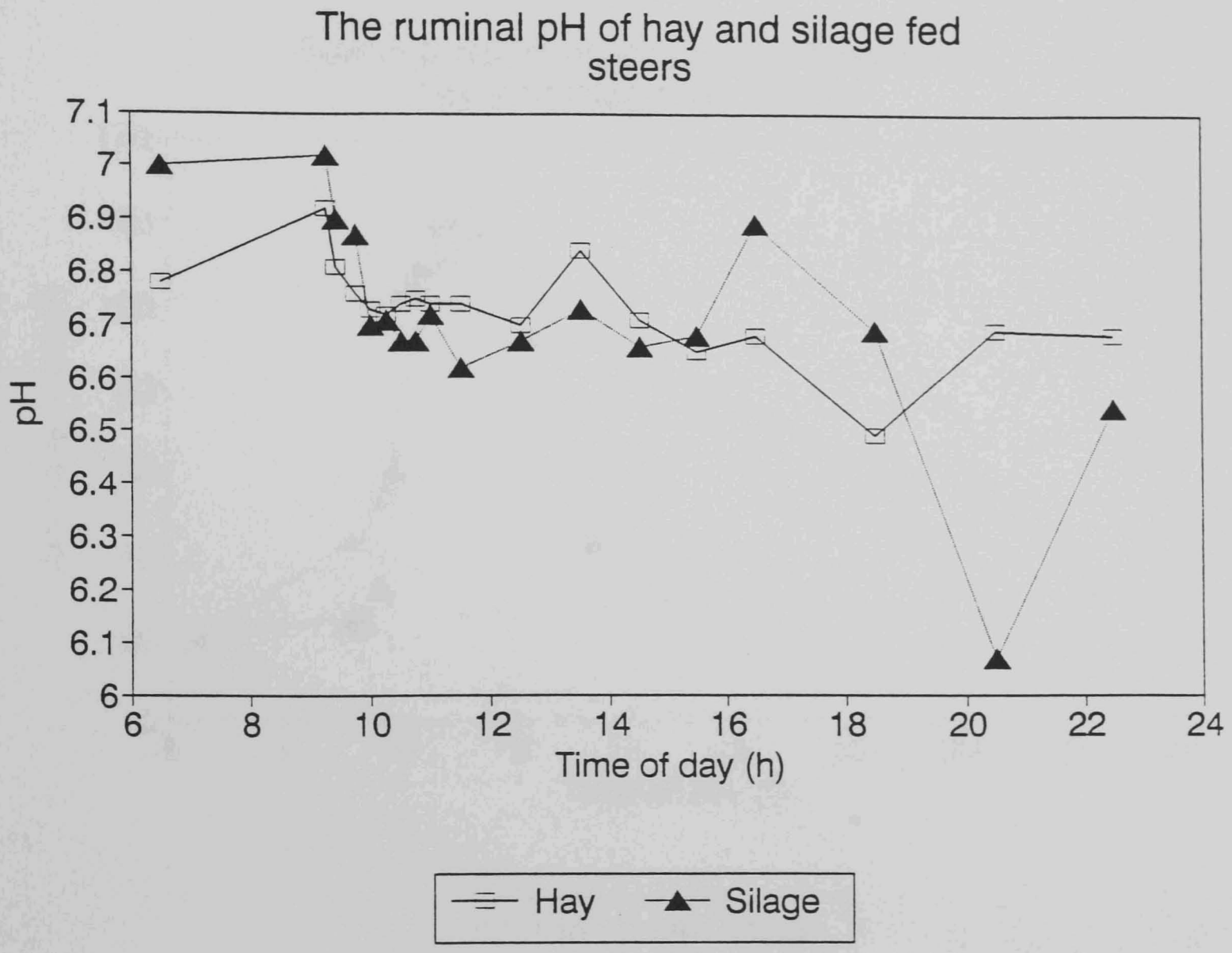
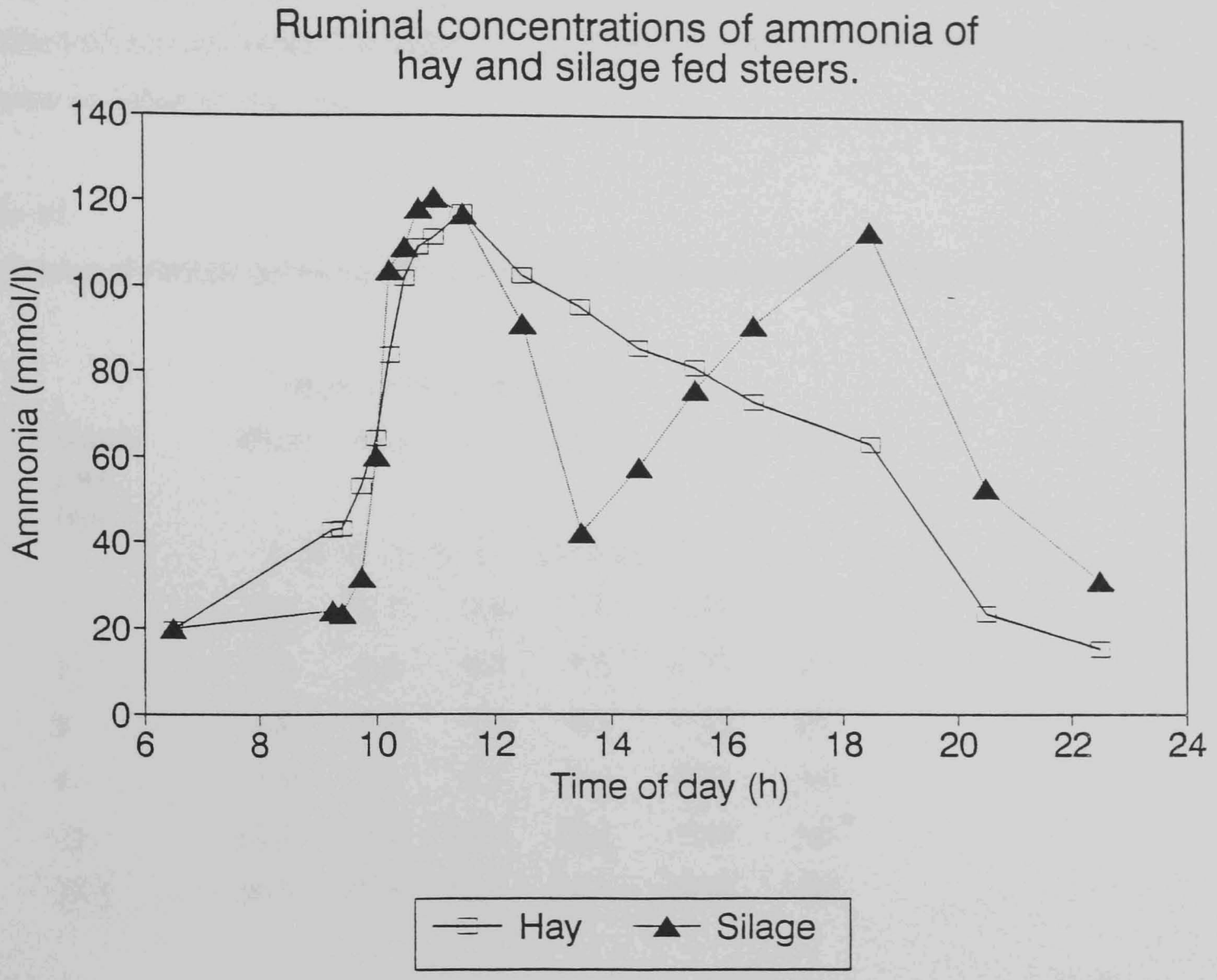

The ruminal pH of hay and silage fed steers
pH
7.1
7
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6
6
8
10
12
14
16
18
20
22
24
Time of day (h)
Hay
Silage

Figure 7


Ruminal concentrations of ammonia of hay and silage fed steers.
Ammonia (mmol/l)
140
120
100
80
60
40
20
0
6
8
10
12
14
16
18
20
22
24
Time of day (h)
Hay
Silage

Ruminal lactic acid concentrations were not significantly different between the two diets and remained low at all times, with the maximum concentration detected being below 4.5 mmol/l.

2. Urea

There was no significant difference ($P > 0.05$) in response to infusions of urea between the cattle offered hay and silage. These data have been included in the appendix tables (A5 and A6) for reference, in the remainder of the text forage will be the word used to describe both hay and silage. The effect of urea infusions on the intake of both hay and silage is shown in Table 16 and Figure 8.

Table 16

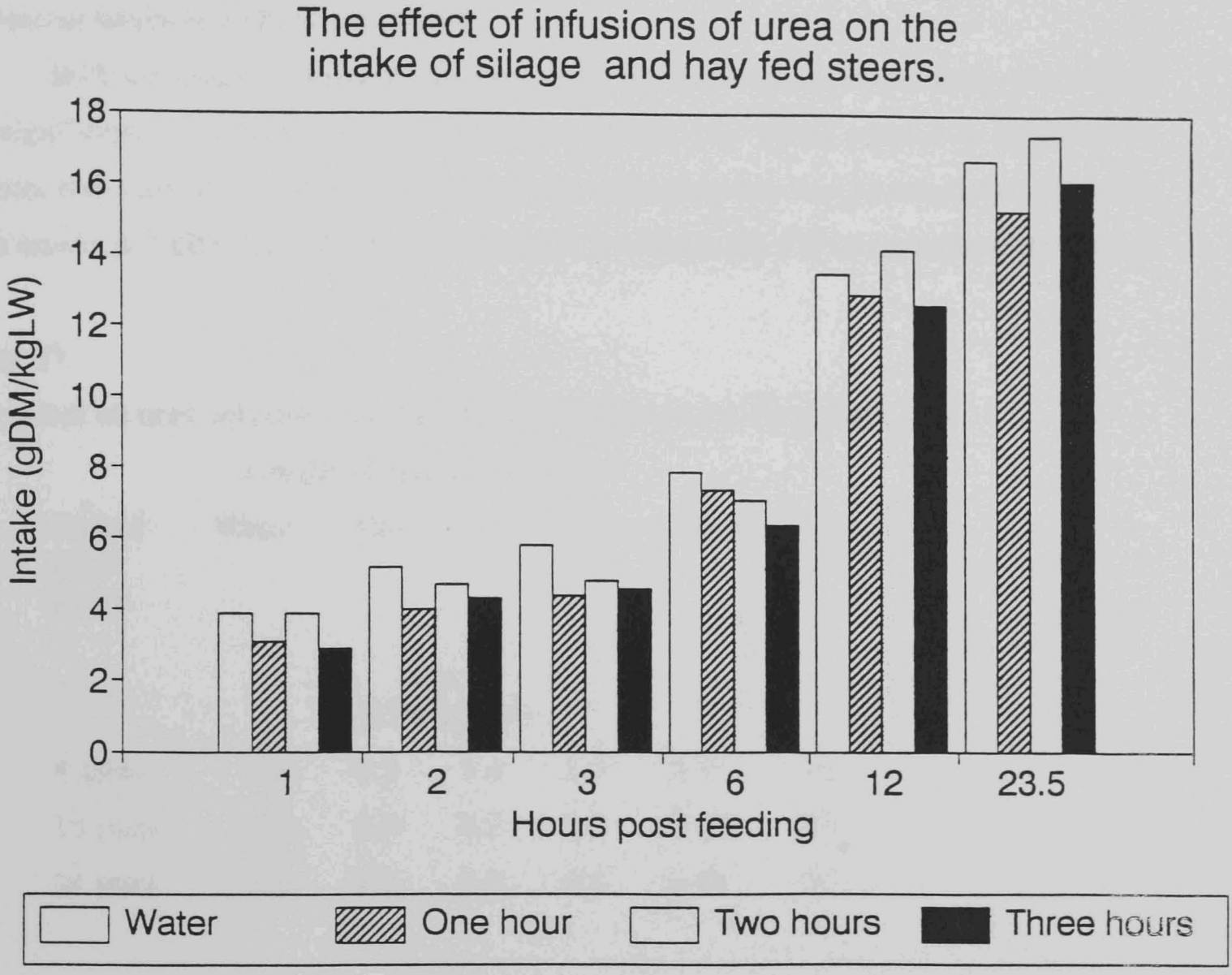
The intake of forage (gDM/kgLW) during the 23.5 hours in which urea was infused.

	Length of urea infusion (h)					
Hours post feed	Water	One	Two	Three	S.E.M.	sig
	I N T A K E (gDM/kgLW)					
1	3.9	3.1[a]	3.9	2.9[a]	0.24	*
2	5.2	4.0	4.7	4.3	0.33	NS
3	5.8	4.4	4.8	4.6	0.35	NS
6	7.9	7.4	7.1	6.4	0.61	NS
12	13.5	12.9	14.2	12.6	0.44	NS
23.5	16.7	15.3	17.4	16.1	0.70	NS

Across rows superscripts indicate a significant difference from the control ($a = P < 0.05$)

Urea infusions had no effect on the total daily intake of the steers when compared to the control infusion of water. Although a significant difference was seen between the total daily intake of hay, 15.5 gDM/kgLW, and the intake of silage, 18.3 gDM/kgLW, measured over this infusion period as would have been expected from the results obtained during the digestibility trial.

Figure 8


The effect of infusions of urea on the intake of silage and hay fed steers.
Intake (gDM/kgLW)
0
2
4
6
8
10
12
14
16
18
1
2
3
6
12
23.5
Hours post feeding
Water
One hour
Two hours
Three hours

At one hour post feeding the intake of the steers during both the three hour infusion (2.9 gDM/kgLW) and the one hour infusion (3.1 gDM/kgLW) were lower ($P<0.05$) than both the intake of the control group and the two hour infusion (both 3.9 gDM/kgLW). However, since intake was not depressed at any other time post feeding, and the two hour infusate had no effect on intake, too much reliance should not be put on this result.

The intake of the control group was slightly higher than that of all the treatments up to six hours post feeding, albeit not significantly, with the three hour infusion of urea depressing intake to 81% of the control.

Both the length and size of the first meal, defined in three ways, were reduced but not significantly ($P>0.05$) by infusion of urea (Table 17). When a pause in eating of 24 minutes was used as the end of meal indicator the three hour infusion of urea reduced the first meal size by 1.7 gDM/kgLW (28.3%) and by 37.5 minutes (28.3%) compared to the control.

Table 17

The effect of urea infusions on the size and length of the first meal.

End of meal pause	Length of urea infusion (h) Water	One	Two	Three	S.E.M.	sig
	Size (gDM/kgLW)					
8 mins	5.0	4.2	4.4	3.9	0.39	NS
16 mins	5.2	4.2	4.7	4.3	0.40	NS
24 mins	6.0	4.6	4.9	4.3	0.45	NS
	Length (mins)					
8 mins	91.0	85.5	73.3	70.0	8.59	NS
16 mins	105.3	77.9	77.3	75.3	10.56	NS
24 mins	115.3	88.9	77.8	77.4	9.68	NS

As with the measurements of intake, the rumen fluid parameters are reported as the effect of the infusions on both silage and hay intake as there was no difference in response to urea infusions between hay- and silage-fed steers.

Intra-ruminal infusions of urea had a significant effect on ruminal ammonia concentrations (Table 18, Figure 9). The peak in rumen ammonia related to the concentration of urea in the infusate. The highest peak was seen at the end of the one hour infusion, 1002 mg/l, although due to the spread of results this was not significant. The elevated levels of ammonia in the rumen were seen for up to 3 hours after the end of each infusion in relation to the control.

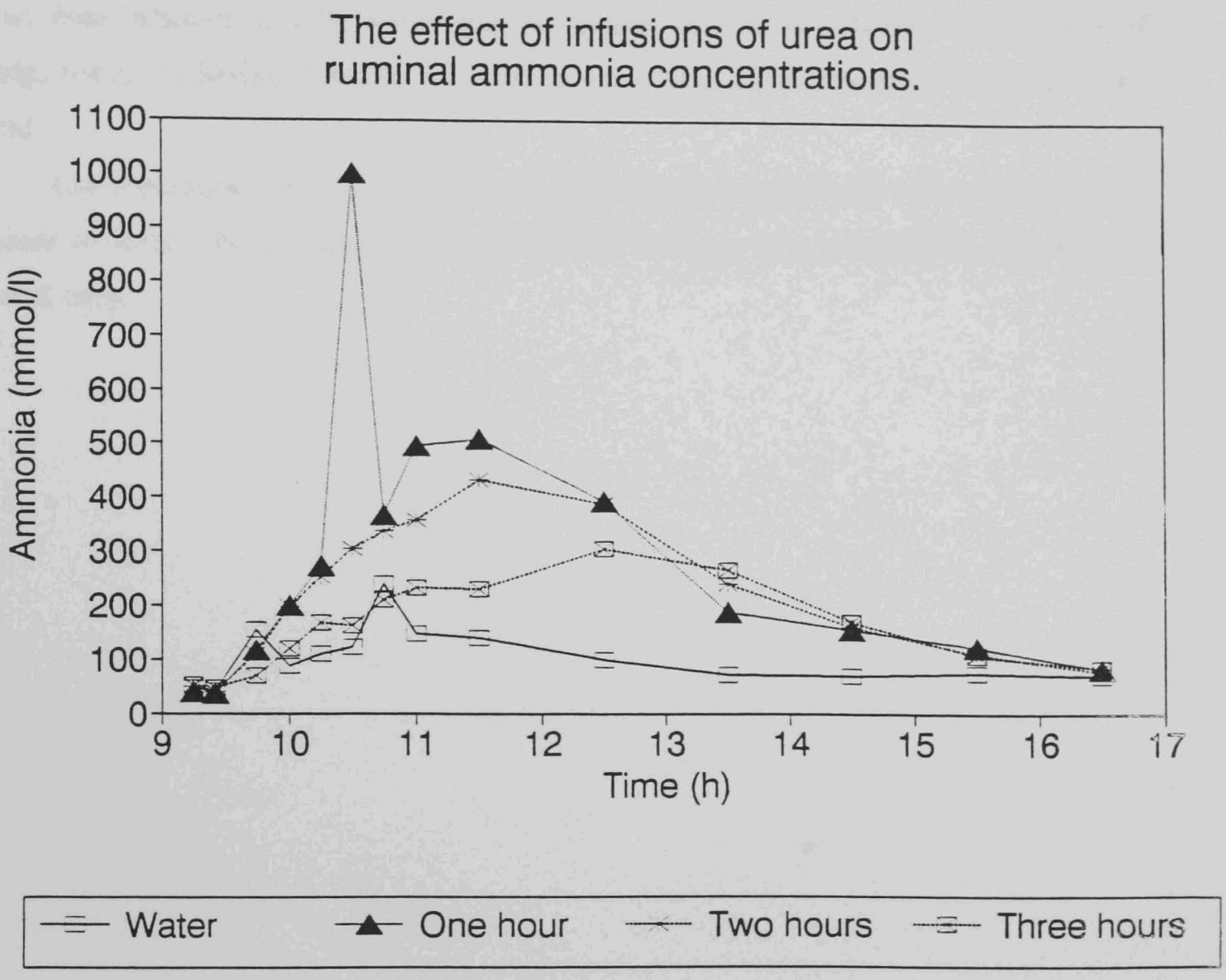
Table 18

The effect of intra-ruminal urea infusions on ruminal ammonia concentrations (mg/l).

		Length of infusion (h).				
Time	Water	One	Two	Three	S.E.M.	sig
		A M M O N I A (mg/l)				
09:15	46	38	49	51	3.5	NS
09:25	42	36	39	47	2.6	NS
09:45	154	116	109	69	46.6	NS
10:00	87	200[b]	198[b]	120	25.3	*
10:15	110	273[b]	253[b]	166	32.2	**
10:30	123	1002	304	163	312.2	NS
10:45	240	367	337	209	69.7	NS
11:00	147	497[c]	357[a]	232	59.2	**
11:30	139	509[b]	432[a]	231	81.8	*
12:30	100	393[a]	388[a]	306	80.3	*
13:30	71	188[a]	241[b]	264[b]	35.3	**
14:30	70	154[a]	159[a]	169[b]	23.3	**
15:30	73	121	109	106	38.4	NS
16:30	68	82	76	85	20.6	NS

Across rows superscripts indicate significant differences from the control (a=$P<0.05$, b=$P<0.01$ and c=$P<0.001$).

Figure 9


The effect of infusions of urea on ruminal ammonia concentrations.
Ammonia (mmol/l)
1100
1000
900
800
700
600
500
400
300
200
100
0
9
10
11
12
13
14
15
16
17
Time (h)
Water
One hour
Two hours
Three hours

The effect of urea infusions on ruminal pH is shown in Figure 10 (Appendix Table A7). The rise in rumen pH reflects the concentration of ammonia in the rumen, with the highest peak following 30 minutes after the end of the one hour infusion at over pH 7.00. Following the treatments, rumen pH returned to similar levels of the control group by 5 hours post feeding.

The ruminal osmolalities following infusions of urea are shown in Figure 11 (Appendix Tables A8 and A9). There was a significant difference between both the one and the two hour infusions and the water infusion between 30 and 90 minutes past the onset of feeding, but no difference between the ruminal osmolality of the three hour infusion or the control.

The concentrations of total, acetic, propionic and butyric acids were unaffected by infusions of urea. The concentrations were similar to those seen pre-infusion and are not reported here.

Figure 10

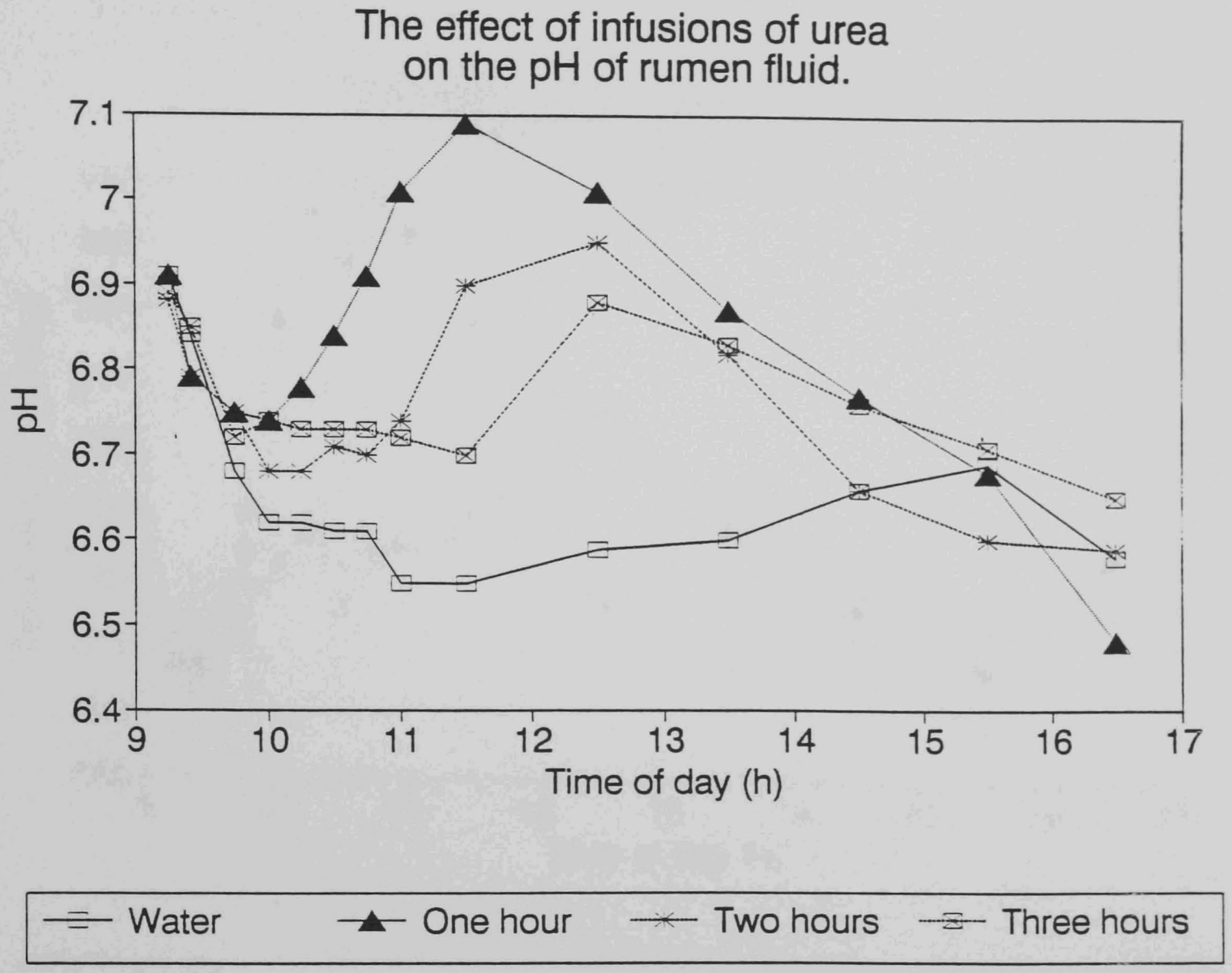
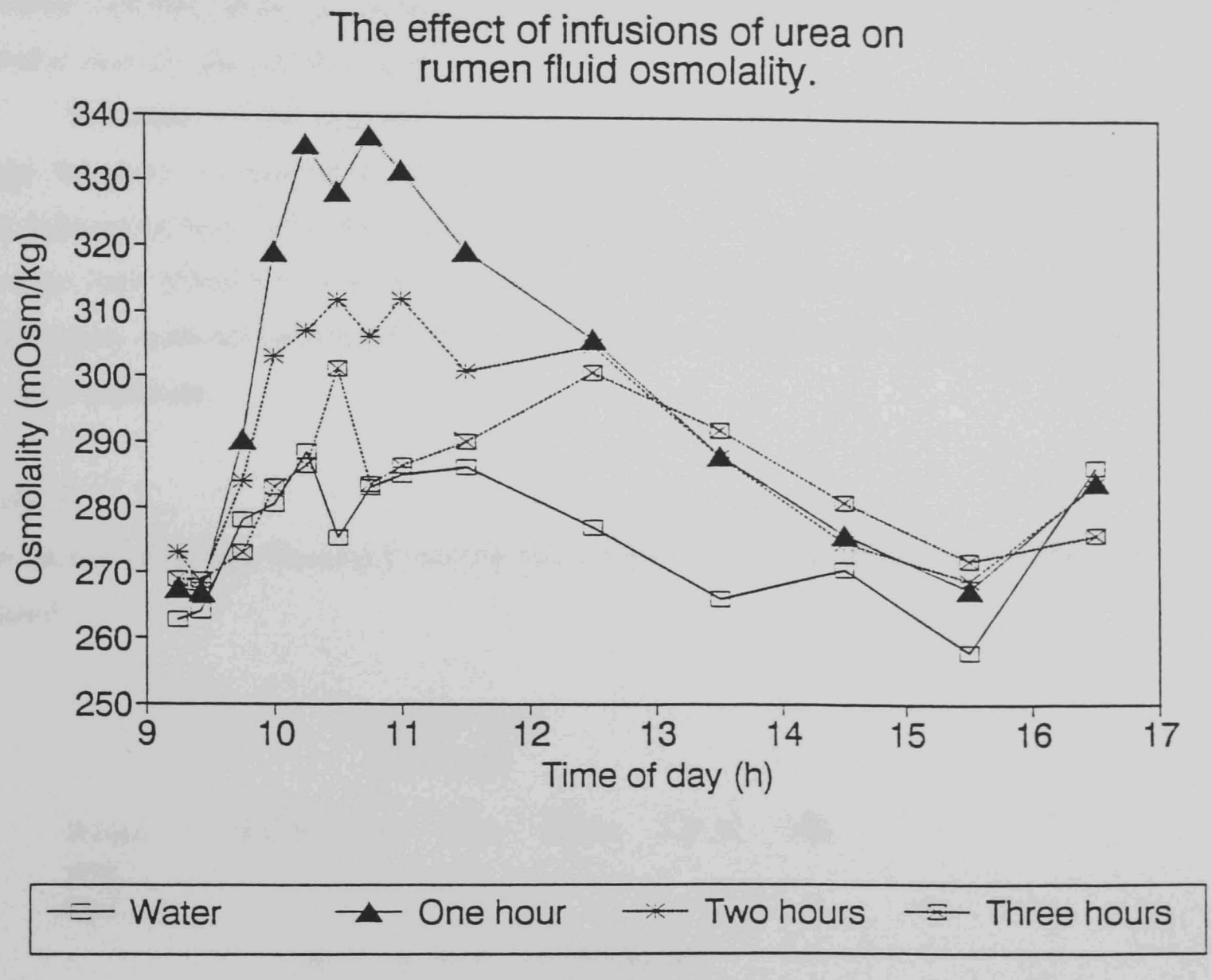

The effect of infusions of urea
on the pH of rumen fluid.
pH
7.1
7
6.9
6.8
6.7
6.6
6.5
6.4
9
10
11
12
13
14
15
16
17
Time of day (h)
Water
One hour
Two hours
Three hours

Figure 11


The effect of infusions of urea on rumen fluid osmolality.
Osmolality (mOsm/kg)
340
330
320
310
300
290
280
270
260
250
9
10
11
12
13
14
15
16
17
Time of day (h)
Water
One hour
Two hours
Three hours

3. Lactic acid

As with infusions of urea there was no significant difference ($P>0.05$) in response to intra-ruminal infusions of lactic acid by cattle offered either silage or hay. These data have been included in the appendix tables (A10-A13, A19, A21, A23) for reference, in the remainder of the text the word forage will be used to describe both hay and silage.

Daily intake of hay, 15.4 gDM/kgLW, was not significantly different from daily silage intake, 16.4 gDM/kgLW, measured over the infusion period. The intake of silage recorded over this period was however 2.1 gDM/kgLW lower than the daily silage intake recorded over the digestibility period.

The effects of the infusions of lactic acid on the voluntary intake of both hay- and silage- fed steers are summarised in Table 19, Figure 12. At one hour post-feeding the two hour infusion of lactic acid caused a reduction in intake of 1.2 gDM/kgLW ($P>0.01$), and the three hour infusion a reduction in intake of 0.9 gDM/kgLW compared to the control. These values were not significantly different ($P<0.05$) from the intakes of the steers on the one hour treatment.

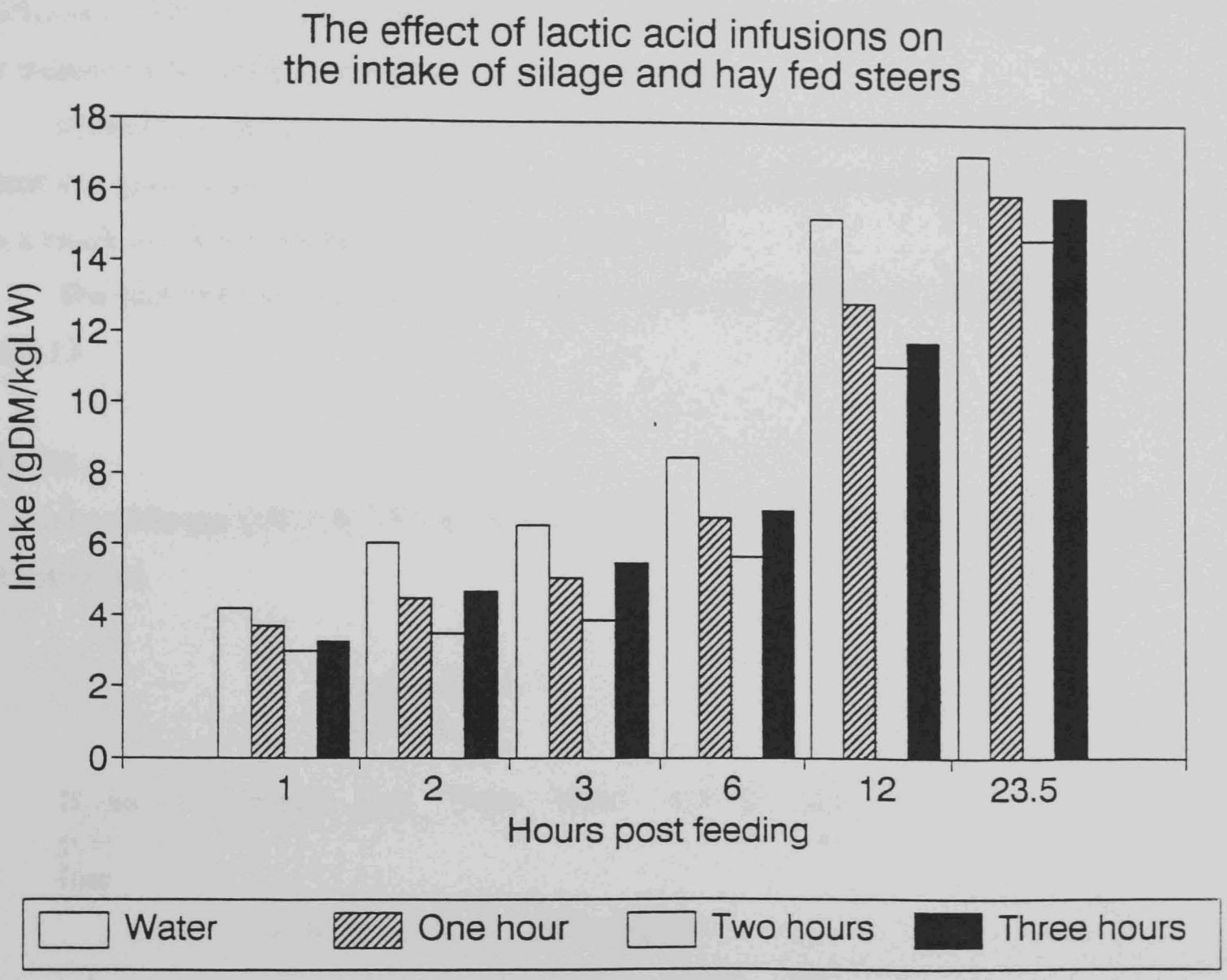
Table 19

The intake of forage measured during the 23.5 hours during which lactic acid was infused.

Hours post feed	Water	Length of lactic infusion (h) One	Two	Three	S.E.M.	sig
	I N T A K E (gDM/kgLW)					
1	4.2	3.7	3.0[b]	3.3[a]	0.23	*
2	6.1	4.5[b]	3.5[c]	4.7[b]	0.29	***
3	6.6	5.1[b]	3.9[c]	5.5[a]	0.32	**
6	8.5	6.8[a]	5.7[c]	7.0[a]	0.45	**
12	15.3	12.9	11.1	11.8	0.76	NS
23.5	17.1	16.0	14.7	15.9	0.70	NS

Across rows superscripts indicate significant differences from the control (a=$P<0.05$, b=$P<0.01$ and c=$P<0.001$).

Figure 12


The effect of lactic acid infusions on
the intake of silage and hay fed steers
Intake (gDM/kgLW)
0
2
4
6
8
10
12
14
16
18
1
2
3
6
12
23.5
Hours post feeding
Water
One hour
Two hours
Three hours

At two hours post feeding all three infusions of lactic acid had significantly reduced the steers' intake. The greatest depression in intake was seen with the two hour infusion which reduced intake by 42.6% ($P>0.001$) of the control treatment.

The intakes of all treatment groups were still reduced at three hours post feeding, the one hour infusion by 23% ($P>0.01$),two hour infusion by 40.9% ($P>0.01$) and the three infusion by 17% ($p>0.05$).

By six hours post feeding the intake of the two hour infusion group remained significantly lower ($P<0.01$) than that of the control group, as did the one and the three hour treatments ($P>0.05$) although not to the same extent.

However, if the intakes between three and six hours are examined (Table 20) it is evident that there is no depression in intake over this period and the reduction at six hours is as a result of a lower intake during the first three hours.

The size and the length of the first meal are shown in Table 21 and illustrated in Figure 13.

Table 20

The intake of forage (gDM/kgLW) at various time periods following intra-ruminal lactic acid infusions

Hours post feed	Length of lactic infusion (h)					
	Water	One	Two	Three	S.E.M.	sig
	I N T A K E (gDM/kgLW)					
0-3	6.6	5.1[b]	3.9[c]	5.5[a]	0.32	**
3-6	2.1	1.7	1.5	2.1	0.34	NS
6-12	6.9	6.2	5.9	5.7	0.81	NS
12-23.5	2.1	2.4	2.7	3.9	0.82	NS

Across rows superscripts indicate significant differences from the control (a=$P<0.05$, b=$P<0.01$ and c=$P<0.001$).

Table 21

The effect of lactic acid infusions on the size and length of the first meal of hay- and silage-fed steers.

	Length of lactic infusion (h)					
End of meal pause	Water	One	Two	Three	S.E.M.	sig
			Size (gDM/kgLW)			
8 mins	5.8	4.9	3.1^{c}	4.4^{a}	0.34	**
16 mins	6.5	4.9^{a}	3.1^{c}	4.8^{b}	0.36	**
24 mins	6.6	5.2^{b}	3.1^{c}	4.8^{c}	0.38	**
			Length (mins)			
8 mins	89.0	83.0	52.0	85.0	11.33	NS
16 mins	109.7	86.3	52.4^{b}	93.5	9.85	*
24 mins	111.2	96.2	52.4^{b}	95.1	10.78	*

Across rows superscripts indicate significant differences from the control (a=$P<0.05$, b=$P<0.01$ and c=$P<0.001$).

As the end of the first meal pause is increased from 8 to 16 to 24 minutes the size and the length of the first meal following the one hour, three hour and the water control infusions gradually increases.

However, following the two hour infusion there is a complete cessation in eating, with the size and length of the first meal staying constant at 3.1 gDM/kgLW and 52 minutes respectively. This occurred at a level of intake much lower than the control group. For example when a 24 minute pause defined the end of the meal the control group had consumed 53.3% more feed than the two hour treatment group.

There was no difference in the measured rumen fluid parameters between the hay- and the silage-fed steers following intra-ruminal infusions of lactic acid. The results of rumen fluid analysis are therefore presented as a mean of both the hay and silage fed steers; where there were significant differences between the treatments the data showing the hay and silage

seperatelyhavebeen included in the appendix.

There was no significant effect of level of infusion of lactic acid upon its concentration in rumen fluid (Table 22, Appendix Table A12, Figure 14), although the more concentrated the lactic acid infusate was, the higher the peak detected in the rumen fluid. The maximum concentration detected was 25.9 mmol/l 15 minutes after the end of the one hour infusion.

Table 22

Ruminal concentrations of lactic acid (mmol/l) following infusions of lactic acid.

	Length of infusion (h)					
Hours post feed	Water	One	Two	Three	S.E.M.	sig
	L A C T I C A C I D (mmol/l)					
09:15	0.2	0.0	0.1	0.1	0.07	NS
09:25	0.1	0.0	0.0	0.0	0.02	NS
09:45	4.7	3.7	3.6	3.0	1.20	NS
10:00	6.2	4.6	7.2	11.1	1.99	NS
10:15	6.3	16.1	14.5	5.2	1.81	NS
10:30	6.2	17.7	14.3	8.8	4.82	NS
10:45	4.1	25.9	11.6	7.9	7.47	NS
11:00	3.6	20.9	8.8	6.8	4.38	NS
11:30	4.4	15.7	9.2	8.5	3.83	NS
12:30	2.3	5.1	8.5	10.5	1.36	NS
13:30	1.6	2.1	3.5	7.3	4.73	NS
14:30	0.5	1.4	1.6	3.0	0.59	NS
15:30	1.3	0.8	1.2	1.4	0.66	NS
16:30	1.1	1.4	1.9	3.4	0.59	NS

Ruminal pH declined in relation to increasing concentration of ruminal lactic acid (Table 23, Appendix Table A13, Figure 15). The lowest ($P > 0.001$) ruminal pH (6.04) in relation to the control, occurred 15 minutes after the end of the one hour infusion, at the same

Figure 13

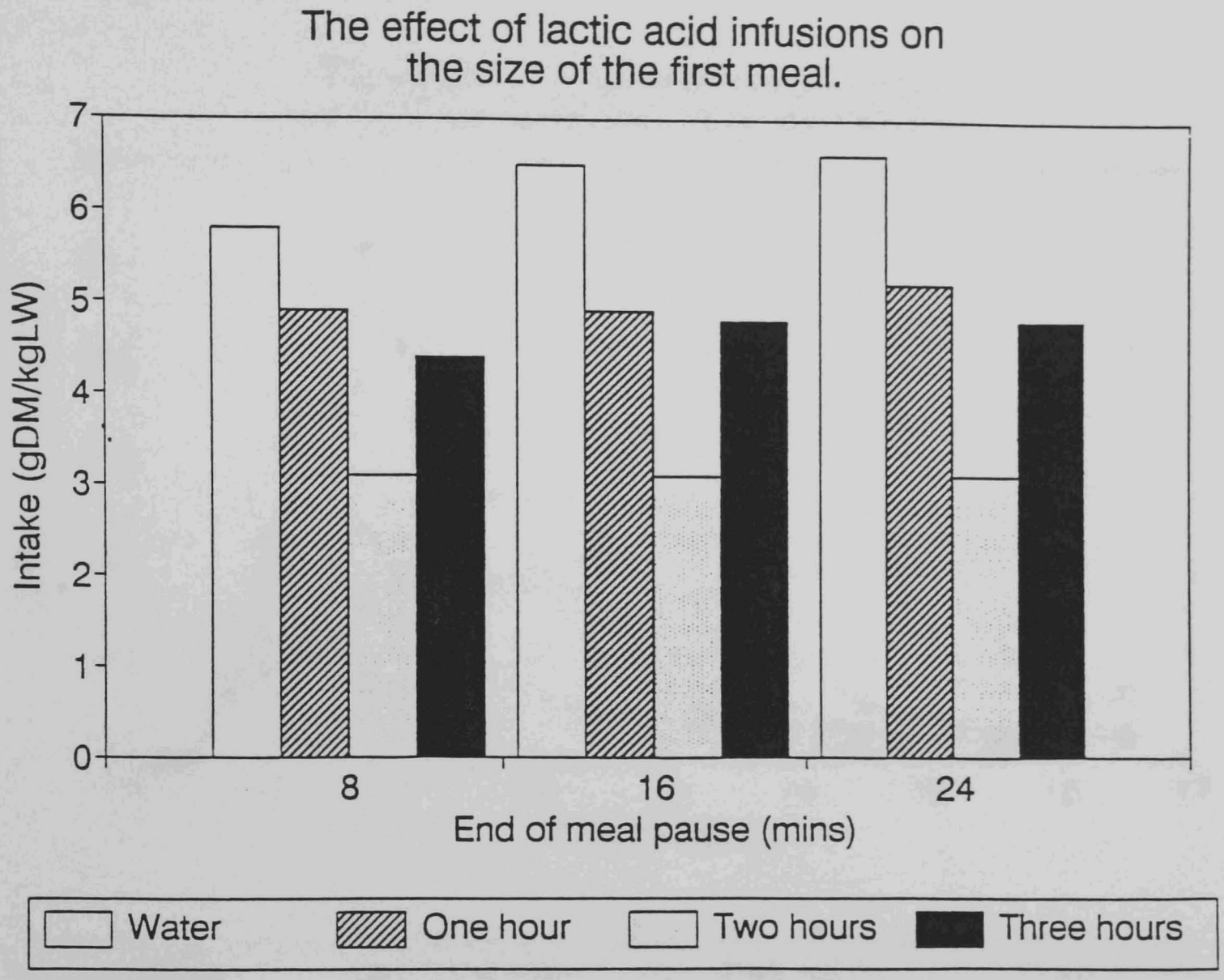
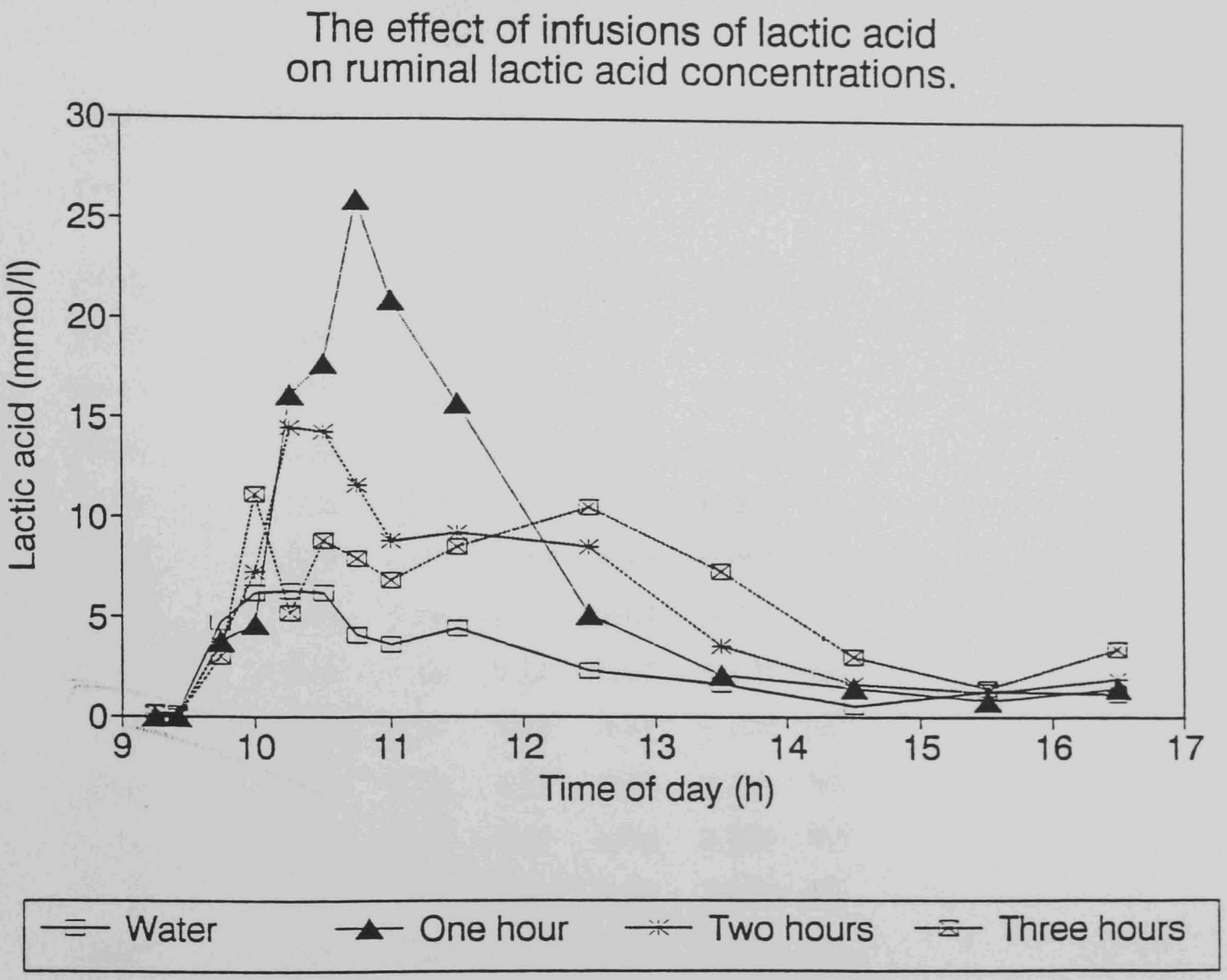

The effect of lactic acid infusions on the size of the first meal.
Intake (gDM/kgLW)
0
1
2
3
4
5
6
7
8
16
24
End of meal pause (mins)
Water
One hour
Two hours
Three hours

Figure 14


The effect of infusions of lactic acid
on ruminal lactic acid concentrations.
Lactic acid (mmol/l)
30
25
20
15
10
5
0
9
10
11
12
13
14
15
16
17
Time of day (h)
Water
One hour
Two hours
Three hours

time that ruminal lactic acid levels were at their maximum. As lactic acid concentrations in the rumen decreased the pH in the rumen returned to similar levels of the control.

Table 23

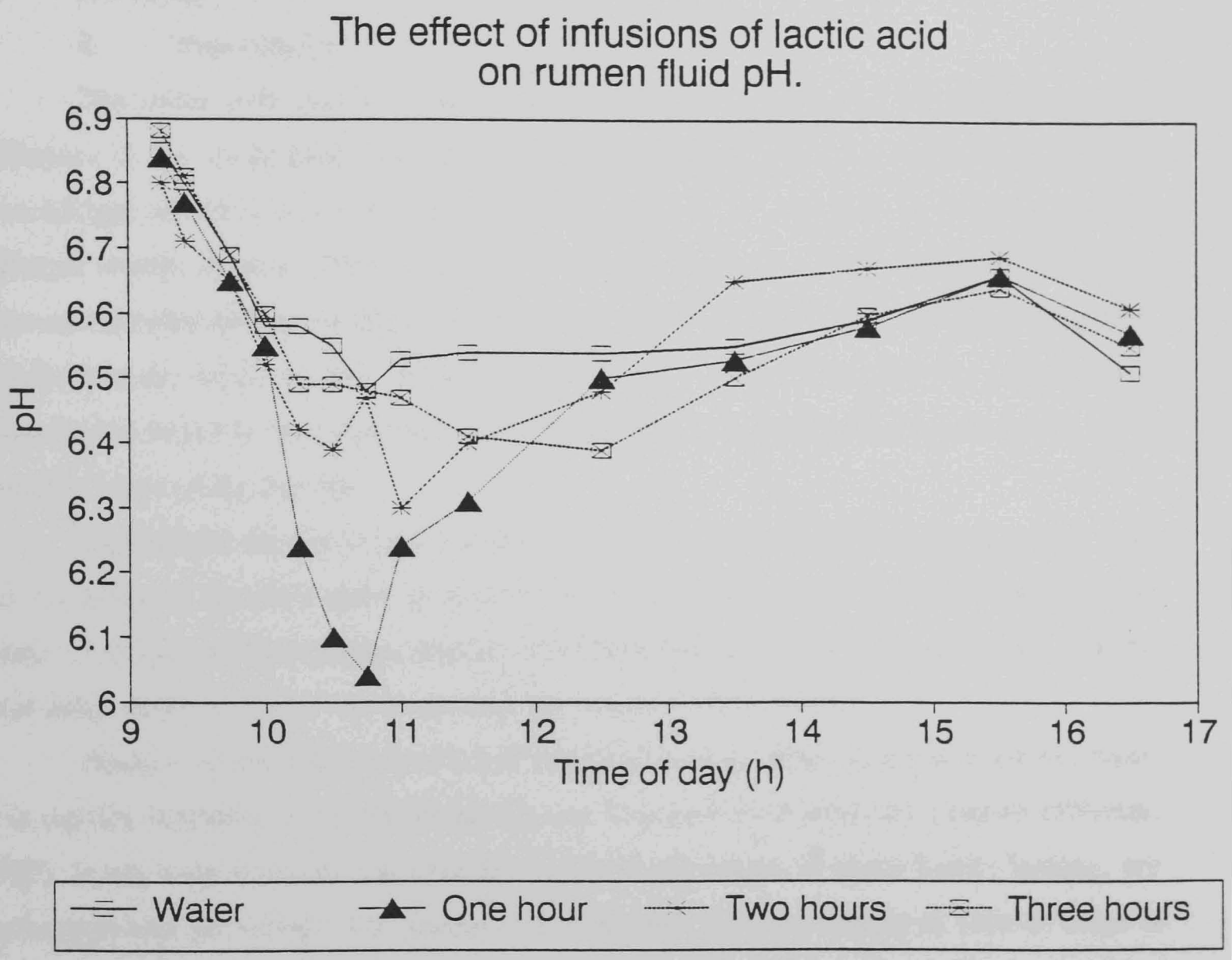
The pH of rumen fluid following intra-ruminal infusions of lactic acid.

	Length of lactic infusion (h)					
Hours post feed	Water	One	Two	Three	S.E.M.	sig
			p H			
09:15	6.85	6.84	6.80	6.88	0.042	NS
09:30	6.80	6.77	6.71	6.81	0.034	NS
09:45	6.69	6.65	6.65	6.69	0.035	NS
10:00	6.58	6.55	6.52	6.60	0.048	NS
10:15	6.58	6.24^{b}	6.42	6.49	0.064	**
10:30	6.55	6.10^{c}	6.39^{a}	6.49	0.069	**
10:45	6.48	6.04^{c}	6.47	6.48	0.074	*
11:00	6.53	6.24	6.30	6.47	0.179	NS
11:30	6.54	6.31	6.40	6.41	0.086	NS
12:30	6.54	6.50	6.48	6.39	0.070	NS
13:30	6.55	6.53	6.65	6.50	0.059	NS
14:30	6.59	6.58	6.67	6.60	0.050	NS
15:30	6.66	6.66	6.69	6.64	0.052	NS
16:30	6.51	6.57	6.61	6.55	0.055	NS

Across rows superscripts indicate significant differences from the control (a=$P<0.05$, b=$P<0.01$ and c=$P<0.001$).

The absolute concentrations of ruminal VFA's, total, acetic, propionic and butyric acids were unaffected by lactic acid infusions (Appendix Tables A.14, A.15, A.16 and A.17). Slight but significant changes in the molar proportions of the acetate, propionate and butyrate occurred between 3 and 6 hours post feeding on all treatments (Appendix Tables A.18-A23). The molar proportions of acetate fell slightly in relation to the control, and subsequently the

Figure 15


The effect of infusions of lactic acid
on rumen fluid pH.
pH
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6
9
10
11
12
13
14
15
16
17
Time of day (h)
Water
One hour
Two hours
Three hours

levels of propionate and butyrate both rose following infusions.

There was no effect of infusion upon rumen fluid osmolality ($P > 0.05$), the values recorded being similar to the levels recorded during the pre-infusion period and so these results have been omitted.

D. Discussion

1. Pre-infusion

The steers were fed two different forms of conserved grass to see if there was any difference in the intake patterns of steers either adapted to a diet containing lactic acid and urea (silage) or a diet devoid of them (hay). In this trial the hay and the silage were from different swards of grass. This is illustrated by their chemical compositions and the intake patterns recorded during the digestibility periods, the total daily intake of silage being 14% higher than the intake of hay. Various workers have shown that when the same crop is conserved as both hay and silage both cattle (Campling, 1966) and sheep (Demarquilly, 1973) consume more of the hay diet.

Throughout the day silage was eaten in smaller more frequent meals than hay, with the exception of the first meal. A similar finding has been reported when comparing the intake of silage and fresh forage (Dulphy and Demarquilly, 1973), although in that trial the total daily intake of silage was lower than the intake of fresh forage.

Because of the high proportion of non-protein N of silage being in a soluble form, it is rapidly degraded in the rumen giving rise to pronounced ammonia patterns (Thomas, 1982). It has been reported that ammonia levels in the rumen of sheep before feeding, are generally lower on a silage diet compared to a hay diet, but rise sharply as soon as silage is consumed (McDonald, 1976). These pronounced ammonia patterns have a detrimental effect on microbial protein synthesis (Beever, 1980) which ultimately leads to limitation of intake, through a slowing in the rate of digestion. In this trial, whilst the concentrations of ammonia before feeding were significantly lower on the silage diet than on the hay diet, the ruminal levels seen post-feeding were similar on each diet and did not reach the levels of 450 mg/l found in the rumen of silage fed-sheep (McDonald, 1976).

Following ingestion of silage the initial increase in total VFA concentration in the rumen occurred more rapidly than with hay, and similar results were reported by Thiago and Gill (1986).

The concentrations of lactic acid in the rumen measured during the pre-infusion period were low at all times. The maximum level detected was 4.5 mmol/l, which compares to a diurnal average value of 0.243 mmol/l reported in the rumen of silage-fed sheep (Gill et al, 1986). .

2. Urea

Intra-ruminal infusions of 0.77 g of urea/kgLW$^{0.75}$ commencing at the time of feeding and proceeding for one, two or three hours did not significantly affect the total daily intake and had virtually no effect on the short-term intake of hay- and silage-fed steers or on the size and length of the first meal. There was no difference in intake in response to urea infusions between cattle eating a high ammonia diet (silage) and those eating a diet not containing ammonia (hay).

The only significant depressions in intake were seen one hour post feeding during the one and three hour infusions. Intake was reduced at this time by 20.5% and 25.6% of the control respectively.

Urea was used in this trial to mimic the effects of additions of ammonia. Ammonia can occur in silages at levels up to 200 g/Kg total-N and high concentrations have been negatively correlated with low intakes of sheep (Wilkins et al, 1971; 1978) and cattle (Lewis, 1981; Rook et al, 1990). Rather than an ammonium salt urea was infused to avoid the complications of infusing a hypertonic solution. Urea is rapidly hydrolysed in the rumen to release ammonia by bacterial urease enzymes (Wallace and Cotta, 1988), one mole of urea liberating two moles of ammonia, but does not have the same osmotic activity as an ammonium salt such as ammonium chloride.

The results of this trial differ from those of Conrad et al (1977). Infusions of either ammonium chloride or urea during meals of a high concentrate ration consumed by goats reduced voluntary intake. However, a reduction in intake was not seen until the amount of urea infused was 3.8% of the dietary DM. This is substantially more than the amount infused in this experiment, where the maximum amount of urea infused (one hour infusion) amounted to only 0.05% of the DM consumed during that time.

In the experiments of Conrad et al the depression in intake seen following infusion of ammonium chloride was double that of urea, although no allowance was made for the osmolality of the ammonium salt in limiting intake. More recently it has been reported that a marked depression in intake occurred when non-lactating dairy cattle fed silage were given

intra-ruminal infusions of urea that brought the total CP content of the diet to 220 gCP/kgDM (Choung, Thomas and Chamberlain, 1989). This is approximately 100 g more of CP than the steers in this trial received.

Ruminal concentrations of ammonia increased in all treatments following infusions of urea. The peak value of 1002 mg/l observed following the one hour infusion was in excess of the concentrations reported by Conrad et al (1977) which ranged from 98 mg/l to 237 mg/l, and above the maximum values detected in the rumen of silage-fed sheep (McDonald, 1976). It has been postulated (Visek, 1968) that reductions in intake occur following increased ammonia concentrations in the rumen due to intra-cellular acidification leading to toxic reactions in the cells located in the tissue near the surface of the rumen. This leads to a decrease in the motility of the rumen, a slowing of digestion and the subsequent limitation on VFI.

It is doubtful whether rumen ammonia per se was responsible for the limitation of intake seen in this trial at one hour post-feeding, as greater concentrations failed to affect voluntary intake.

In conclusion it would appear that intra-ruminal infusions of urea have little effect on the intake of silage- and hay- fed steers at a level of 0.77 g/kgLW$^{0.75}$. Although silages with high ammonia concentrations have been negatively correlated with silage intake, Clancey et al (1977) could only attribute 40% of the depressions in intake seen following infusions of silage extract to ammonia. In addition Barry et al (1978) found that ammonia N as a proportion of total-N accounted for only 37% of the decrease in OM intake of sheep. It would appear that other compounds within silage produced in the same conditions as ammonia by putrefactive micro-organisms are likely to be involved in limitation of intake, such as the group of amine compounds used in experiment 1 (Barry et al, 1978; Buchanan-Smith, 1986).

3. Lactic acid

Short-term intra-ruminal infusions of 2.26 g lactic acid/KgLW$^{0.75}$ depressed the voluntary intake of steers up to six hours post feeding. There was no significant difference between the effect of lactic acid on intake of cattle either adapted to a regular intake of lactic acid (eating silage) or those not adapted (eating hay).

The greatest effect of infusion was seen when lactic acid was administered for two hours. Intake of the animals on this treatment was significantly lower than that of the control group up to six hours post feeding and a complete cessation in eating was seen after the first

meal. Since each infusate contained the same quantity of lactic acid, and it was only the duration of administration that varied between treatments, this result was somewhat surprising. It would have been expected that the greatest depression in intake would occur following the more concentrated one hour infusion or with the continual intra-ruminal infusion of lactic acid over a three hour period, not with the intermediate two hour infusion.

Lactic acid has been shown to be one of the fermentation end-products of silage involved in the limitation of voluntary intake, both by statistical correlations and by direct additions to the diet or the rumen. Wilkins et al, (1971) found a negative correlation between the lactic acid content of grass silages fed to sheep and voluntary intake. However, in a later study (Wilkins et al, 1978) that included maize and legume silages no correlation was found.

The result of this trial is in agreement with Montgomery et al (1963) who infused 360 g of lactic acid into the rumen of lactating Holstein cows for 4 hours and decreased hay intake by 5%. Continual intra-ruminal infusion of lactic acid depressed the intake of sheep fed dried grass when the amount of lactic acid administered exceeded 1.2 moles/day (Wilkins and Valdemoro, 1973). Additions of lactic acid to grass silage, bringing the total lactic acid content to 11.3% of the DM have been shown to depress the intake of sheep by 21.9% (McLeod et al, 1970) and a depression of 12% has been seen when 50 g lactic acid/kgDM has been added to silage fed to cattle (Thomas, Gill and Austin, 1980).

All these results differ from the work of Morgan and L'Estrange (1977) who compared the effect of either additions of lactic acid to the feed or infusions directly into the rumen upon voluntary intake of sheep offered a pelleted grass meal diet.

Additions to the feed of 600, 800, 1000 mmol/kgDMI had no effect on voluntary intake whilst infusions of 1000 mmol/kgDMI depressed daily intake by 32.3%. The reduction in intake only being observed following infusions was attributed to problems of administration since infusions progressed continuously whilst meals were intermittent. It was proposed that localised acid 'hot spots' on the rumen epithelium may have inhibited rumen motility, rather than lactic acid directly effecting VFI and it was concluded that the levels found within silages are unlikely to limit voluntary intake. Whilst rumen motility was not monitored in this trial, the animals did not appear to be in discomfort caused by the infusion acids burning the rumen wall, as no kicking or rubbing of the rumen was witnessed.

The total amount of lactic acid reaching the rumen, from both infusions and the diet, in this trial was approximately 120 g/kgDM, which is below the physiological maximum reported in grass silages, 180 g/kgDM (Chamberlain et al, 1983). However the amount of

lactic acid encountered in the short-term (during and immediately post infusion) following the one and the two hour infusion would be in excess of levels found in grass silages, whilst the three hour infusion would provide similar levels to the maximum concentrations.

The mode of action of lactic acid in reducing voluntary intake is unclear. It has been reported that reticulo-ruminal epithelial receptors are activated by VFA's after exposure of the luminal surface to DL-lactic acid (Critchlow, 1988) and Forbes and Barrio (1992, in press) concluded that the depressing effect of lactic acid on VFI may be indirectly related to inhibition of reticulo-ruminal motility.

Total ruminal concentrations of lactic acid remained low at all times, peaking at 25 umol/ml 15 minutes after the end of the one hour infusion. Lactic acid is rapidly metabolised within the rumen and has a half-life as short as 25 mins, with D(+) and L(-) isomers being metabolised at the same rate (Chamberlain et al, 1983). Only 10% of dietary lactic acid is believed to be absorbed intact from the rumen (Gill et al, 1986) and this would account for the low levels reported here and in other trials.

The absolute concentrations of the total ruminal VFA's, acetic, propionic, or either n- or iso- butyric acids did not change significantly in relation to the control following infusion of lactic acid.

Lactic acid within the rumen is predominantly metabolised to acetic and propionic acids with the production of a small amount of butyric acid, the molar proportions of the end-products varying with the amount of lactic acid present. On silage diets where the concentration of lactate is low and the rumen pH is 6.5 the molar proportions of acetate, propionate and butyrate have been 0.62, 0.35 and 0.03 (Gill et al, 1986). However, on high lactate diets the predominant end-products of metabolism have been reported to be propionate and butyrate (Chamberlain et al, 1983).

The molar proportion of acetic acid in the rumen following lactic acid infusions was seen to decrease between 3 and 6 hours post feeding, whilst the molar proportions of propionate and butyrate were higher than that of the control following infusion. These changes in the molar proportions of Ac, Pr and Bu agree with the work of Chamberlain et al (1983), who found propionate and butyrate increased following lactic acid infusion and concluded that propionate was the major end-product of lactic acid metabolism although the results from this trial do not show anywhere near the magnitude of change in molar proportions seen by Chamberlain et al, and the molar proportions are similar to the values reported by Gill et al, (1986).

The dissociation constant of lactic acid (pka = 3.7) is lower than that of any of the three primary acids (e.g. Ac, pka = 4.7) found in the rumen and the fall in pH seen within the rumen following infusion of lactic acid was to be expected. The depression in rumen pH was in line with the concentration of the infusate, the most marked decrease following the one hour infusion. At no time did rumen pH fall below pH 6.0, and a value of pH 5.0 has been reported as being necessary before pH per se affects voluntary intake (see Forbes, 1986). However, even at pH 6.0 it is likely that the activity of cellulolytic bacteria is impeded leading to a slowing of digestion in the rumen and a subsequent increase in retention time which would ultimately lead to a depression in voluntary intake (Campling, 1970; Leek, 1986). However as the pH following the two hour infusion did not fall by the greatest margin and was not significantly lower than the rumen pH of the control group it would appear that pH per se is not the primary cause of intake limitation following infusions of lactic acid.

In conclusion, while short-term intra-ruminal infusions of lactic acid did decrease voluntary intake, the effect did not appear to differ between cattle adapted to a regular intake of lactic acid (eating silage) and those not adapted (eating hay). The effect did not appear to be mediated by rumen lactic acid, VFA's or pH, and the fact that the greatest depression in intake was seen following the infusion of the intermediate concentrate infusate could not be explained.

Experiment 3

The effect of intra-ruminal infusions of lactic acid or a combination of acetic and propionic acids on the voluntary intake of silage by lactating dairy cows.

A. Introduction

In the previous experiment it was seen that intra-ruminal infusions of lactic acid at a rate equivalent to 32 g/kgDMI depressed the voluntary intake of silage- and hay- fed steers, the greatest depression being seen when the acid was administered over a two hour period. Although no firm conclusions could be drawn as to how lactic acid caused this depression in voluntary intake, it is widely accepted that lactic acid is rapidly metabolised in the rumen to form acetic and propionic acids (Gill et al, 1986; Chamberlain et al, 1983) both of which have been shown to limit VFI of ruminants.

The aim of this study was to investigate the effect of intra-ruminally infusing either lactic acid or a combination of acetic and propionic acids on the voluntary intake of dairy cows fed silage or modified silage diets.

The dairy cows, in mid lactation, were fed silage ad libitum with a supplement of concentrates at a flat rate throughout the trial. The silages were modified prior to being fed to the cows to see if this alleviated any depressions in intake caused by short-term organic acid infusions. Firstly, exogenous lactic acid was added to the silage to investigate whether cows became tolerant to silages with a high acid content. The second treatment involved feeding silage which had previously been modified by the addition of 500 g of fishmeal, since Thomas et al (1980) found that the mixing of fishmeal with silage prior to feeding reduced the depression in intake observed when lactic acid was added to the silage. Thirdly the silage was fed without any additions to it.

Lactic acid was infused at a rate of 32 g/kgDMI over a two hour period, the same rate and level as that which caused the largest depression in intake of steers during experiment 2. To investigate whether any reduction of intake caused by infusion of lactic acid into the rumen is as a result of its subsequent metabolism in the rumen, acetic and propionic acids were infused together in the molar proportions to which 32 g lactic acid/kgDMI is likely to be metabolised. On a silage based diets, which generally contain low levels of lactic acid, the ratio of the end-products of its metabolism are believed to be 62:35, Ac:Pr (Gill et al, 1986).

B. Experiment 3.1

A preliminary investigation to compare two methods of administration of lactic acid on the voluntary intake of silage by dairy cows.

The purpose of the main experiment was to investigate whether additions of fishmeal or lactic acid to silage prior to it being fed would reduce the depressions in voluntary food intake seen following infusions of organic acids. It was hypothesised that cows fed a silage diet with very high levels of lactic acid may become tolerant to a high acid intake. Subsequently the reductions in voluntary food intake seen following short-term infusions of organic acids would be attenuated if not alleviated completely.

The aim of this preliminary trial was to compare two methods of increasing the intake of lactic acid by the cows, either by direct additions to the silage prior to it being fed or by continual intra-ruminal infusion, and to decide on a method of administration for the main experiment. Both infusions of lactic acid directly into the rumen (Montgomery et al, 1963; Wilkins and Valdemoro, 1973; Morgan and L'Estrange, 1977) and dietary additions of lactic acid (Thomas et al, 1980; McLeod et al, 1971) have been shown to reduce voluntary food intake in ruminants.

1. Materials, methods and measurements.

Three dairy cows in late lactation, already adapted to a silage based ration, were used in the trial. They were individually housed and offered silage ad libitum in two equal batches at 09:00 and 16:00 h. A cereal based concentrate feed was offered in two 1.5 kg allotments, 30 minutes after each silage feed.

Lactic acid was added (for method of mixing see Section B) to the silage at two levels, either 30 g/kgDM (a similar level to the amount infused per kg consumed in experiment 2) or 50 g/kgDM (the level used by Thomas et al (1980) to reduce intake of steers). Lactic acid was infused at the same two levels, but based on the DM intake of the cows in the previous three days, not the amount of DM offered. The infusates were made up to 6 litres with water and infused for 12 hours at a rate of 500 ml/hour. From data obtained in experiment 2 steers were shown to consume 80% of their daily ration in the first twelve hours it was offered. It was decided that all of the acid should be infused in this period so as to avoid infusing into the rumen at times when the cows were unlikely to be eating.

A higher level of lactic acid was not administered to avoid exceeding the total

maximal concentrations of lactic acid commonly found in grass silages which are in the region of 150-180 g/kgDM. It was known from analysis of cored samples taken from the silage to be used in the main trial that the concentration of lactic acid was approximately 120 g/kgDM.

The experimental protocol is summarised in Table 24, with all three of the cows being treated in the same way. Total daily intake of silage was the only parameter measured during the course of this trial, being recorded on the final day of each period.

Table 24 **Experimental protocol.**

Period	Day No	Treatment
1	1	Control
2	2-4	Lactic acid infused 30 g/kgDMI
3	5-7	Lactic acid infused 50 g/kgDMI
4	8-10	Control
5	11-13	Lactic acid added to silage 30 gkg/DM
6	14-16	Lactic acid added to silage 50 gkg/DM
7	17-19	Control

2. Results and discussion.

The analysis of silage used in this trial is summarised in Table 25.

Table 25

The chemical composition of the silage

pH		3.90
DM	(g/kg)	290.01
total-N	(g/kgDM)	19.1
NH_3N	"	0.78
lactic acid	"	70.3
acetic acid	"	12.9
propionic acid	"	1.34
i-butyric acid	"	0.69
n-butyric acid	"	1.21

The silage had undergone a predominantly lactic acid fermentation, with low concentrations of butyric acid and ammonium-N. The lactic acid concentration of 70.3 g/kgDMI was lower than that of the silage used in the main experiment, but not untypical of an ADD-F treated silage.

The average intake of silage was reduced by both levels of lactic acid in relation to the control, regardless of method of administration (Table 26). These results are compared by direct comparison, they have not been subjected to statistical analysis.

The higher level of lactic acid caused the greatest reduction in intake in both cases. Following the infusion, intake was reduced to 95.6% of the control, whilst following additions to the silage intake was reduced to 96.1% of the control.

Table 26

Average daily intake (gDM/kgLW) following lactic acid administration.

Treatment	Intake (gDM/kgLW)
Control	18.4
Infusions	
30 g/kgDMI	18.2
50 g/kgDMI	17.6
Dietary additions	
30 g/kgDMI	18.2
50 g/kgDMI	17.7

All these results are based on the average of the three dairy cows. The control result is based on the average of the three animals on three separate days.

Despite finding that long-term intra-ruminal infusions caused a slightly greater depression in VFI than dietary additions it was decided that for the main trial the method of administration of exogenous lactic acid would be to mix it with silage prior to offering to the cows.

Additions to the diet create a more realistic 'on-farm' situation than long-term intra-ruminal infusions. It also ensured that the cows only encountered exogenous lactic acid when they voluntarily consumed silage (except during the short-term infusions) and avoided

complications of infusing into an 'empty' rumen, at times when the cows were not eating.

C. Experiment 3.2

D. Materials and Methods

1. Animals

Six lactating dairy cows in the first or second lactation were fitted with T-piece duodenal cannulae under general anaesthesia between three and five months before they were due to calve. Two weeks after having calved the cows were each fitted with a rumen cannula , with an internal diameter of 6", under local anaesthetic. The cows were tethered by neck yokes throughout the experiment in individual standings on a bedding of sawdust. They were milked, in their standings, twice daily at 07:00 and 15:30 h.

2. Experimental design

The experiment ran for 15 weeks, from between weeks 9 and 12 of lactation until weeks 24 and 27 (Table 27).

Table 27

Calving dates, initial liveweight and weeks of lactation on experiment.

Animal	Calving	Weeks of lactation on expt.	Initial liveweight (kg)	Expt. group
#7	10/11/89	9-24	597	2
#20	26/10/89	11-26	615	2
#24	12/10/89	10-25	619	1
#32	04/10/89	11-26	594	1
#35	29/09/89	12-27	485	1
#52	10/11/89	9-24	608	2

The six cows were split into two groups of three according to their calving dates. Cows #32, #35 and #24 formed one group and #7, #20 and #52 the other. The two groups of cows were each used in a 3x3 latin square of dietary treatments, each dietary period contained a 3x3 latin square of intra-ruminal infusions of lactic acid, acetic and propionic

acids and a control infusion of water.

The cows were randomly allocated to one of the three silage treatments; silage, silage plus 32 g lactic acid/KgDM offered or silage plus 500 g fishmeal. All the cows followed the same experimental protocol throughout the trial summarized in Table 28.

Table 28 **Experimental protocol**

Day	Treatment
1-14	Diet adaptation period
15-23	Eight day digestibility period
23	Rumen sampling
23-25	Rumen emptying
26-35	Infusion period

The cows were adapted to the diets over a 14 day period, and measurements were made on days 15-35 of each dietary period. On day 36 the silage diets were abruptly changed and the experimental procedure was then repeated.

3. Feeds and Feeding

Silage, or modified silage, was fed ad libitum (intake+15%) throughout the experiment. In addition to silage the cows were fed a flat rate of 6 kg per day of cereal concentrates throughout the experiment. At all times water and minerals were available from automatic drinking bowls and licking blocks respectively and 100 g of mineral supplement (Dairy Super, FSL Bell Ltd., Corsham, Wiltshire.) was added to the silage prior to feeding.

Fresh silage was offered in two equal amounts twice daily at 09:00 and 16:00 h, concentrates were offered 30 minutes after the silage, from buckets mounted on the side of the cow standing; no measurement of rate of concentrate intake was made.

The silage was cut on 19 June 1989 from a sward of perennial ryegrass (Lolium perenne, cv Endura) using a precision chop forage harvester and ensiled in a clamp silo after treatment with Add-Safe (BP Nutrition (UK) Ltd, Northwich, Cheshire) at a rate of 3.45 l/tonne. The silo was opened at the end of December 1989 and fresh silage was taken from the clamp every day.

Where the silage required treating by mixing with either exogenous lactic acid or

fishmeal, this was carried out the evening before the silage was due to be fed. Pre-weighed amounts of silage were emptied and spread evenly onto a piece of polythene sheet, 1 x 2.5 metres. Half of the fishmeal or lactic acid was then sprinkled over the silage, thoroughly mixed using a silage fork, the remainder of the treatment applied, and a final mixing carried out. The silage was then returned to the appropriate feed bin, covered and left to stand in the cattle shed until feeding. To keep the overall diet of silage and concentrates iso-nitrogenous two types of concentrate feeds were fed during the trial, either a high (compound H) (barley 383 kg/t, wheat 383 kg/t, soya 67 kg/t and fishmeal 167 kg/t) or a low (compound L) (barley 460 kg/t, wheat 460 kg/t, soya 80 kg/t) protein pellet. All animals received the low protein ration in the mornings and the cows on the silage plus fishmeal diet were also fed this ration in the afternoon. The cows on the other two silage treatments were fed the high protein ration in the afternoons.

4. Measurements

In vivo whole tract digestibility of the rations was measured over days 15-23 of each dietary period. Milk samples were taken on days 1 (p.m.), 2 (a.m), 4 (p.m.), 5 (a.m.), 7 (p.m.) and 8 (a.m.) of the digestibility period and analysed for fat, protein and lactose. The rate and pattern of roughage intake were recorded on each infusion day and on three days during the digestibility measurements. Rumen samples were taken on day 23 of the trial at 08:45, 08:55, 09:15, 09:30, 09:45, 10:00, 10:15, 10:30, 11:00, 12:00, 13:00, 14:00, 15:00 and 16:00 h and at the same time on each infusion day.

i. Rumen emptying

On days 23, 24 and 25 the total rumen contents of each cow were removed, weighed, sub-sampled for chemical analysis and replaced at three different times to establish levels of gut fill. The times were 18:00 h on day 23, 11:00 h on day 24 and 07:30 h on day 25 of the trial.

The lids on top of the feed bins were closed during the evacuations to prevent the cows eating and although they had continual access to water during this period they did not drink at that time. The rumen cannulae bungs were removed from each cow simultaneously and the digesta removed by hand and placed into a large metallic bin immediately below the cannula. Every twentieth sample was put into a smaller container which was sub-sampled once all digesta were removed to provide material for laboratory analysis. The liquid phase

at the bottom of the rumen was removed using a small plastic beaker and as with the more solid digesta every 20th sample was placed into the sample vessel. As each bin was filled it was weighed and immediately the last bin was weighed the digesta were returned to the rumen. The contents of the bins were returned to the rumen in the reverse order of their removal. The smaller sample vessel was also weighed, mixed and 3 x 400 g trays of digesta were taken and dried at 105°C for 48 hrs. A 500 g sample was freeze dried and ground to pass through a 1 mm screen before being analysed for NDF, ash and OM content. Any remaining digestawere returned to the rumen and the bung replaced, before the feed shutter was removed to allow the cows access to their food.

5. Infusions

A 3x3 latin square design of infusions was carried out during each dietary period. The three infusates were lactic acid (LC), a mixture of acetic and propionic acids (AP) and a control infusion of water (W). The infusions commenced when fresh silage was offered at 09:00 h, and lasted for two hours, the infusates administered at a rate of 900 ml per hour. Lactic acid was infused at a level of 32 g/kgDMI, based on the intake of silage during the digestibility period. On diets containing low concentrations of lactic acid such as silage based rations, lactic acid is metabolised in the rumen to form acetic and propionic acids in the molar proportions 0.62 and 0.35 (Gill et al, 1986). The amount of acetic and propionic acids infused was therefore based on the amount of each of these acids likely to be produced from an infusion of 32 g/kgDMI lactic acid, 13.2 g acetic acid/kgDMI and 9.2 g propionic acid/kgDMI. The control infusion was 1800 ml of water.

6. Statistical analysis.

The data obtained during the digestibility study comparing the difference between the parameters measured on the three different silage based diets were analysed by analysis of variance using Genstat 5.0, release 1.3, which took into account diet, cow, period and square effects. The data obtained during the infusion periods were analysed separately also by analysis of variance using Genstat 5.0, which took into account the effects of infusions, diets, cows, periods, subperiods and squares and the interaction between them. This enabled the effect of infusions on the intake of the three diets to be considered both separately and together.

E. Results

The chemical analysis of the silage is shown in Table 29 and the compound feeds and fishmeal in Table 30. The silage had a relatively high DM (312.8 g/kgDM) which made compaction difficult and resulted in a high amount of wastage around the shoulder of the clamp as the experiment proceeded into the spring months when the environmental temperature increased. It had a low pH (3.86) having undergone a predominantly lactic acid fermentation, with low levels of butyric acid detected, although a relatively high proportion of total-N was in the form of ammonia-N, 16%.

Table 29

The chemical composition of the silage.

pH		3.86
DM	(g/kg)	312.80
OM	(g/kgDM)	928.00
total-N	"	20.95
insol-N	"	10.56
NH_3N	"	3.35
NDF	"	509.30
ADF	"	302.80
Lactic acid	"	122.20
Acetic acid	"	11.34
Propionic acid	"	1.23
iso-butyric acid	"	0.76
n-butyric acid	"	0.98

These results are based on the analysis of twelve samples.

Compound L had a higher NDF and OM content than compound H as a result of the higher inclusion rates of barley and wheat in the ration and the lower level of fishmeal. The nitrogen content of the two feeds differed as intended. The total nitrogen obtained from the compound feeds offered to cattle fed the silage without any addition of fishmeal was 177 g. The total nitrogen available from the low protein compound feed and the 500 g of fishmeal

added to the silage was 193.5 g. However, silage was offered ad libitum, as intake plus 15%, therefore assuming that fishmeal was uniformly mixed with the silage, the amount of nitrogen consumed from the fishmeal and the compound feeds was approximately 185.3 g per day.

Table 30

The chemical composition of the two compound feeds and the fishmeal.

		H	L	Fishmeal
DM	(g/kg)	865.8	863.0	915.1
OM	(g/kgDM)	931.7	972.5	747.5
total-N	"	35.9	23.1	109.8
NDF	"	195.9	258.1	-
ADF	"	54.2	58.3	-
starch	"	345.2	387.3	
EE	"	-		86.6

Results of H and L analysis based on twelve bulk samples, analysis of fishmeal based on six samples.

The digestibility of the organic matter in the dry matter, the D-value is reported in Table 31. There was no effect of additions of either lactic acid or fishmeal to the silage on the D-value of the total diet.

The average milk yield, composition and component yield measured over the digestibility periods of the trial are summarised in Table 32.

Table 31

The digestibility of organic matter in the dry matter of the three diets.

DIET				
S	F	L	S.E.M.	sig
0.672	0.664	0.662	0.0122	NS

(The D-value was unaffected by the omission of data collected from animal #32.)

Table 32

The effect of diet on milk yield, composition and the component yield of milk during the digestibility periods.

	DIET				
	S	F	L	S.E.M.	sig
Yield (kg/d)	20.04	19.68	20.13	0.795	NS
Composition (g/kg)					
fat	47.03	46.12	47.70	0.063	NS
protein	35.26	36.56	35.46	0.206	NS
lactose	46.22	46.96	45.93	0.030	NS
Component yield (kg/d)					
fat	0.917	0.913	0.929	0.0376	NS
protein	0.700	0.689	0.716	0.0273	NS
lactose	0.924	0.913	0.943	0.0419	NS

There was no significant effect of additions of lactic acid or fishmeal to the silage prior to it being fed on any of the three measured parameters. No attempt was made to include milk data obtained during the infusion period, as it is unlikely that these short-term treatments would have any distinguishable effect on yield or composition.

There was no significant effect of additions of either fishmeal or lactic acid to the silage on the short-term or total daily intake of silage by the cows measured during the digestibility period (Table 33, Figure 16). The cows fed lactic acid treated silage had a 5% lower daily intake than cows fed the untreated silage, similar to the result obtained during the preliminary trial.

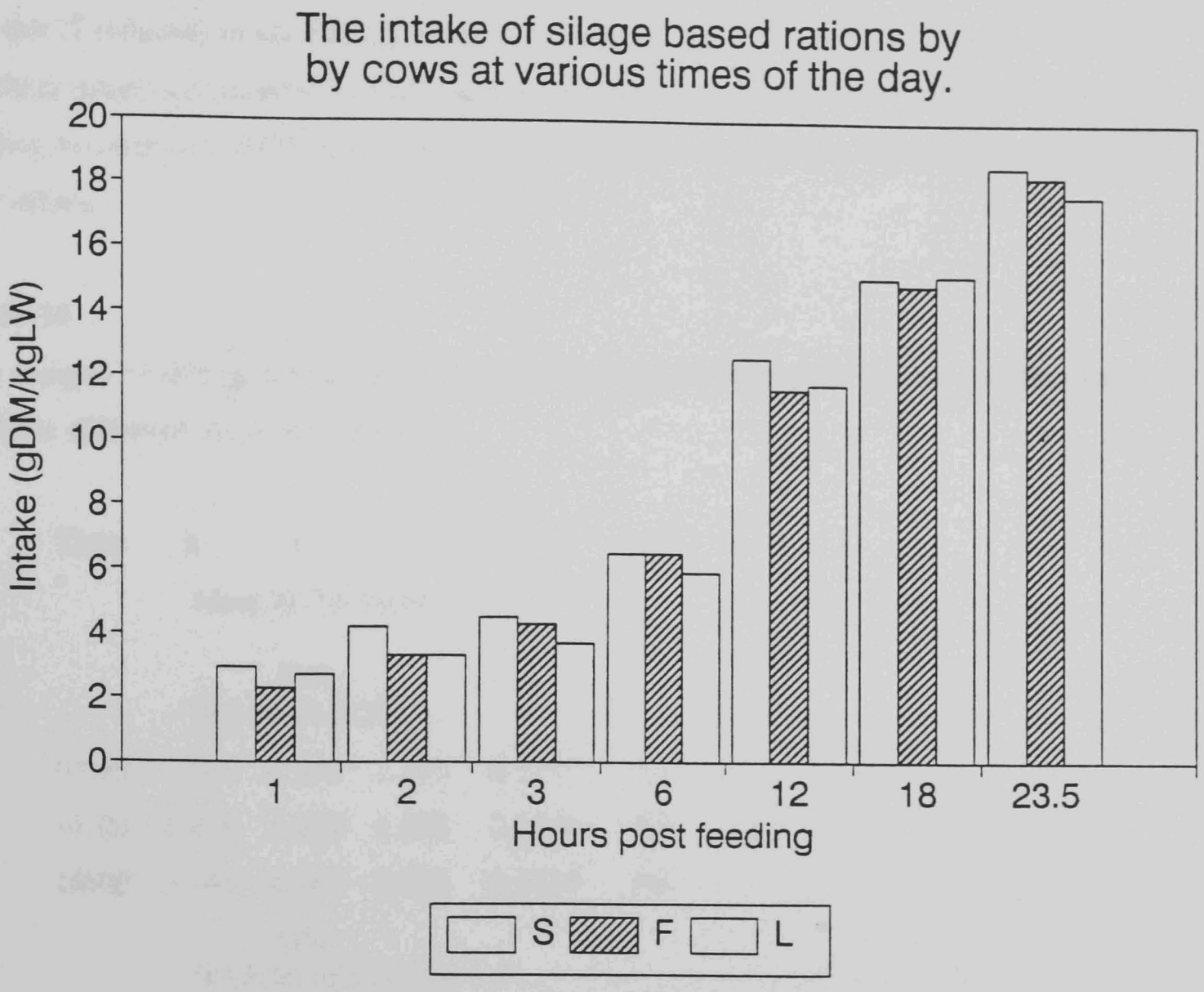
Table 33

The intake (gDM/kgLW) of silage, or silage modified by the addition of fishmeal or lactic acid by lactating dairy cows at various times of the day.

Hours past a.m. feeding.	Diet S	F	L	S.E.M	sig
		I N T A K E (gDM/kgLW)			
1	2.9	2.3	2.7	0.25	NS
2	4.3	3.3	3.3	0.48	NS
3	4.5	4.3	3.7	0.41	NS
6	6.5	6.5	5.9	0.59	NS
12	12.6	11.6	11.8	0.81	NS
18	15.1	14.9	15.2	0.86	NS
23.5	18.6	18.3	17.7	0.57	NS

It was originally intended to record the pattern of intake over these three days, however, due to corruption of the floppy discs onto which intake patterns were recorded it has been impossible to analyse these fully. It should be noted that no attempt was made to record intake patterns of concentrate feeds as previous, unpublished, work conducted at Hurley has demonstrated that they are eaten extremely rapidly. Indeed this was noticed in this trial and in all cases the concentrate allocation of each cow was totally consumed.

Figure 16


The intake of silage based rations by
by cows at various times of the day.
Intake (gDM/kgLW)
20
18
16
14
12
10
8
6
4
2
0
1
2
3
6
12
18
23.5
Hours post feeding
S
F
L

The rumen pool sizes of DM, OM and NDF are reported in Table 34 and illustrated in Figure 17. The results are expressed in terms of gDM/100kgLW to reduce the effect of animal size.

There was no significant ($P > 0.05$) effect of dietary treatment on the rumen pool sizes of DM, OM or NDF in the rumen measured at 07:30, 11:00 and 18:00 h. However, the weight of all three parameters was always slightly lower on the lactic acid treated silage diet and this is reflected in the slightly lower total daily intake of these cows. The volume of all the three measured parameters in the rumen increased from a minimum recorded at the pre-feeding evacuation at 07:30 h and peaked at the 18:00 h evacuation, 9 hours after silage was first offered.

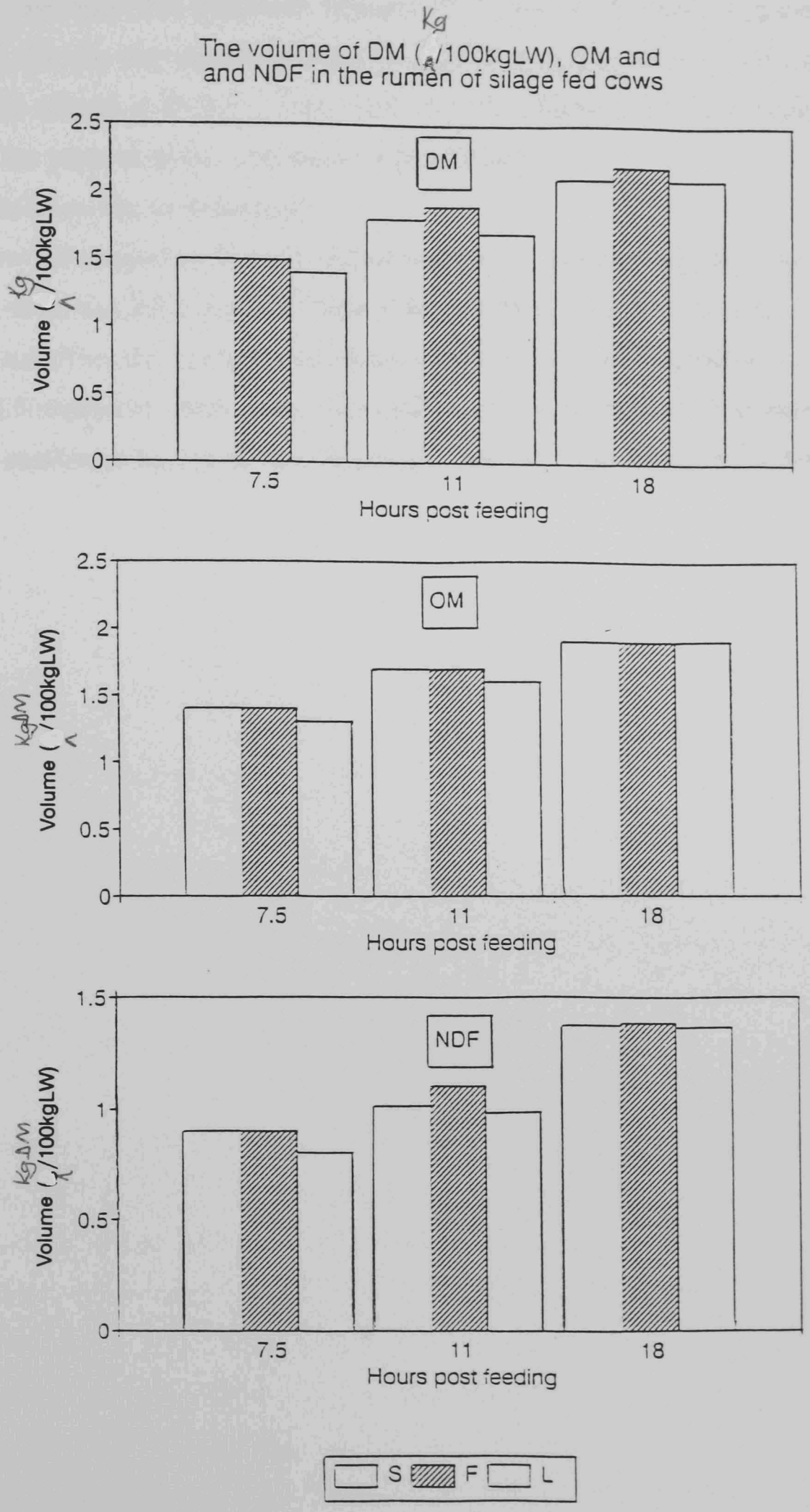
Table 34

The weight of DM (g/100kgLW), OM and NDF (gDM/100kgLW) in the rumen of cows at three different times of the day.

Time	S	F	L	S.E.M	sig
	Mass in the rumen				
	DM (kg /100kgLW)				
07:30	1.516	1.520	1.373	0.7310	NS
11:00	1.844	1.872	1.692	0.9200	NS
18:00	2.144	2.188	2.080	0.1210	NS
	OM (kgDM/100kgLW)				
07:30	1.395	1.395	1.257	0.0693	NS
11:00	1.693	1.723	1.557	0.0843	NS
18:00	1.940	1.910	1.893	0.1161	NS
	NDF (kgDM /100kgLW)				
07:30	0.899	0.903	0.824	0.0620	NS
11:00	1.076	1.100	0.980	0.0836	NS
18:00	1.376	1.378	1.361	0.0805	NS

(There was no effect of omission of data collected from animal #32.)

Figure 17


The volume of DM (kg/100kgLW), OM and and NDF in the rumen of silage fed cows
DM
Volume (kg/100kgLW)
2.5
2
1.5
1
0.5
0
7.5
11
18
Hours post feeding
OM
Volume (kgDM/100kgLW)
NDF
Volume (kgDM/100kgLW)
S
F
L

The concentrations of acetic and butyric acids in the rumen fluid were unaffected by the dietary treatments (Appendix Tables B.1 and B.3) and both remained fairly constant throughout the day. Propionic acid levels were slightly higher when the untreated silage diet was fed compared to the other two treatments between 09:15 and 12:00 hours (Appendix Table B.2). However, this was only significant at 09:45, the first sampling time after the a.m. concentrate ration was offered at 09:30 h. Since there was no difference in silage intakes during this time for the purpose of this trial these slight differences in propionic acid concentration can be discounted.

Rumen pH was not affected by diet and with all treatments a reduction in pH occurred after the concentrate ration was fed (Appendix Table B.4). Additions of lactic acid to the diet prior to feeding did not affect the ruminal concentration of lactic acid, which remained low at all times (below 2.5 mmol/ml). Both rumen osmolality or the concentration of ammonia in the rumen were unaffected by the dietary treatments and all these results have been omitted.

Intra-ruminal infusions of both lactic acid and a combination of acetic and propionic acids reduced the intake of silage fed dairy cattle (Table 35, Figure 18).

Table 35

The pattern of silage intake (gDM/kgLW) at various times from the onset of infusions of LC, AP and W.

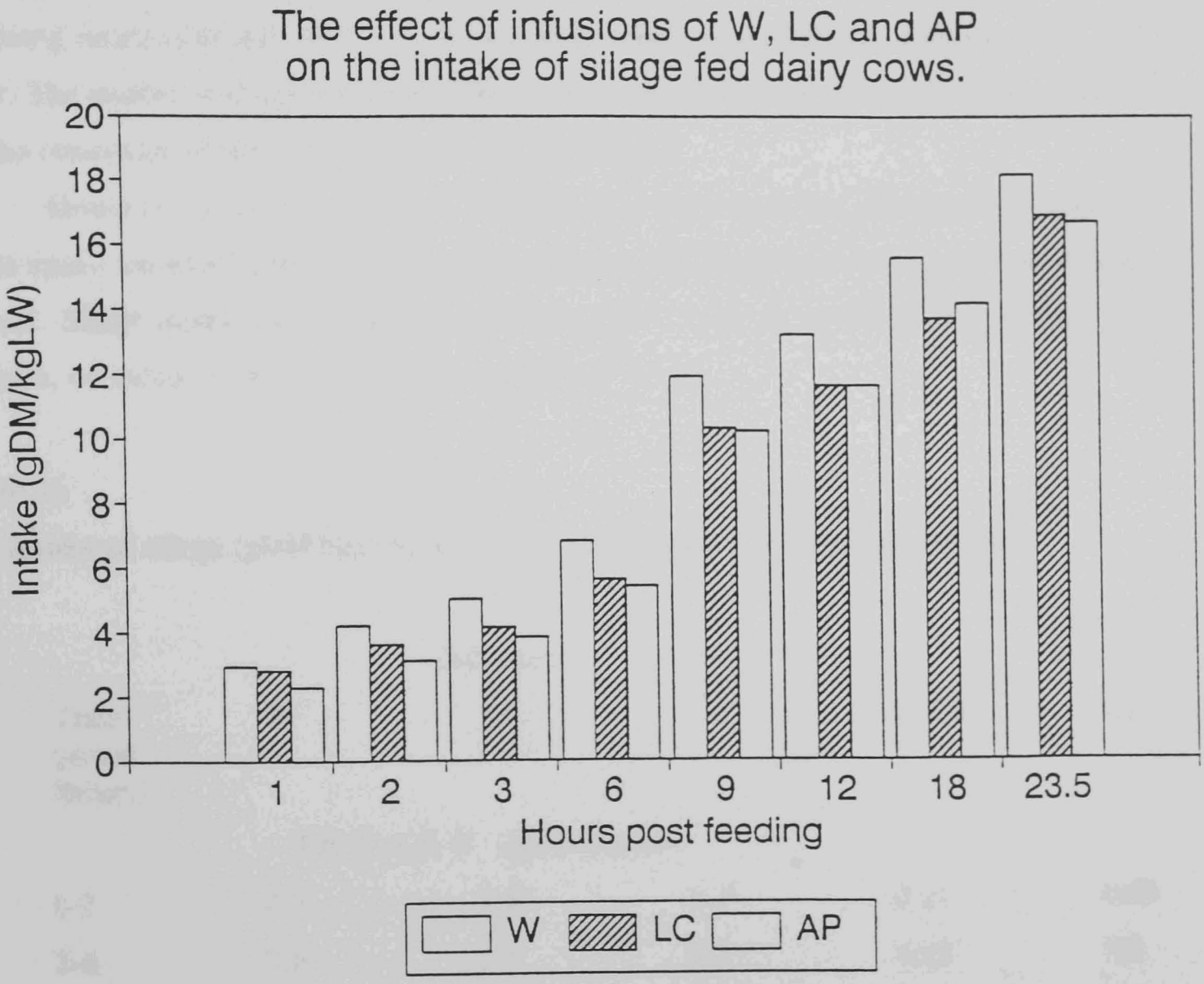
	Infusion				
Hours post feeding	W	LC	AP	S.E.M.	sig
	I N T A K E (gDM/kgLW)				
1	2.9	2.8	2.3	0.17	NS
2	4.2	3.5[a]	3.1[b]	0.21	0.029
3	5.0	4.1[a]	3.8[b]	0.24	0.046
6	6.8	5.6[a]	5.4[b]	0.33	0.018
9	11.9	10.3[b]	10.2[b]	0.35	0.013
12	13.2	11.6[b]	11.6[b]	0.32	0.023
18	15.6	13.7[b]	14.2[a]	0.48	0.011
23.5	18.2	16.9[a]	16.7[a]	0.41	0.042
Average no. of meals	14.6	13.4	15.0	0.09	NS
Average meal size	1.29	1.33	1.19	0.06	NS

Across rows superscripts indicate a significant difference from the control (a=P<0.05 and b=P<0.01).

(#32 omitted from these results, S.E.M. calculated with n=15)

However, there was no difference in intake in response to these infusions of cattle fed silage alone or with the addition of either 50 g/kgDM lactic acid or 500 g fishmeal, therefore when discussing the effects of infusion on intake the word silage refers to all three diets. Data from cow #32 have been omitted from the comprehensive analysis of intake patterns during the infusion periods owing to the cow's lameness. Although the cow was able to eat her daily ration she was reluctant to stand up of her own accord and her daily pattern of intake was peculiar. In addition, data recording her intake pattern were difficult to interpret as her head was continually resting on the side of her standing which affected the monitoring of

Figure 18


The effect of infusions of W, LC and AP
on the intake of silage fed dairy cows.
Intake (gDM/kgLW)
20
18
16
14
12
10
8
6
4
2
0
1
2
3
6
9
12
18
23.5
Hours post feeding
W
LC
AP

the feed weights by the balances. Where there was no effect on the significance of the results of other measured parameters by inclusion of data obtained from #32 it was not omitted.

The intake of silage was depressed following infusions of both lactic acid and the acetic/propionic acid mix in relation to the control. The combination of acetic and propionic acids generally depressed intake to a greater extent than infusions of lactic acid although the intakes following these treatments were not significantly different from each other. At two hours post feeding the intake following the LC treatment was 86% of the control and following infusion of AP 74%, and these results were not statistically different from each other. The intakes of silage remained significantly lower following the organic acid infusions for the remainder of the day compared with the control.

However, it can be seen from Table 36 that the depressing effects of the acids on silage intake are short-term. Intake was only depressed in the two hours when the acids were infused. Silage intake was unaffected by the organic acids ($P > 0.05$) in the 4 hours post infusion, or indeed in the 21.5 hours to the end of the day.

Table 36

The intake of silage (gDM/kgLW) at various time periods following infusions.

Time period (hours)	Infusion W	LC	AP	S.E.M.	sig
	I N T A K E (gDM/kgLW)				
0-2	4.2	3.5[a]	3.1[b]	0.21	0.029
2-6	2.6	2.0	2.2	0.23	NS
6-12	6.3	6.1	6.3	0.30	NS
12-23.5	5.5	5.7	5.0	0.40	NS

Across rows superscripts indicate a significant difference from the control (a=$P < 0.05$ and b=$P < 0.01$).

(#32 omitted from these results, S.E.M. calculated with n=15)

Organic acid infusions had no effect on the number or size of meals (a pause of 8 minutes defined the inter-meal interval) consumed during the course of the day in relation to the control. The first and main meal of the day was dramatically reduced by intra-ruminal infusions of AP both in terms of size and length (Table 37, Figures 19 and 20), whether the end of a meal was defined as a pause in eating of 8, 16, or 24 minutes.

Lactic acid infusions caused a non-significant reduction in the size (0.3 gDM/kgLW) and length (4.2 minutes) when a pause in eating of 8 minutes signalled the end of a meal. When this inter-meal interval was increased to 16 and 24 minutes both the size and the length of the meal were significantly reduced compared to the control infusion of water.

Table 37

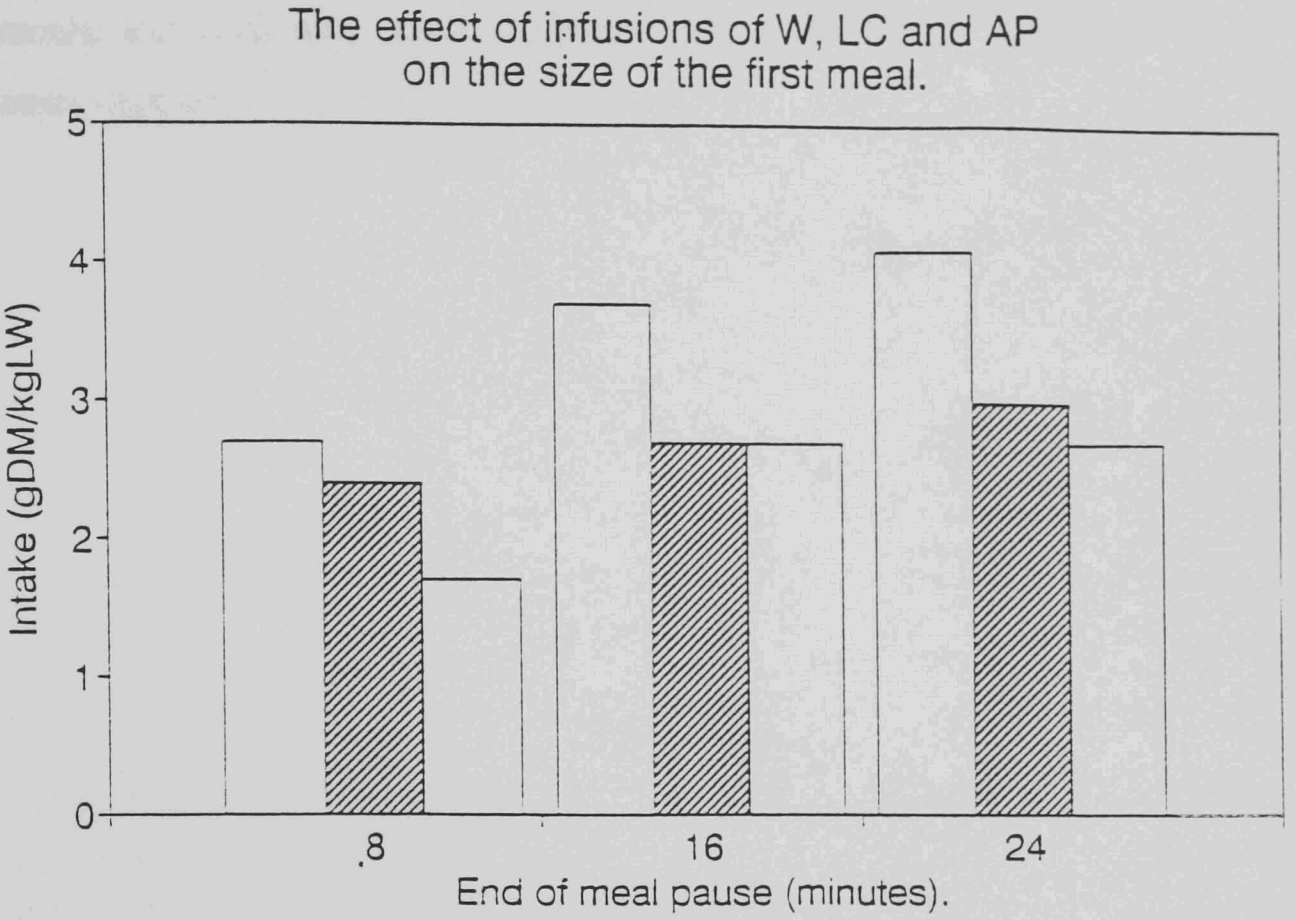
The effect of infusions of LC, AP and W on the size (gDM/kgLW) and length (minutes) of the first meal of silage fed dairy cows.

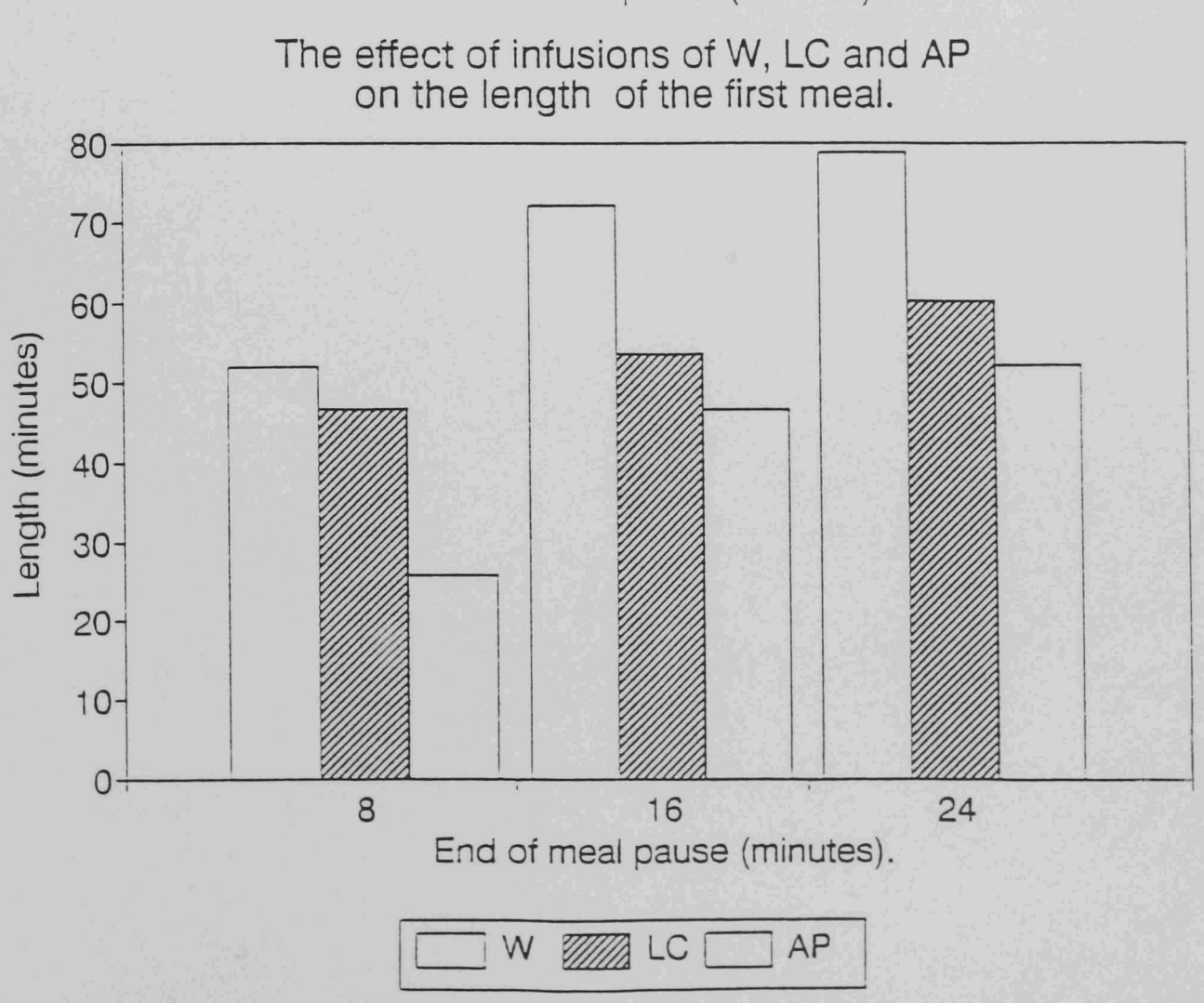
		Infusion			
End of meal pause	W	LC	AP	S.E.M.	sig
		Size (gDM/kgLW)			
8 minutes	2.7	2.4	1.7[b]	0.25	0.024
16 minutes	3.7	2.7[a]	2.7[a]	0.25	0.047
24 minutes	4.1	3.0[b]	2.7[c]	0.22	0.002
		Length (minutes)			
8 minutes	51.9	46.7	25.9[a]	6.29	0.004
16 minutes	72.1	53.5[a]	46.4[b]	5.26	0.008
24 minutes	78.7	60.2[b]	52.1[c]	4.51	0.001

Across rows superscripts indicate a significant difference from the control (a=$P<0.05$, b=$P<0.01$ and c=$P<0.001$).

(#32 omitted from these results, S.E.M. calculated with n=15)

Figures 19 and 20





There was no difference in the effect of the infusions of organic acids upon rumen fluid VFA concentrations, lactic acid, pH, osmolality or ammonia concentration of cows fed any of the three silage diets at any time during the sampling period. The results from the analysis of rumen fluid parameters, unless otherwise stated, include data obtained from animal #32, as omission of these results had no overall effect on the significance of the results.

Both ammonia and osmolality concentrations in the rumen were unaffected by organic acid infusions (Appendix Tables B.5 and B.6).

Ruminal pH declined to significantly lower levels than those of the control (Table 38, Figure 21) on all three diets during the two hours in which LC and AP were infused, but returned to similar levels of the control group by one hour post infusion. Both the LC and AP infusions caused similar reductions in pH, the minimum value being recorded 90 minutes from the start of the infusion at 5.63 and 5.9 respectively. This decline in pH was to be expected owing to the nature of the infusates.

Table 38

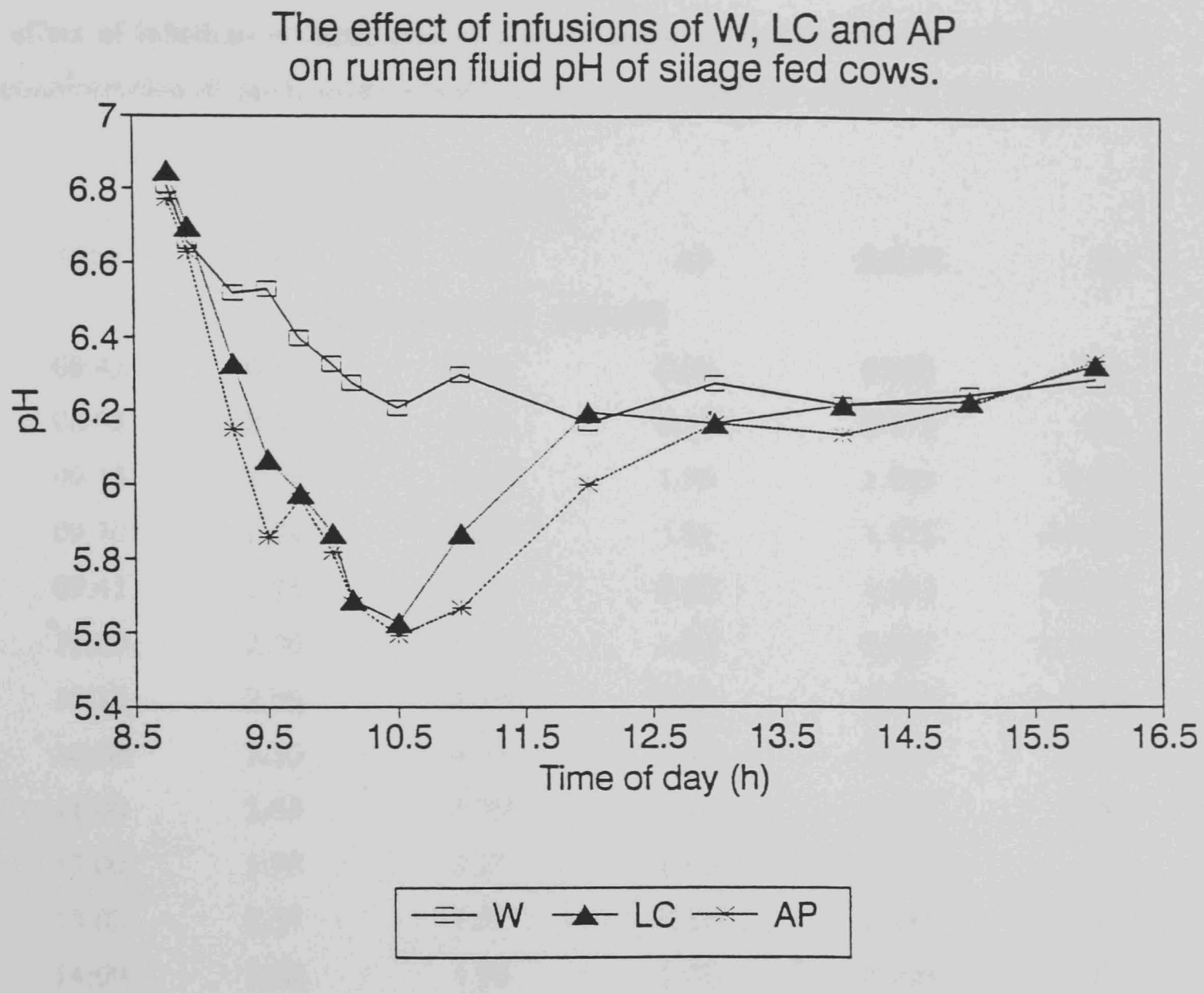
The effect of infusions of lactic acid or a combination of acetic and propionic acids on rumen fluid pH of silage fed dairy cows.

		INFUSION			
Time	W	LC	AP	S.E.M.	sig
		pH			
08:45	6.81	6.85	6.77	0.055	NS
08:55	6.66	6.70	6.63	0.066	NS
09:15	6.52	6.33	6.15^{b}	0.065	0.01
09:30	6.53	6.07^{c}	5.86^{c}	0.069	<0.001
09:45	6.40	5.98^{c}	5.98^{c}	0.069	0.013
10:00	6.33	5.87^{b}	5.82^{c}	0.086	0.008
10:15	6.28	5.69^{c}	5.68^{c}	0.075	0.001
10:30	6.21	5.63^{b}	5.59^{b}	0.115	<0.001
11:00	6.30	5.87^{b}	5.67^{c}	0.096	<0.001
12:00	6.17	6.20	6.00	0.069	NS
13:00	6.28	6.17	6.17	0.068	NS
14:00	6.22	6.22	6.14	0.091	NS
15:00	6.25	6.23	6.22	0.076	NS
16:00	6.29	6.33	6.34	0.056	NS

Across rows superscripts indicate a significant difference from the control (b=P<0.01 and c=P<0.001).

#32 has been omitted from the analysis of data, n=15

Figure 21


The effect of infusions of W, LC and AP
on rumen fluid pH of silage fed cows.
pH
7
6.8
6.6
6.4
6.2
6
5.8
5.6
5.4
8.5
9.5
10.5
11.5
12.5
13.5
14.5
15.5
16.5
Time of day (h)
W
LC
AP

Levels of lactic acid in the rumen were significantly higher during the LC infusion compared to both the control and the AP groups (Table 39, Figure 22) but returned to similar levels of the other treatments post infusion.

Table 39

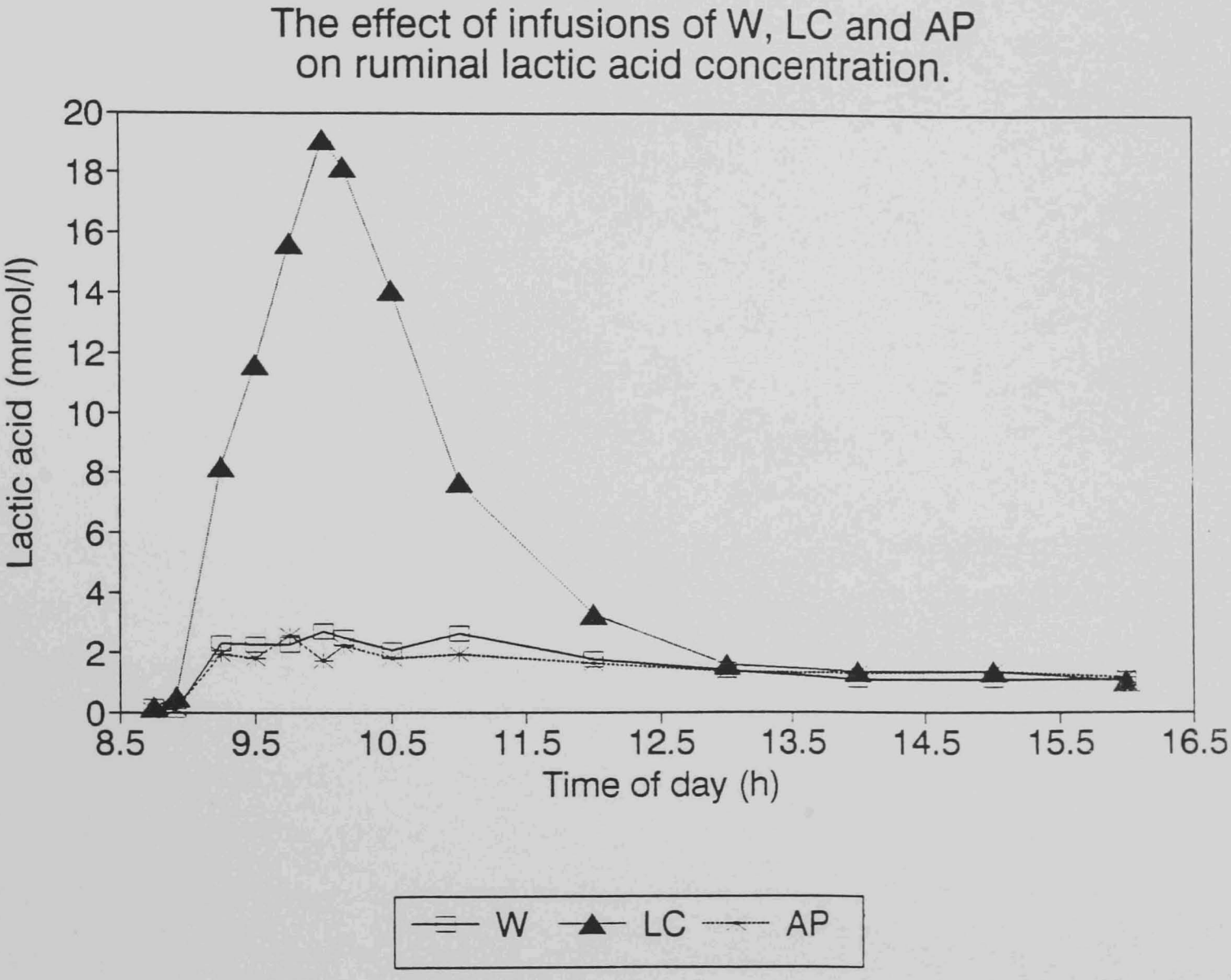
The effect of infusions of lactic acid or a combination of acetic and propionic acids on the concentration of lactic acid (mmol/l) in the rumen fluid of silage fed dairy cows.

	INFUSION				
Time	W	LC	AP	S.E.M.	sig
	Lactic acid conc. (mmol/l)				
08:45	0.16	0.18	0.08	0.086	NS
08:55	0.07	0.53	0.12	0.175	NS
09:15	2.29	8.18[b]	1.95	1.340	0.002
09:30	2.24	11.60[c]	1.81	1.475	<0.001
09:45	2.25	15.64[c]	2.56	1.692	<0.001
10:00	2.70	19.10[c]	1.70	1.847	<0.001
10:15	2.50	18.20[c]	2.20	1.504	0.001
10:30	2.10	14.10[c]	1.80	1.594	0.001
11:00	2.63	7.70[b]	1.95	0.938	0.001
12:00	1.75	3.27	1.60	0.479	NS
13:00	1.37	1.57	1.33	0.249	NS
14:00	1.08	1.36	1.29	0.200	NS
15:00	1.06	1.34	1.36	0.317	NS
16:00	1.06	1.03	1.17	0.213	NS

Across rows, superscripts indicate a significant difference from the control (b=P<0.01 and c=P<0.001)

The levels of acetic acid and propionic acids in the rumen rose during intra-ruminal infusions of AP (Appendix Tables B.7 and B.8) and remained elevated until the infusion ceased.

Figure 22


The effect of infusions of W, LC and AP
on ruminal lactic acid concentration.
Lactic acid (mmol/l)
20
18
16
14
12
10
8
6
4
2
0
8.5
9.5
10.5
11.5
12.5
13.5
14.5
15.5
16.5
Time of day (h)
W
LC
AP

There was no significant difference in the ruminal concentrations of acetic, propionic and butyric acids (Appendix Table B.9) between the control group and the LC group at any time post feeding. The molar proportions of acetic acid increased following infusion AP, whilst that of butyric acid decreased and that of propionic acid was unaffected (Appendix Tables B.10, B.11, B.12).

F. Discussion

Three different silage based rations were fed to dairy cows during the experiment to investigate whether inclusion of either fishmeal or lactic acid would affect the long term voluntary intake of silage or modify intake during periods of intra-ruminal infusions of organic acids. Short-term intra-ruminal infusions of lactic acid and the products of its digestion in the rumen depressed the voluntary intake of silage fed dairy cows during the two hours in which the organic acids were infused. There was no difference in response to organic acid infusion of cows fed either straight silage, a silage with increased levels of lactic acid (50 g/kgDMI) or a silage mixed with 500 g of fishmeal prior to being offered. Indeed, there was no significant effect of mixing fishmeal or lactic acid with silage prior to feeding on voluntary intake measured during the pre-infusion periods.

This is in contrast to work carried out with growing steers fed silage ad libitum, with no concentrate supplement feed, where inclusion of fishmeal at a rate of 50 g/kgDMI increased absolute intake (Thomas et al, 1980). The elevation in silage intake caused by additions of fishmeal has been shown to be lower when good quality (Gill et al, 1987) compared to poor quality (Gill and England, 1984; Garstang, Thomas and Gill, 1979) silage is fed.

The silage used in this experiment was reasonably well preserved, indicated by a low pH (3.86) due to a predominantly lactic acid fermentation and consequently low concentrations of both butyric acids and ammonia were present. The response to fishmeal in the above trials was as a supplementary feed. It is well established that protein deficient diets are associated with low intakes in ruminant animals (see Forbes, 1986) and on most grass silage diets the quantity of protein absorbed from the small intestines is relatively low (Beever, 1980). Supplementation of silage with concentrate feeds containing high levels of crude protein has been shown to reduce the substitution rate of dairy cows (Reeve, Baker and Hodson, 1986). Therefore to avoid this trial becoming an investigation into protein supplementation the concentrate feeds were balanced to be iso-nitrogenous. From the intakes recorded over the digestibility period it would appear that inclusion of fishmeal in the silage as opposed to the concentrate supplement does not have an effect on the daily intake of dairy cows in mid lactation.

Only a slight, non significant, reduction in intake of 5% was seen when the level of lactic acid in the silage was raised to over 160 g/kgDM, towards the maximum (180 g/kgDM) likely to be encountered on a silage diet (Chamberlain et al, 1983). This result is similar to

the finding of the preliminary trial. Additions of lactic acid to silage have been shown to have varying results in different species. McLeod et al (1970) found that raising the lactic acid level of a silage diet to 11.3% of the DM depressed intake by sheep by 22%, whilst additions of lactic acid to a pelleted grass diet to a level of 9.0% of the DM had no significant effect on the intake by sheep. Additions of lactic acid at a rate of 50 g/kgDM offered to silage prior to feeding to beef steers reduced the intake by 12% (Thomas et al, 1980).

To investigate the role of physical fill on silage intake total rumen contents were removed from the animals at three times during the day, pre-feeding at 07:30, at 11:00 after the first and main meal of the day and at 18:00, two hours after the p.m. silage was offered. It was evident that the amount of digesta in the rumen peaked at 18:00 in terms of DM, OM, and NDF. It would appear that some other factor apart from 'physical gut fill' was involved in the cessation of the first meal and short-term silage intake in general.

These results are in agreement with the work of Campling (1966) who found that non-lactating dairy cows offered a hay or silage (of similar digestibility) for 5 hours, stopped eating the silage at a lower weight of digesta in the rumen. Similar results have been obtained with young steers fed either hay or silage at restricted levels (20 gDM/kgLW); the weight of digesta in the rumen following the end of the first meal and at peak fill of silage was lower than that of hay (Thiago and Gill, 1986). Waldo et al (1966) compared the weight of rumen digesta of heifers following the ingestion of a variety of hays and silages with D-values ranging from 53 to 72, and found the weight of digesta in the rumen of the silage fed cows was constantly lower than that of hay fed cows. The conclusion that can be drawn from all these experiments and from the evacuations carried out in this trial is that factors apart from rumen fill limit the voluntary intake of silage.

It should be noted that the D-valuesof the diets used in this trial were around 0.66. This is at the top end of the range (0.67) at which Conrad et al (1964) suggested that ruminant intake was controlled by physical factors, and above which the importance of metabolic regulation of VFI increases. Whilst a D-value of 0.67 should not be regarded as a clear-cut value where the importance of physical fill on the limitation of intake abruptly changes to a metabolic control, it is apparent that in this trial it was not only physical gut fill that was restricting the intake of silage during the day. Gill et al (1988) suggested that when silages diets are fed to dairy cows both types of intake limitation, physical and metabolic, may be operating simultaneously in the control of individual meal size, but their relative importance may vary at different times of the day.

Whilst there was significant change in the amount of digesta in the rumen that may be involved in intake limitation, there was little change in any of the measured rumen parameters that may be implicated in the metabolic control of short-term voluntary silage intake recorded during the pre-infusion periods.

During infusions of both lactic acid and a combination of acetic and propionic acids intake was reduced on all three diets but no interaction between the diets and infusions was seen. Infusion of AP reduced intake to a greater extent than LC at two hours post infusion, and in terms of the first and main meal of the day, although there was no significant difference in the intake response between the two treatments. Daily intake was unaffected by the organic acid infusions, nor were the meal patterns defined by the number and size of meals consumed over the day.

The depression in silage intake caused by the lactic acid infusion is in agreement with the result obtained during experiment 2, where the same infusion of lactic acid reduced the intake of hay and silage fed steers. Very little work has been conducted to investigate the effects of these three organic acids on silage intake of dairy cows. However, work with hay-fed lactating dairy cows showed that intake was reduced by 5% following intra-ruminal infusions of lactic acid (Montgomery et al, 1963).

The depression in intake following the infusion of AP was similar to the results obtained by Mbanya (1988) in a comprehensive study into the role of acetic and propionic acids in the limitation of silage intake at The University of Leeds. Working with the sodium salts of the two acids he found that when infused alone, 12 moles of sodium acetate infused for three hours reduced intake by 50.6% whilst 4-8 moles of propionate reduced intake by 54%. When these two acids were infused together at the same level in a separate trial silage intake of cows at a later stage of lactation was reduced by 13%. It should be noted that no measure of rumen fluid osmolality was recorded in these trials. The levels of acetic and propionic acids used in this trial are lower than those used by Mbanya, but not surprisingly they are above the level of 69 g of acetic acid used to reduce the voluntary intake of silage fed sheep by 46.4% (Wilkins et al, 1971).

It was anticipated that the response to acid infusions would differ across the diets. Additions of lactic acid to silages reduced the intake of silage fed beef steers but were alleviated by the subsequent addition of fishmeal to the silage (Thomas et al, 1980). This was attributed to fishmeal lowering the protein:energy ratio of the diet, and increasing the amount of protein reaching the small intestines. Since lactic acid is predominantly metabolised to

acetic and propionic acids in the rumen of silage fed ruminants (Gill et al, 1986), adding it to silage may alter the protein:energy ratio which subsequent additions of fishmeal to the diet would reverse. However, in light of these results it would have been expected that infusions of AP into the rumen would not have depressed the intake of the silage/fishmeal fed dairy cows which was not the case.

Ruminal lactic acid concentrations rose following infusion of LC, reaching a maximum of 19.1 umol/ml, but returned to levels similar to the control group immediately after the infusion ceased. There was no apparent difference in the increase in the concentration or molar proportions of acetic and propionic acids (the end-products of lactic acid metabolism) in the rumen following infusion of either water or lactic acid, although propionate levels were slightly higher after infusion of LC. Infusions of AP did result in significant increases in ruminal acetic and propionic acid concentrations and this was accompanied by a significant drop in ruminal pH.

It would be difficult to conclude however that it was the ruminal concentrations of acetic and propionic acids that were responsible for the limitation of voluntary intake, since ruminal pH declined to a similar level during infusion of LC when no concurrent increase in acetic and propionic acids were detected. It would therefore appear that in this trial ruminal pH was playing a major role in the regulation of short-term intake.

The pH fell to minimum values of 5.63 (LC) and 5.59 (AP), thirty minutes before the end of the infusion, and after the cessation of the first meal. These values are extremely low and below the levels at which cellulolysis is impeded, and lower than the values recorded in experiment 2. This difference was probably due to the feeding of the concentrate feed 30 minutes after the a.m. silage, the rapid digestion of which would have assisted the decline in pH. It is documented that pH receptors are present in the wall of the anterior rumen and reticulum, sensitive to the rumen pH irrespective of the acid applied (Critchlow and Leek, 1981) and the reduction of rumen pH below pH 6 can reduce voluntary food intake (see Baile and Forbes, 1974).

In conclusion whilst two hour infusions of both lactic acid and acetic and propionic acids limit the voluntary intake of silage fed cows in the short-term, these reductions in intake would appear to be as a result of the decline in ruminal pH as opposed to the presence of high concentrations of any specific acids (see Forbes and Barrio, 1992 (in press)). The response to organic acid infusion was the same whether the cows were already adapted to a silage diet with a high lactic acid content, one with a protein supplement mixed through it or silage fed

on its own.

Experiment 4

The effect of three levels of intra-ruminal lactic acid infusions on the voluntary intake of silage-fed steers.

A. Introduction

In experiment 2 the effect of intra-ruminally infusing 32 g of lactic acid/kgDMI over various time periods on the voluntary intake of hay- and silage-fed steers was investigated. It was found that when lactic acid was given over two hours the amount of roughage eaten was reduced in the short-term, although no difference in response was observed between the two diets. The same amount of lactic acid depressed the short-term intake of silage-fed dairy cattle in experiment 3.

In this trial the effect of intra-ruminally infusing different amounts of lactic acid over a two hour period on voluntary intake of silage and concurrent changes in rumen fluid parameters was investigated using Friesian steers. This is in contrast with experiment 2 where the effect on VFI of varying the length of time of administration of one level (32 g) of lactic acid was investigated. The rumen fluid samples were to be analysed for VFA's and ammonia content using traditional wet chemistry analytical procedures. The samples were then to have been scanned using NIRS so that the NIRS wavelength spectra could be correlated with the analytical results. This would have enabled the development of NIRS for use in the rapid analysis of rumen fluid samples. However, the losses of the relevant equipment, due to financial constraints at I.G.E.R., have prevented this work from being undertaken.

B. Materials and methods

1. Animals

Four Friesian steers that had previously been fitted with rumen, duodenal and ileal cannulae had an average initial liveweight of 388 kg and were housed throughout the experimental periods in modified cow standings (See experiment 1). Before the trial commenced the steers were kept in individual resting pens with aslattedfloor similar to that in the standings.

2. Feeds and feedings

Silage was fed ad libitum (previous days intake + 15%) as the sole feed throughout the experiment. Fresh feed was offered at 09:00 h and 16:00 h and was withdrawn from the

animals at 08:30 h, 23.5 hours after first being offered. Water was available at all times from automatic drinking bowls. The steers had continual access to mineral licking blocks (Baby Red Rockies, Tithebarn.). In addition 100 g of mineral supplement was added to the silage at feeding.

The silage was cut on the 21 May 1990 from a sward of perennial ryegrass (Lolium pe renne, cv Endura) and ensiled in a clamp silo after treatment with Add-Safe at 3.6 l/tonne. Fresh silage was removed from the clamp daily and immediately offered to the steers.

3. Experimental design.

The four steers were used in a 4x4 latin square to investigate the effects of three levels of lactic acid infusion on the intake of steers in relation to a control infusion of water.

The steers were adapted to the experimental silage over the course of 14 days whilst in the resting pens, before being moved to the modified cow standings where they remained for the following 25 days of the trial.

4. Measurements

In vivo whole tract digestibility was recorded between days 1 and 11 of the experimental period. Total daily intake was recorded throughout the digestibility period and the rate and pattern of silage intake were recorded on each day of infusion. Rumen samples were taken on days of infusion at 08:45, 08:55, 09:15, 09:30, 09:45, 10:00, 10:15, 10:30, 11:00, 12:00, 13:00, 14:00, 15:00 and 16:00h.

5. Infusions

The 4 x 4 latin square of infusions consisted of a control infusion of water and three levels of lactic acid infusions 16 (L), 32 (M) and 48 (H) g/kgDMI. The infusions commenced when fresh silage was offered at 09:00 h and lasted for two hours at a rate of 900 ml per hour. Thirty two grams of lactic acid/kgDMI had previously reduced the intake of silage and hay fed steers (experiment 2), and dairy cattle fed modified silage rations (experiment 3) when infused over two hours. The other two levels of lactic acid infusion were either half the strength of this level, infusion L, or half as strong again, infusion H.

6. Statistical analysis

All data obtained during the trial were analysed by ANOVA using Genstat 5, release 1.3. Where there appeared to be a dose related depression in intake following lactic acid infusion the data were analysed further. Using GLM (General Linear Model, Minitab, Release 7.1), covariance analysis was performed in which the difference between animals was accounted for before regression analysis was performed between the intake data and the amount of lactic acid infused.

C. Results

The chemical composition of the silage is shown in Table 40. The silage had a relatively high dry matter (321.0 g/kg) and was well preserved, having a lactic acid content of 81.6 g/kgDM and a low concentration of butyric acid (0.7 g/kgDM) and ammonia-N (0.09 g/ g total N).

The silage had an <u>in vivo</u> D-value of 0.69 and this was reflected in the good average daily intake of 19.3 gDM/kgLW, measured over the eight day digestibility period.

Table 40

The chemical composition of the silage.

pH		3.96
DM	(g/kg)	321.0
OM	(g/kgDM)	930.8
total-N	"	24.1
NH_3N	"	2.28
NDF	"	561.1
ADF	"	307.9
lactic acid	"	81.6
acetic acid	"	11.5
propionic acid	"	1.8
i-butyric acid	"	0.6
n-butyric acid	"	0.1
D-value		0.69

Compared with the control infusion (water), individual intra-ruminal infusions of the three concentrations of lactic acid (L, M and H) did not significantly ($P > 0.05$) reduce either the total daily intake or the short-term intake of the steers (Table 41, Figure 23). However, regression analysis showed that total daily intake and short-term intake up to three hours post feeding were significantly depressed with increasing concentration of lactic acid.

Table 41

The effect of intra-ruminal infusions of lactic acid on the intake (gDM/kgLW) of silage fed steers at various times post feeding.

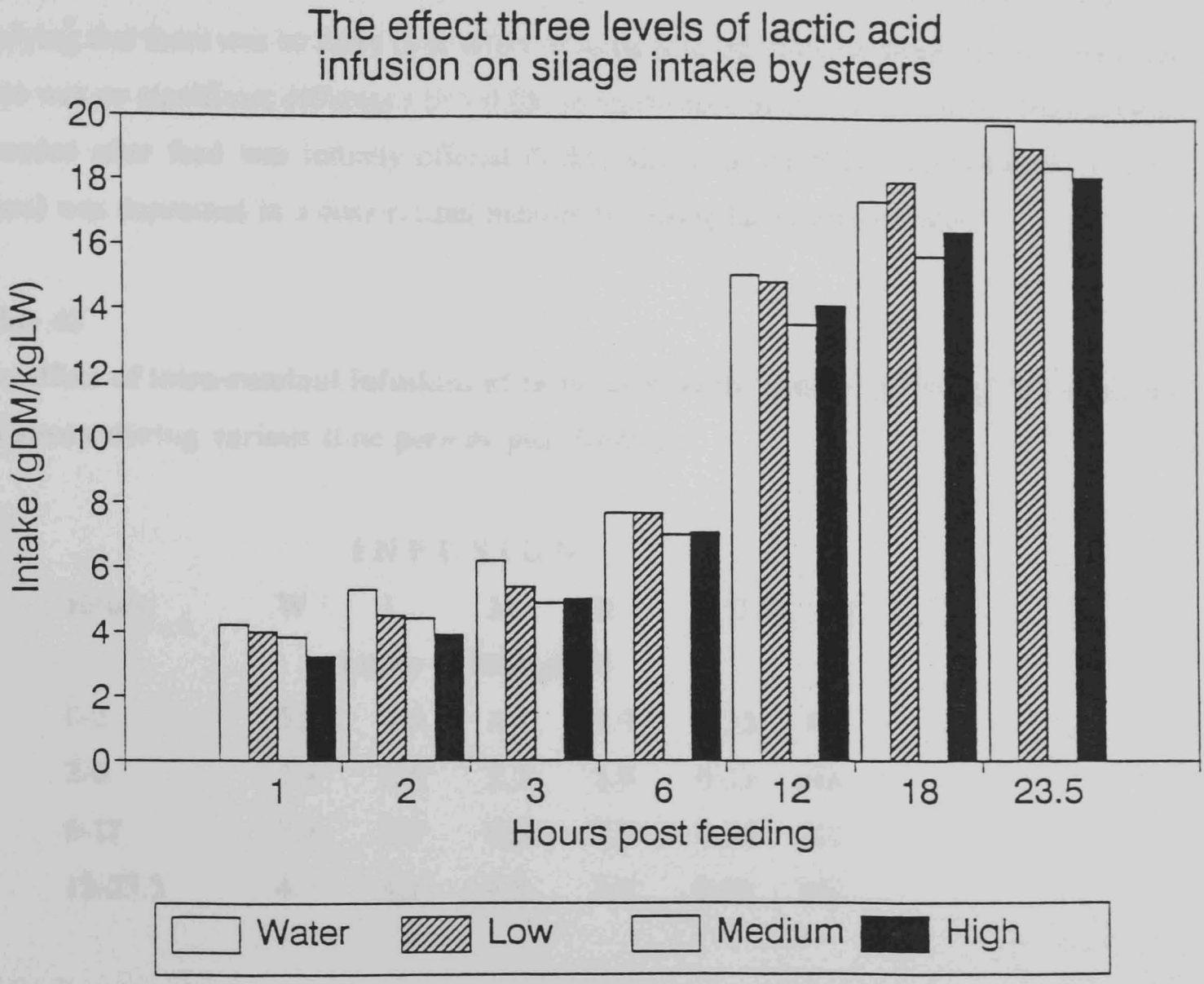
	INFUSION					
Hours post feed	W	L	M	H	S.E.M.	sig
	Intake (gDM/kgLW)					
1	4.2	4.0	3.8	3.2	0.38	NS
2	5.3	4.5	4.4	3.9	0.33	NS
3	6.2	5.4	4.9	5.0	0.38	NS
6	7.7	7.7	7.0	7.1	0.48	NS
12	15.1	14.9	13.6	14.2	0.57	NS
18	17.4	18.0	15.7	16.5	0.50	NS
23.5	19.8	19.1	18.5	18.2	0.21	NS

Regression Equations

Hours post feed	Equation	sig
1	$y = 4.26 - 0.020\ (\pm 0.0088)\ x$	0.046
2	$y = 5.27 - 0.028\ (\pm 0.0054)\ x$	0.001
3	$y = 6.18 - 0.029\ (\pm 0.0076)\ x$	0.004
6	$y = 7.65 - 0.018\ (\pm 0.0104)\ x$	0.110
12	$y = 15.18 - 0.026\ (\pm 0.0133)\ x$	0.080
18	$y = 17.40 - 0.027\ (\pm 0.0167)\ x$	0.142
23.5	$y = 19.67 - 0.034\ (\pm 0.0091)\ x$	0.004

Where y = silage intake (gDM/kgLW) and x = g lactic acid/kgDMI infused.

Figure 23


The effect three levels of lactic acid infusion on silage intake by steers
Intake (gDM/kgLW)
0
2
4
6
8
10
12
14
16
18
20
1
2
3
6
12
18
23.5
Hours post feeding
Water
Low
Medium
High

At the end of the infusion period, two hours after feeding, the intake of silage was reduced by 15%, 17% and 26% of that of the control group by infusions L, M and H respectively. Intakes remained significantly depressed in a dose related manner following lactic acid infusions up to three hours post feeding.

There was no significant difference ($P > 0.05$) in the intakes between treatments during the four hours immediately following the end of the infusion period (Table 42) implying that there was no carry over effect of lactic acid infusions on silage intake. Although there was no significant difference ($P > 0.05$) in intake seen in any of the various time periods recorded after feed was initially offered (Table 42), total daily intake (measured at 23.5 hours) was depressed in a dose-related manner following lactic acid infusion.

Table 42

The effect of intra-ruminal infusions of lactic acid on the intake (gDM/kgLW) of silage fed steers during various time periods post feeding.

	INFUSION					
Hours	W	L	M	H	S.E.M.	sig
	Intake (gDM/kgLW)					
0-2	5.3	4.5	4.4	3.9	0.33	NS
2-6	2.4	2.5	2.7	2.9	0.33	NS
6-12	7.4	7.9	6.6	7.5	0.54	NS
12-23.5	4.7	4.1	4.8	3.9	0.51	NS

Regression Equations

Hours	Equations	sig
0-2 Hours	$y = 6.18 - 0.029\ (\pm\ 0.0076)\ x$	0.004
2-6 Hours	$y = 2.42 + 0.009\ (\pm\ 0.0071)\ x$	0.234
6 -12 Hours	$y = 7.49 - 0.007\ (\pm\ 0.0137)\ x$	0.621
12-24 Hours	$y = 4.64 - 0.010\ (\pm\ 0.0145)\ x$	0.507

Where y = silage intake (gDM/kgLW) and x = g lactic acid/kgDMI infused.

The three levels of lactic acid infusion had no effect on the daily pattern of silage intake by the steers, defined by the number of meals, their average size and length (Table 43). The end of a meal was defined as a pause in eating of greater than 8 minutes, which ensured continuity with the results of the other trials. The total time spent eating was reduced by all the treatment infusions in relation to the control, the greatest being a reduction of 50 minutes, following infusion H, but again this was not significant ($P > 0.05$).

Table 43

The effect of infusions of lactic acid on the number of meals, the time spent eating (minutes), the average meal size (gDM/kgLW) and average meal length (minutes).

	INFUSION					
	W	L	M	H	S.E.M.	sig
No of meals	14.8	15.0	12.3	14.3	1.02	NS
Av. meal size	1.4	1.3	1.5	1.4	0.09	NS
Av. meal length	19.7	19.6	23.9	19.5	2.09	NS
Time spent eating	321	276	281	271	14.4	NS

Following individual intra-ruminal infusions of lactic acid there was no significant reduction in the size and length of the first meal, whether it was defined in a pause in eating of 8, 16 or 24 minutes (Table 44). However, regression analysis showed that the size of the first meal, when defined by a pause in eating of 8, 16 or 24 minutes, was significantly reduced in a dose-related manner by increasing concentrations of lactic acid infusion. The average length of the first meal generally decreased with increasing concentration of lactic acid infusion but this was non significant.

Table 44

The effect of intra-ruminal infusions of lactic acid on the size and length of the first meal.

End of meal pause	Infusion W	L	M	H	S.E.M.	sig
			Size (gDM/kgLW)			
8 minutes	4.1	3.8	3.5	3.3	0.24	NS
16 minutes	4.7	4.3	3.9	3.3	0.38	NS
24 minutes	4.7	4.4	3.9	3.7	0.37	NS
			Length (minutes)			
8 minutes	50.0	42.6	39.4	35.4	7.82	NS
16 minutes	50.8	52.5	49.0	35.4	7.92	NS
24 minutes	50.8	55.6	52.0	40.8	7.66	NS

Regression Equations

End of meal pause	Equation		sig
		Size (gDM/kgLW)	
8 minutes	$y =$	4.05 - 0.016 ($\pm$ 0.006) x	0.026
16 minutes	$y =$	4.71 - 0.030 ($\pm$ 0.008) x	0.005
24 minutes	$y =$	4.71 - 0.026 ($\pm$ 0.009) x	0.012

Where y = meal size (gDM/kgLW) and x = g lactic acid/kgDMI infused.

		Length (minutes)	
8 minutes	$y =$	45.51 - 0.137 ($\pm$ 0.201) x	0.510
16 minutes	$y =$	54.44 - 0.312 ($\pm$ 0.212) x	0.171
24 minutes	$y =$	54.86 - 0.210 ($\pm$ 0.642) x	0.234

Where y = meal length (minutes) and x = g lactic acid/kgDMI infused.

The ruminal concentrations of lactic acid significantly increased within the rumen during infusion of all three levels of lactic acid (Table 45 and Figure 24).

Table 45

The effect of lactic acid infusions on the ruminal concentration of lactic acid (mmol/l).

	I N F U S I O N					
Time	W	L	M	H	S.E.M.	sig
	Lactic acid concentration (mmol/l)					
08:45	0.39	0.28	0.06	0.21	0.168	NS
08:55	0.12	0.06	0.03	0.17	0.055	NS
09:15	1.02	2.89	8.72[b]	7.01[b]	1.627	0.049
09:30	1.44	5.73	18.05	17.88	4.300	NS
09:45	1.58	8.08	20.80[a]	33.18[c]	4.922	0.016
10:00	1.07	10.26	15.73	41.48[c]	6.826	0.026
10:15	0.49	9.02	20.05[b]	28.42[b]	4.898	0.029
10:30	0.44	7.55	17.45	21.95	5.220	NS
11:00	0.33	11.35	12.22	14.76	4.777	NS
12:00	0.15	9.97	7.13	11.71	4.000	NS
13:00	0.43	2.87	6.11	8.08	3.671	NS
14:00	0.32	0.43	1.23	4.04	2.100	NS
15.00	0.25	2.56	1.24	1.08	0.681	NS
16:00	0.22	0.75	1.63	1.03	0.490	NS

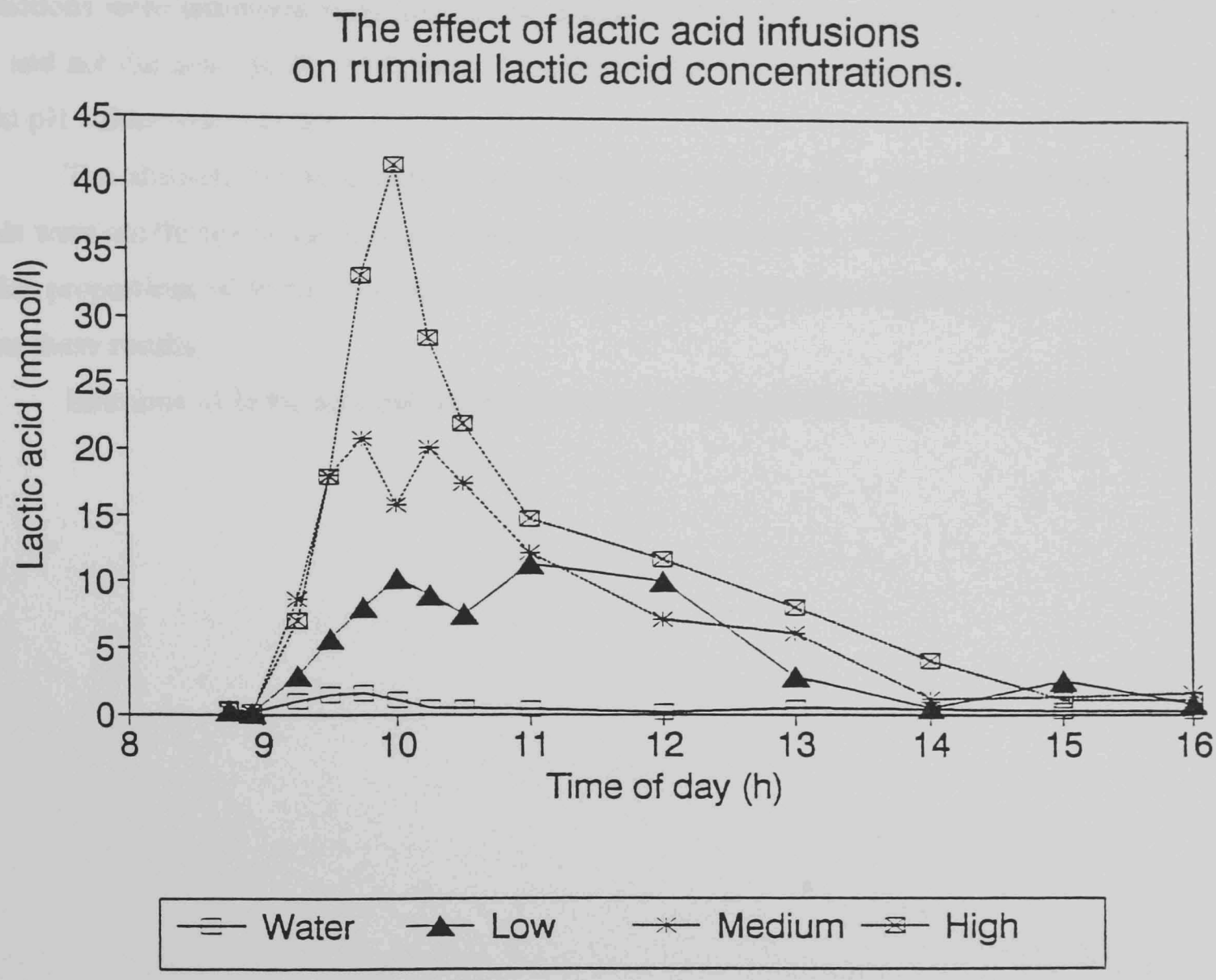
Across rows superscripts indicate a significant difference from the control (a=P<0.05, b=P<0.01 and c=P<0.001).

Regression equations where there was a significant effect at the time points above.

Time	Equation	sig
09:15	$y = 2.991 + 0.538\ (\pm 0.180)\ x$	0.012
09:45	$y = -0.076 + 0.717\ (\pm 0.211)\ x$	0.006
10:00	$y = 3.021 + 0.476\ (\pm 0.161)\ x$	0.013
10:15	$y = 3.581 + 0.254\ (\pm 0.147)\ x$	0.057

Where y = lactic acid concentration (mmol/l) and x = g lactic acid/kgDMI infused.

Figure 24


The effect of lactic acid infusions
on ruminal lactic acid concentrations.
Lactic acid (mmol/l)
45
40
35
30
25
20
15
10
5
0
8
9
10
11
12
13
14
15
16
Time of day (h)
Water
Low
Medium
High

These remained elevated in the three hours after the end of the infusions. At one hour post feeding, during the middle of infusion H, the highest concentration of lactic acid, 41 mmol/l, was recorded.

Following intra-ruminal infusions of lactic acid, rumen fluid pH declined (Table 46, Figure 25) in relation to the control. Rumen pH was significantly reduced forty five minutes after the onset of the three treatment infusions in relation to the control. However, the reductions were somewhat surprising as the greatest fall in pH occurred following infusion M, and not the more acidic infusion H. Two hours after the end of the infusions all rumen fluid pH values were similar.

The absolute concentrations of ruminal VFA's, total, acetic, propionic and butyric acids were unaffected by lactic acid infusions (Appendix Tables C.1, C.2, C.3 and C.4). The molar proportions of these acids were not affected by the infusions and have been omitted from these results.

Infusions of lactic acid did not affect rumen fluid osmolality (Appendix Table C.5).

Table 46

The effect of intra-ruminal infusions of lactic acid on rumen fluid pH.

	INFUSION					
Time	W	L	M	H	S.E.M.	sig
			pH			
08:45	6.59	6.66	6.73	6.73	0.078	NS
08:55	6.73	6.72	6.65	6.68	0.045	NS
09:15	6.52	6.43	6.39	6.54	0.089	NS
09:30	6.59	6.25	6.27	6.43	0.076	NS
09:45	6.57	6.20^{b}	6.26^{b}	6.30^{a}	0.072	0.043
10:00	6.53	6.20	5.68	6.26	0.333	NS
10:15	6.57	6.13	5.54	6.20	0.385	NS
10:30	6.52	6.18	5.50	6.26	0.396	NS
10:45	6.47	6.06	5.41	6.22	0.410	NS
11:00	6.43	6.31	5.97	6.56	0.194	NS
11:30	6.44	6.37	6.05	6.47	0.167	NS
12:00	6.44	6.42	6.10	6.51	0.123	NS
13:00	6.46	6.44	6.37	6.58	0.090	NS
14:00	6.51	6.59	6.46	6.58	0.053	NS
15:00	6.50	6.45	6.52	6.57	0.074	NS
16:00	6.39	6.40	6.45	6.49	0.103	NS

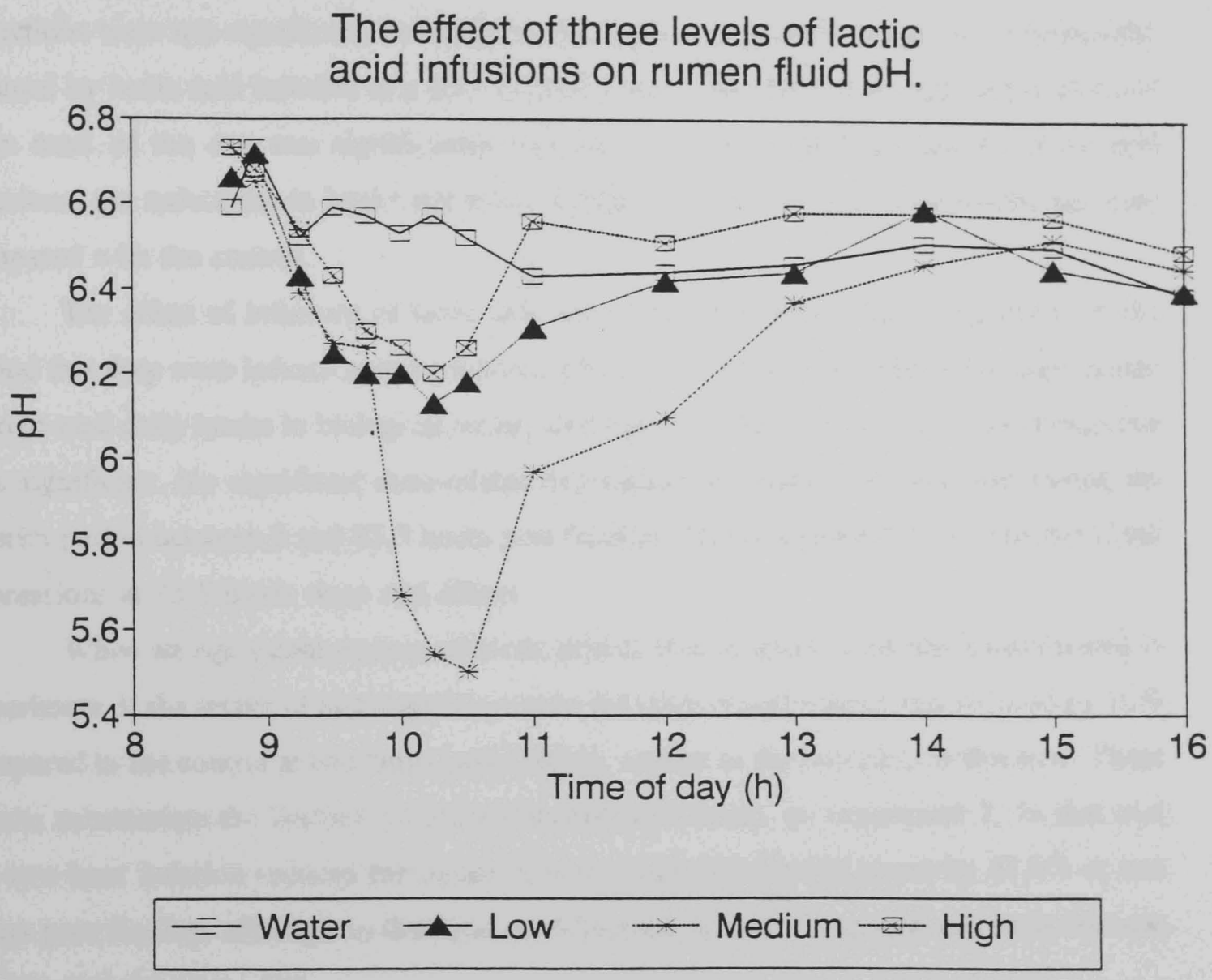
Across rows, superscripts indicate a significant difference from the control (a=$P<0.05$ and b=$P<0.01$).

Regression equation at the time point above where there was a significant effect.

Time	Equation	sig
09:45	$y = 6.444 - 0.0048\ (\pm\ 0.0026)\ x$	0.096

Where y = rumen fluid pH and x = g lactic acid/kgDMI infused.

Figure 25


The effect of three levels of lactic acid infusions on rumen fluid pH.
pH
6.8
6.6
6.4
6.2
6
5.8
5.6
5.4
8
9
10
11
12
13
14
15
16
Time of day (h)
Water
Low
Medium
High

D. Discussion

Intra-ruminal infusions of three levels of lactic acid significantly reduced the short-term and the total daily intake of silage fed steers in a dose related manner. Infusions L, M and H caused reductions in intake of 15.1%, 17% and 26.5% respectively in relation to that of the control at the end of the two hour infusion period. When analysed individually these reductions were not significant, but analysed by regression analysis intake was significantly reduced by lactic acid infusion in a dose related manner. In addition the size of the first and main meal of the day was significantly reduced in a dose related manner by lactic acid infusions, the reductions in intake not being significant when the individual treatments were compared with the control.

The effect of infusions of lactic acid were only short-term, depressing intake in the period that they were infused and the following hour. It is difficult to explain the depressions seen in total daily intake in biological terms, although the reduction seen in intake at this time was significant. No significant dose-related depressions in intake were observed during the interim period between 3 and 23.5 hours post feeding. This would have been expected if the depressions at 23.5 hours were real effects.

When an equivalent amount of lactic acid to that of infusion M was administered in experiment 3, the intake of lactating dairy cattle fed silage based rations was reduced by 16% compared to the control at two hours post feeding, similar to the reduction in this trial. These results substantiate the findings of those obtained with steers, in experiment 2. In that trial the two hour infusion reduced the intake of both silage and hay fed steers by 42.6% at two hours post feeding, although in that trial no difference in intake response was seen between the hay and the silage diet.

The size of silage meals tends to be smaller and the frequency greater than those of hay (Thiago and Gill, 1986) when fed to young cattle, this limitation being caused by silage fermentation end-products in general (Gill et al, 1988). Infusions of a similar level of lactic acid to that of infusion M, into the rumen of goats during bouts of eating of concentrates reduced meal size and resulted in a lower total daily intake (Conrad et al, 1977). The pattern of intake in this trial was unaffected by the lactic acid infusions with the number, size and length of meals unaffected by lactic acid infusions. However, the total time spent eating was up to 16% lower than the control following infusion H (321 compared with 271 minutes), although this was not significant. In the work of Conrad et al a 9% reduction (non significant)

in the total daily time spent eating was reported following lactic acid infusion.

The total amount of lactic acid administered with infusion H was in excess of the maximum value likely to be encountered on a high lactic acid silage, 180 g/kgDM (Chamberlain et al, 1983). The amount of lactic acid obtained from the silage and infusion L was below this level, whilst that obtained from infusion M was equivalent to it. Expressed as moles, the amount of lactic acid infused in this trial ranged from 1.14 moles, infusion L, to over 4.19 moles with infusion H, the exact value depending on the individual animal. Wilkins and Valdemoro (1973) found that as little as 1.5 moles of lactic acid infused over a 24 hour period reduced the intake of silage fed sheep.

In this present study pH fell below 6.0 during infusion M to a level of 5.41, 15 minutes before the end of the infusion. However, the more concentrated infusion H did not produce such a dramatic decline in rumen fluid pH. This may suggest it was a problem of taking rumen fluid samples in close proximity to the end of the infusion line that caused this reduction. This should not have occurred since the sampling probe was weighted to drop down in the rumen away from the infusion line. Depressions in intake caused by infusions of lactic acid have been attributed to problems of administration of an acid into the rumen. Morgan and L'Estrange (1977) concluded that lactic acid did not affect voluntary intake of sheep, where depressions in intake were seen following infusions it was said to be as a result of acid 'hot spots' on the rumen wall.

The amount of lactic acid in the rumen was related to the concentration of the infusate, with levels being elevated up to three hours after the end of the infusion. There was no increase in the concentration of the three primary volatile fatty acids, Ac, Pr and Bu following lactic acid infusions, a similar result to that obtained in experiments 2 and 3. The main end-products of lactic acid metabolism are acetic and propionic acids (Gill et al, 1986; Chamberlain et al, 1983), the relative proportions depending on the concentration of lactic acid in the diet. In experiment 2 increases in the molar proportions of propionic and butyric acids and a decrease in the proportion of acetic acids were observed, whilst in this present study no differences in the molar proportions of any acids were recorded.

The results of this trial support the findings of experiment 2 in that intra-ruminal infusions of lactic acid significantly reduced the short-term intake of silage fed-steers, althougth it was not possible to conclude the method by which lactic acid reduces intake.

Experiment 5

The use of NIRS to monitor rumen fluid composition

A. Introduction

During the course of these postgraduate studies numerous rumen fluid samples have been analysed to determine the concentrations of various ruminal metabolites in order to investigate their involvement in the regulation of voluntary food intake. The assays employed to measure these parameters are extremely time consuming when dealing with such a large number of samples as were generated during these trials. For example, the analysis of one rumen fluid sample by gas liquid chromatography (GLC) to determine VFA concentration took up to 25 minutes to complete, whilst determination of the amine content took over 5 hours and this did not include the sample preparation time.

Near infra-red spectroscopy (NIRS) has been widely used in many ares of Agricultural Science over the last 20 years as a tool for the rapid analysis of feedstuffs. In addition successful correlations have been obtained between the nutritive value of a feed and NIRS spectra, such as dry matter digestibility (Norris et al, 1976). A preliminary study was conducted to assess the viability of using NIRS to monitor changes in rumen fluid composition and to specifically measure the concentration of rumen fluid volatile fatty acids.

B. Materials and methods

Twenty three rumen fluid samples that were taken from the lactating dairy cows during experiment 3.2 and had previously been centrifuged, acidified and analysed for VFA concentrations by a method of GLC (Fussell and McCalley, 1987) were used in this study. The samples were scanned on an NIRSystems 6250 monochromator in 'transmission' mode.

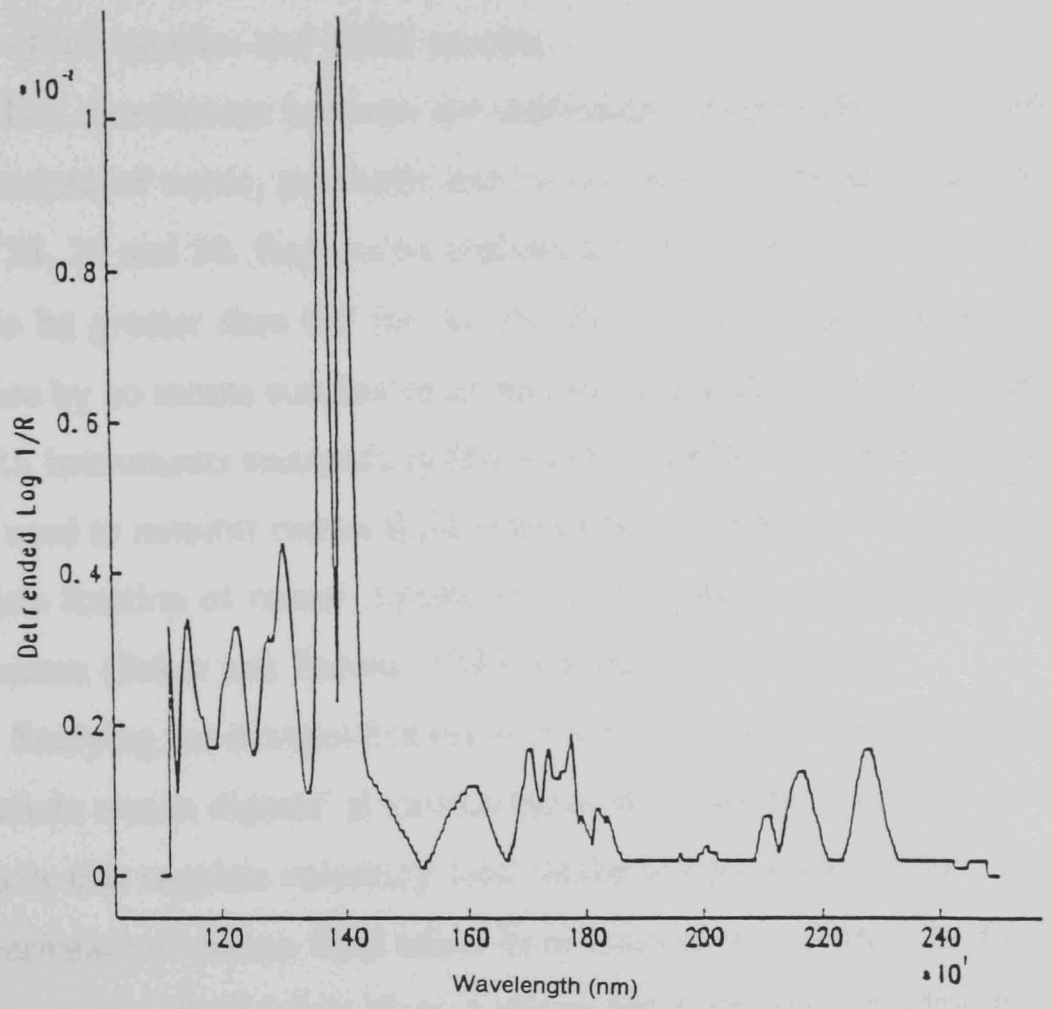
C. Results and discussion

The spectra were treated with the mathematical transformations of standard normal variate (SNV) and de-trending (Barnes et al, 1989). These treatments are applicable to independent samples to remove the scatter effects, path length variations and differences in the baseline shift generally due to variations in particle size in samples. All of these problems interfere with the successful analysis of spectral data.

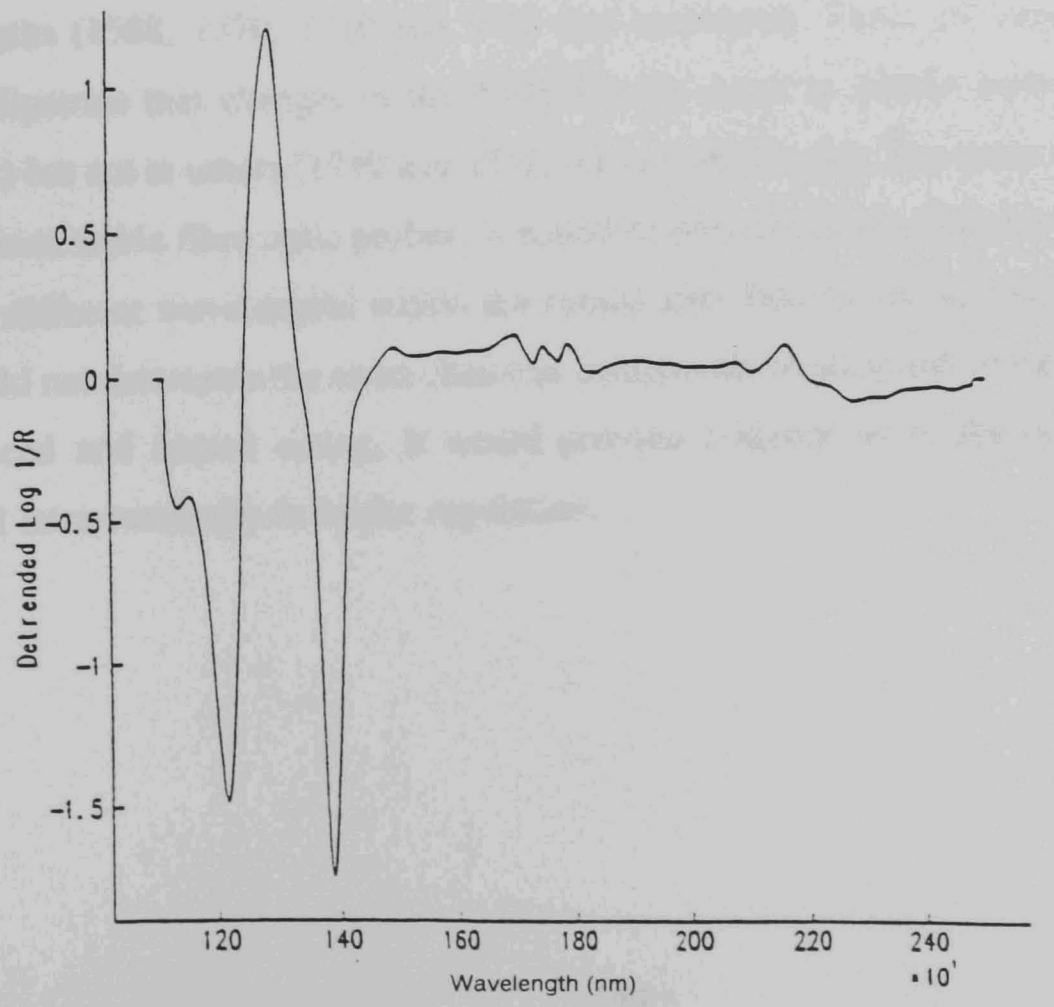
The detrended log 1/R of the mean 23 spectra is shown in Figure 26, along with the standard deviations in Figure 27.

Figures 26 and 27

Standard deviation of log 1/R of the 23 samples



Mean Log 1/R of the 23 samples



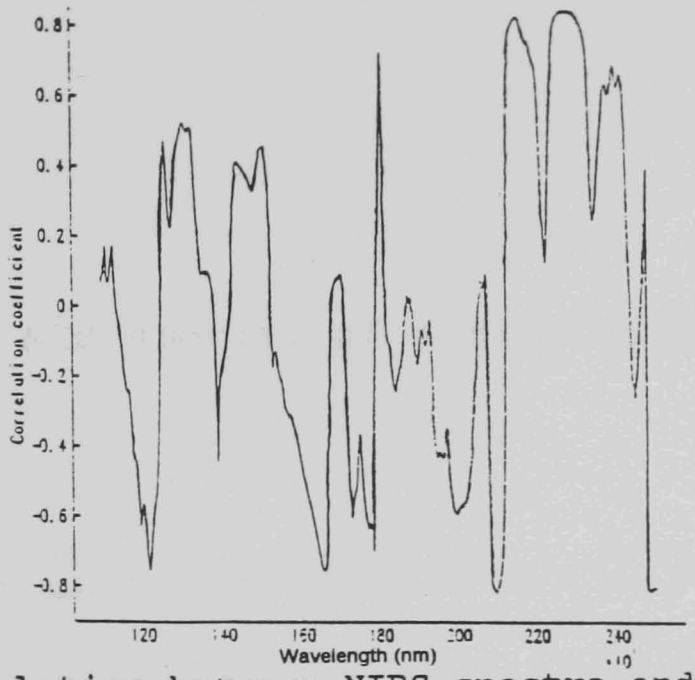
These figures illustrate that variations exists between the spectra produced from the different samples and identifies at what wavelengths spectral differences can be found. These differences within the data set mean that correlations can be made between the wet chemical analysis of the samples and NIRS spectra.

The correlations between the individual wavelengths of the NIRS spectra and the concentrations of acetic, propionic and butyric acids in the samples are shown in Figures 28, 29 and 30. Regression analysis for this small set of data (23 samples) showed R^2 values to be greater than 0.8 for Ac and Pr, whilst not quite so high (0.4) for Bu. These results are by no means conclusive as approximately 100 samples are needed to calibrate and test NIRS instruments successfully (Shenk et al, 1976). However, they do suggest that NIRS may be used to monitor rumen fluid composition. NIRS scans have already been made of the particulate fraction of rumen digesta and used to study the kinetics of cell wall degradation in the rumen (Baker and Barnes, 1990; Givens et al, 1990).

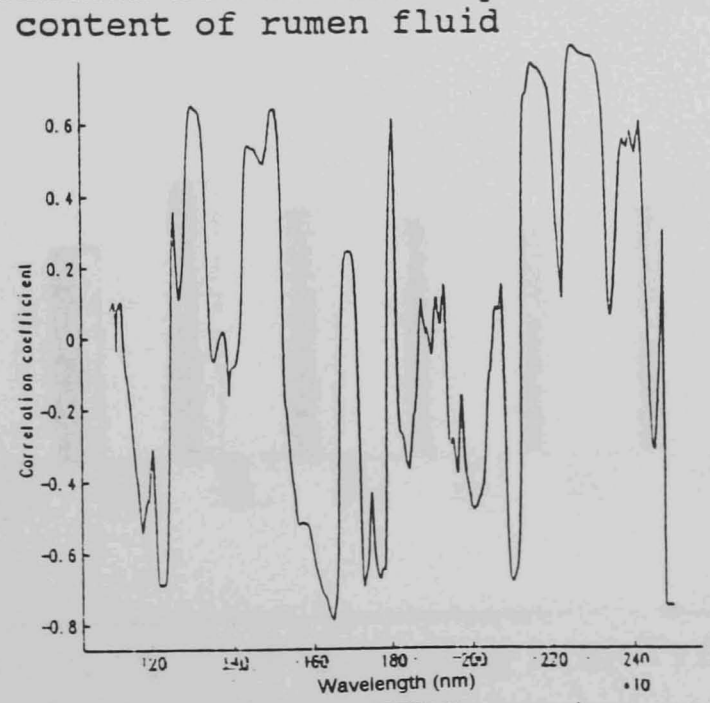
Studying the changes that occur at various wavelengths of the NIRS spectra produced from 'whole rumen digesta' at various times after feeding may increase our understanding of the factors that regulate voluntary food intake in ruminants. Further to the NIRS analysis of the supernatant of rumen fluid taken from dairy cows, changes in the NIRS spectral values of whole rumen fluid taken from a silage fed steer were examined. Rather than try and correlate the spectra to measured rumen fluid parameters the samples were scanned using a fibre optic probe (Perstorp Analytical, Bristol) and the changes in the spectral values at four wavelengths (1568, 1570, 1710 and 1712 nm) monitored. These are summarised in Figure 31 and illustrate that changes in the NIRS spectra occur at certain wavelengths (1568 and 1570 nm) but not at others (1710 and 1712) throughout the day. In a more detailed study, and using rumen stable fibre optic probes, it would be possible to examine the changes that occur at many different wavelengths within the rumen after feeding throughout the day. Although this would not determine the exact chemical compounds peaking and troughing as the animals commenced and ceased eating, it would provide evidence as to the types of compounds involved intra-ruminally in intake regulation.

Figures 28, 29 and 30

Correlation between NIRS spectra and acetic acid content of rumen fluid



Correlation between NIRS spectra and propionic acid content of rumen fluid



Correlation between NIRS spectra and butyric acid content of rumen fluid

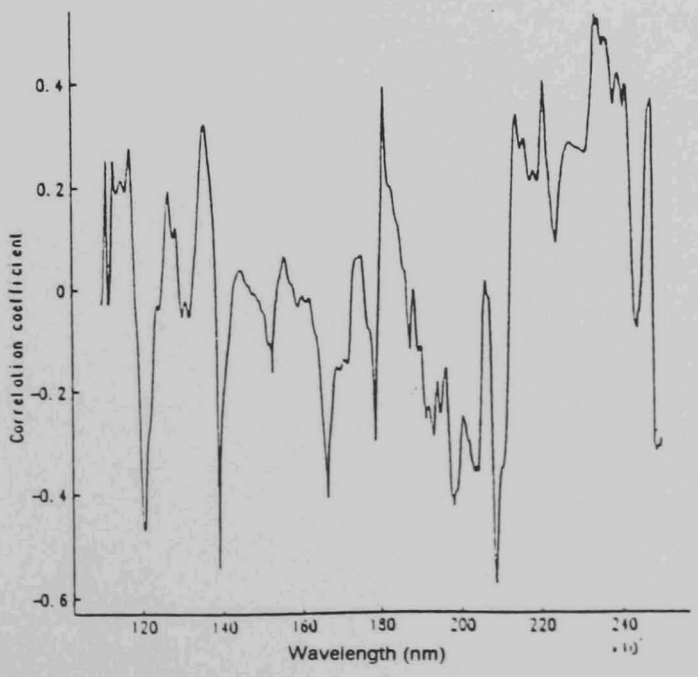
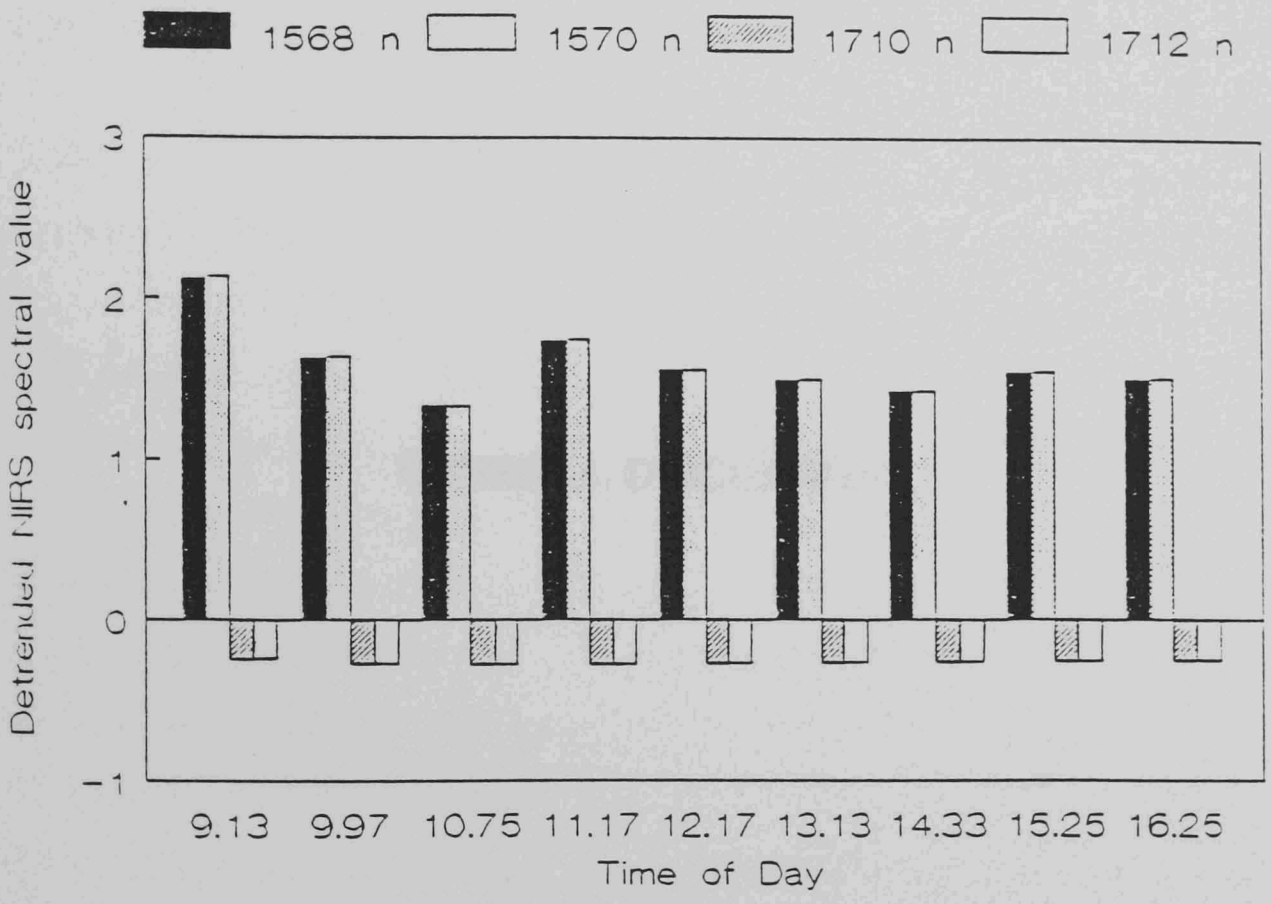


Figure 31


Changes in NIR spectra of rumen fluid with time
1568 n
1570 n
1710 n
1712 n
Detrended NIRS spectral value
3
2
1
0
-1
9.13
9.97
10.75
11.17
12.17
13.13
14.33
15.25
16.25
Time of Day

SECTION D

GENERAL DISCUSSION

General Discussion

The experiments reported in this thesis have investigated the role of short-term intra-ruminal infusions of the end-products of silage fermentation on the daily pattern of roughage intake by cattle.

It is well documented that the intake of ensiled grass is lower than that of the corresponding fresh (Demarquilly, 1973) and dried herbage (Campling, 1966).

Long-term intake limitation of silage has been attributed to a reduction in the nutritive value of the feed caused by the fermentation of water soluble carbohydrates and the degradation of soluble proteins during the ensiling process. This can result in poor microbial synthesis in the rumen when silage is given as the sole feed (Beever, 1980) which ultimately leads to a depression in long term voluntary feed intake (Austin and Gill, 1981).

Although the reasons for this are not fully understood, it would appear that silage intake, at least in the short-term, is primarily regulated by metabolic factors such as the end-products of silage fermentation rather than physical factors such as gut fill (Gill et al, 1986).

The compounds investigated in the course of these trials represent those found in both well preserved (lactic acid) and poorly preserved (urea, to mimic silage ammonia) silages, with the amines reported as occurring in both types of silages (Woolford, 1984).

In the experiments reported in this thesis it is acknowledged that the levels of some of the infusates used have been in excess of those normally found on silage diets, such as the highest concentrations of urea and lactic acid and that of the amines administered in experiment 1. However, these individual fermentation end-products are only one of a number of compounds found in silages that are implicated in intake regulation. Infusions of concentrations greater than the normal physiological levels have been justified in order to induce a depression in intake (Gill et al, 1988).

A considerable amount of work was conducted in the 1960's to determine the amount of amines present in silages (Neumark et al, 1964; MacPherson and Violante, 1966) with little since. The results of analysis of silages made at Hurley in 1987 and 1988 (experiment 1) have showed that they still occur today despite improvements in ensiling techniques over the last 25 years. The greatest quantity of total amines are found in a notoriously poorly preserved silage (Baker et al, 1991) and the single most common amine is Gaba.

Infusions of Gaba and putrescine alone at concentrations above those normally found in silage reduced the intake (non-significantly) of silage fed cattle up to 6 hours past feeding.

The depression in intake seen when these amines were infused in combination was almost double that of when they were infused alone, suggesting possible additivity in the capability of amines to reduce intake. There was no indication of how silage amines might mediate any reduction in intake, although Gaba is a known neurotransmitter involved in the balancing of the satiety and hunger centres of the brain (Morley, 1980).

Gaba has been shown to depress the intake of sheep fed alfalfa pellets when infused alone (Buchanan-Smith, 1982) and sheep fed lucerne silage when infused in combination with other amines including putrescine and the organic acids acetic and butyric (Buchanan-Smith and Phillip, 1986).

It is unlikely that only Gaba and putrescine would be found in a silage. The conditions that favour their production would also give rise to others such as cadaverine, tyramine, histamine and alpha-amino butyric acid. Whilst the involvement of amines in the regulation of silage intake is implied by the results of experiment 1, this is not conclusive. More detailed work needs to be carried out as to the effect of amines on VFI. However, this research may be limited by the very high cost of many of these compounds in the current agricultural economic climate.

Silage ammonia levels have been negatively correlated with the intake of silage by the three major UK classes of ruminant: sheep (Wilkins et al, 1971; Wilkins 1978), dairy cattle (Lewis, 1981) and beef cattle (Rook et al, 1990).

Infusions of urea (experiment 2), to mimic silage ammonia, had no effect on the intake of silage- and hay-fed steers. Intra-ruminal infusions of ammonium salts and urea reduced the intake of concentrate-fed goats (Conrad et al, 1977), although the amount of urea needed to affect intake was substantially more than the amount used here and no account was taken of the osmotic effects of ammonium salts on feed intake.

Whilst ammonia concentrations of silage provide information as to the quality of fermentation, it would appear that silage ammonia per se is not a primary inhibitor of silage intake. Only 0.37 of the reduction in organic matter intake of sheep could be attributed to ammonia-N (Barry et al, 1978) and it has been suggested that it is the products of decarboxylation rather deamination that are the important nitrogenous silage compounds limiting voluntary intake. Where high levels of ammonia are detected this is an indication of clostridial fermentation and the likely presence of amine compounds, these not being routinely analysed for.

Lactic acid infusions into the rumen of silage- and hay-fed steers (experiment 2) and dairy cattle fed silage based rations (experiment 3) significantly depressed short-term intake.

This was substantiated by the findings of experiment 4 in which the intake of silage fed steers was depressed in a dose related manner by increasing levels of lactic acid infusion. Long-term intake of hay fed cows (Montgomery et al, 1963) and silage-fed sheep (Wilkins and Valdemoro, 1973) has been depressed by long-term infusions of lactic acid.

In experiment 2 no difference in response was observed between steers adapted to a high lactic (silage) or a low lactic (hay) diet. However, the results were peculiar in the fact that it was the intermediate concentration (32 g/kgDMI) of lactic acid that invoked the greatest depression in intake with no apparent explanation. This was in terms of both the intake at various times post feeding and the size of the first (and main) meal of the day.

Modifying the silage prior to feeding by increasing the lactic acid content by 50 g/kgDM or by mixing with 500 g fishmeal did not affect the response of cows to lactic acid infusions in experiment 3. Additions of lactic acid to silage reduced the intake of growing steers, which was subsequently alleviated by the addition of fishmeal prior to feeding (Thomas et al, 1980).

The method by which lactic acid reduces voluntary intake is unclear. Lactic acid is known to have a relatively short half life in the rumen of 25 minutes (Chamberlain et al, 1983), being metabolised to acetic and propionic acids the relative proportions depending on the diet fed (Gill et al, 1986).

The epithelial receptors in the rumen reported as being sensitive to acetic acid (Leek and Harding, 1975) are also believed to respond to lactic acid and when stimulated reduce reticulorumen motility (Gregory, 1987). It is believed that larger quantities of lactic acid than VFA's are needed to stimulate these receptors (Upton, 1976) and that lactic acid may induce a response by damaging the epithelial lining of the membrane surrounding them making them more susceptible to VFA's (Gregory, 1987).

Owing to the fact that larger quantities of lactic acid are needed to stimulate these receptors in comparison to VFA's and the fact that lower levels of lactic acid than of acetic acid were detected in the rumen in all experiments, it is unlikely that lactic acid per se was limiting voluntary feed intake.

The main end-products of lactic acid metabolism in the rumen are acetic and propionic acids (Chamberlain et al, 1983; Gill et al, 1986). On silage based diets, acetic acid is the predominant of the two main end-products (Gill et al, 1986). This was not evident from the results of these trials. In experiment 2 a decrease in the molar proportions of acetic acid and an increase in the molar proportions

of propionic and butyric acids was seen following lactic acid infusion, whilst no changes were seen in the proportions of acids following infusions in experiment 4. It would be difficult to comment on the fate of the infused lactic acid in experiment 3. The feeding of a concentrate feed 30 minutes after the start of infusion was likely to have masked any detectable production of volatile fatty acids from lactic acid metabolism, similar levels of propionate and acetate being detected in the rumen of the control and the lactic acid infused animals.

Infusions of acetic and propionic acids, in the molar proportions that 32 g of lactic acid is likely to be metabolised to, resulted in a depression in intake seen greater than that caused by lactic acid, although the difference was not significant ($P > 0.05$). The depressions in intake that occurred in experiment 3 were attributed to a fall in rumen pH, which fell to a mean of 5.6 following both infusions. Below pH 6.0, cellulolysis in the rumen is impeded and reticulorumen motility is affected via stimulation of receptors sensitive to pH, irrespective of the acid applied to them (Critchlow and Leek, 1981). The reduction in intake seen in experiment 4 was not attributed to a fall in rumen fluid pH, since the greatest decrease in ruminal pH was seen following the medium and not the most acidic infusion.

Whilst lactic acid appears to depress the short-term intake of both beef and dairy cattle fed silages and hay, the method by which intake is limited is not fully understood.

Rumen fluid osmolality has been cited as a factor involved in the regulation of feed intake, with osmolality above 400 mOsm/kg depressing the intake of sheep (Bergen, 1972), this figure being above the normal physiological range. The role of osmolality in the regulation of silage intake is unclear. Depressions in the intake of sheep were caused by infusions of either maize silage extracts or NaCl with similar osmolalities, the level of reduction in intake being proportional to the osmolality of the infusate (Phillip et al, 1981).

These results contrast with the findings of these present trials. Infusions of urea (experiment 2) significantly increased the ruminal osmolality of hay and silage fed steers, up to a maximum value of 340 mOsm/kg, although no difference was obtained between the diets, and no depressions in intake occurred. Rumen fluid osmolality was unaffected by both amine infusions (experiment 1) and lactic acid infusions in all experiments, implying that the osmolality of these compounds has little or no part in the control of silage intake.

The role of physical factors, such as gut fill, in the regulation of silage intake is not considered to be of primary importance, especially in the control of cessation of individual meals. However, it should not be discounted entirely since insertions of water filled balloons into the rumen depressed the intake of highly digestible silage (Fahran and Thomas,

1978). In experiment 3, maximum gut fill of silage fed dairy cows in terms of OM, DM and NDF was observed to occur at 18:00 h, 9 hours after the silage was first offered, implying that physical fill was not a primary factor in the regulation of short-term intake. A similar finding was observed by Thiago and Gill (1986) who later suggested that whilst the physical factors and fermentation end-products operate to regulate individual meals of silage, the relative importance may vary throughout the day (Gill et al, 1988) with physical fill assuming more importance later in the day.

Initially one of the main objectives of this research project was to employ the technique of NIRS in an investigation of silage intake, using the samples and experimental data collected at Hurley in previous years. Direct correlation between NIRS spectra and voluntary silage intake would enable rapid prediction of a silage ingestibility. However, calibration of an NIR instrument requires approximately 100 samples (Shenk et al, 1976) and only 20 samples with appropriate intake data were obtained. In addition these samples were dried and ground, the standard form of sample presentation for traditional NIRS methods. Many of the chemical compounds likely to be involved in the limitation of silage intake are volatile (e.g. VFA's, and ammonia) and are lost in any drying processes. Subsequently, any predictions of intake based on the analysis of dried samples are likely to be inaccurate. Modified NIRS machinery capable of scanning fresh silages was not available during the experimental stages of this thesis, although it was initially anticipated that such equipment could be borrowed from the manufacturer.

Monitoring the changes in the concentration of compounds in the rumen following silage ingestion will provide more information about the control of silage intake than either intra-ruminal infusions or statistical correlations of intake and chemical composition. Traditionally this would have involved collecting and sampling rumen fluid samples extremely frequently. The use of a rumen stable fibre optic probe coupled to a NIRS instrument will allow for continual monitoring of rumen fluid composition, enabling quantification of metabolites within the rumen at the end or start of an individual meal.

The control of silage intake is believed to be multifactorial with a number of fermentation end-products acting simultaneously to limit voluntary silage intake, these being more important than physical fill. The experiments reported in this thesis have shown that lactic acid and two of the amines found in silages, putrescine and Gaba, reduce the intake of forage fed cattle when infused intra-ruminally, whilst ammonia appears to have little effect on silage intake limitation. Further work needs to be undertaken to identify the role of other

fermentation end-products such as other amine type compounds (cadaverine, tyramine, tryptamine and alpha amino-n-butyric acid) and organic acids (butyric acid) not investigated in these trials. The effect of silage fermentation end products on intake limitation is likely to be additive and to fully understand the interactions between different metabolites, experiments need to be carried out where the effects on intake of cocktails of silage end-products are investigated.

SECTION E

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SECTION F

APPENDIX TABLES

Table A.1
The effect of diet on the total ruminal VFA concentration (mmol/l).

Sample Time	Hay	Silage
09:15	77.2	74.9
09:30	78.6	72.8
09:45	79.9	83.4
10:00	88.9	94.3
10:15	88.7	103.6
10:30	78.0	101.7
10:45	79.4	104.5
11:00	78.8	107.9
11:30	79.4	98.6
12:30	78.9	96.9
13:30	74.9	86.9
14:30	76.1	91.9
15:30	79.1	92.1
16:30	90.1	98.6
18:30	84.5	112.4
20:30	90.8	121.9
22:30	93.7	112.9
06:30	88.7	80.9

Table A.2
The effect of diet on the ruminal VFA concentration of acetic, propionic and butyric acids (mmol/l).

Sample Time	Hay	Silage	Hay	Silage	Hay	Silage
	Acetate		Propionate		Butyrate	
09:15	55.3	50.8	14.4	14.5	5.3	7.0
09:30	56.1	46.9	15.1	13.7	5.4	6.7
09:45	57.0	56.4	14.9	16.7	5.5	8.0
10:00	63.1	61.0	16.9	20.1	6.2	10.5
10:15	60.9	64.8	16.6	23.5	6.0	11.8
10:30	55.1	63.1	15.1	23.3	5.4	11.7
10:45	56.1	64.3	15.6	21.3	5.4	12.1
11:00	55.5	54.1	15.5	25.5	5.3	13.0
11:30	55.5	58.8	15.9	23.4	5.5	12.3
12:30	55.5	59.3	15.3	21.7	5.6	11.8
13:30	53.3	53.9	14.3	18.8	5.1	10.6
14:30	53.8	56.2	14.6	20.3	5.3	11.3
15:30	55.7	55.5	15.3	21.0	5.7	11.5
16:30	60.2	57.9	16.4	23.2	6.2	13.2
18:30	60.0	64.6	17.4	27.0	6.2	15.6
20:30	64.5	68.8	18.6	25.5	6.6	14.7
22:30	65.4	70.8	18.9	23.3	6.6	12.8
06:30	62.9	54.4	15.8	17.1	5.9	7.9

Table A.3
The effect of diet on ruminal pH.

Sample Time	Hay	Silage	S.E.M	sig
	pH			
09:15	6.92	7.02	0.315	NS
09:30	6.81	6.90	0.630	NS
09:45	6.76	6.87	0.286	NS
10:00	6.73	6.70	0.428	NS
10:15	6.72	6.71	0.510	NS
10:30	6.74	6.67	0.934	NS
10.45	6.75	6.67	0.345	NS
11.00	6.74	6.72	0.473	NS
11:30	6.74	6.62	0.263	NS
12:30	6.70	6.67	0.197	NS
13:30	6.84	6.73	0.349	NS
14:30	6.71	6.66	0.382	NS
15:30	6.65	6.68	0.176	NS
16:30	6.68	6.89	0.386	NS
18:30	6.49	6.69	0.310	NS
20:30	6.69	6.07	0.898	NS
22:30	6.68	6.54	0.652	NS
06:30	6.78	7.00	0.611	NS

Table A.4
The effect of diet on ruminal osmolality.

Sample Time	Hay	Silage	S.E.M	sig
	mOsm/kg			
09:15	226.5	261.8	7.60	NS
09:30	271.7	260.0	6.01	NS
09:45	273.5	272.2	20.80	NS
10:00	277.0	280.0	7.85	NS
10:15	279.8	286.2	5.03	NS
10:30	286.2	290.7	10.20	NS
10:45	282.3	291.2	15.50	NS
11:00	286.7	286.7	24.45	NS
11:30	269.3	259.0	14.06	NS
12:30	263.5	267.3	8.49	NS
13:30	261.2	264.2	3.18	NS
14:30	260.7	273.8	8.49	NS
15:30	252.8	261.2	13.53	NS
16:30	265.2	272.0	7.94	NS
18:30	256.0	271.3	2.51	NS
20:30	255.3	258.8	9.18	NS
22:30	241.5	255.5	17.16	NS
06:30	245.0	258.2	16.52	NS

Table A.5
The effect of intra-ruminal infusions of urea on the intake of hay and silage at various times post feeding

Hours post feeding	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
		Total Daily Intake (gDM/kgLW)					
1	Hay	3.9	2.8	3.8	2.7	0.32	NS
	Silage	4.0	3.4	4.0	3.1		
2	Hay	4.7	4.5	5.0	4.2	0.89	NS
	Silage	5.6	3.4	4.3	4.4		
3	Hay	5.5	5.2	5.0	4.7	0.50	NS
	Silage	6.1	3.6	4.7	4.6		
6	Hay	7.8	6.7	7.2	6.4	0.87	NS
	Silage	8.0	8.2	7.0	6.5		
12	Hay	13.2	12.6	13.3	12.8	0.62	NS
	Silage	13.9	13.3	15.2	12.5		
23.5	Hay	14.9	15.2	15.9	15.8	0.98	NS
	Silage	18.4	15.5	18.8	16.5		

Table A.6
The effect of intra-ruminal infusions of urea on the size and length of the first meal of hay and silage fed steers.

End of meal pause	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
		Size (gDM/kgLW)					
8 mins	Hay	4.7	5.0	4.6	3.9	0.55	NS
	Silage	5.3	3.4	4.2	3.8		
16 mins	Hay	5.1	5.3	5.3	4.2	0.57	NS
	Silage	5.3	3.4	4.3	4.4		
24 mins	Hay	6.1	5.5	5.2	4.1	0.64	NS
	Silage	5.9	4.0	4.8	4.5		
		Length (minutes)					
8 mins	Hay	89.1	109.1	82.8	76.3	12.14	NS
	Silage	92.8	54.4	61.5	63.6		
16 mins	Hay	118.0	113.4	84.7	84.4	14.93	NS
	Silage	95.7	57.6	71.8	68.5		
24 mins	Hay	122.2	115.5	85.1	85.1	13.69	NS
	Silage	110.1	69.0	74.0	71.7		

Table A.7
The effect of intra-ruminal infusions of urea upon rumen fluid pH of hay and silage fed steers.

Time	Length of infusion (h). Water	One	Two	Three	S.E.M	sig
		p H				
09:15	6.91	6.91	6.88	6.89	0.163	NS
09:30	6.84	6.79	6.79	6.85	0.064	NS
09:45	6.68	6.75	6.75	6.72	0.051	NS
10:00	6.62	6.74	6.68	6.75	0.073	NS
10:15	6.62	6.78	6.68	6.73	0.068	NS
10:30	6.62	6.84	6.71	6.73	0.071	NS
10:45	6.61	6.91	6.70	6.73	0.087	NS
11:00	6.55	7.00	6.74	6.72	0.083	NS
11:30	6.55	7.09	6.90	6.70	0.085	NS
12:30	6.59	7.01	6.95	6.88	0.013	NS
13:30	6.60	6.87	6.82	6.83	0.095	NS
14:30	6.66	6.77	6.66	6.76	0.077	NS
15:30	6.69	6.68	6.60	6.71	0.060	NS
16:30	6.58	6.48	6.60	6.65	0.063	NS

Table A.8
The effect of intra-ruminal infusions of urea upon rumen fluid osmolality (mOsm/kg) of hay and silage fed steers.

Time	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
		Osmolality				
09:15	262.9	267.5	273.2	269.0	6.24	NS
09:30	264.0	266.9	268.4	268.9	3.36	NS
09:45	278.1	290.4	284.0	273.2	4.16	NS
10:00	280.6	319.1^{b}	303.1	283.2	5.15	***
10:15	288.6	335.6^{b}	307.1	286.5	6.01	***
10:30	275.3	328.4^{b}	311.7^{a}	301.3	6.63	**
10:45	283.1	337.0^{c}	306.2^{b}	283.6	5.53	***
11:00	285.0	331.7^{b}	312.4^{a}	286.5	7.16	***
11:30	286.3	319.3^{a}	301.4	290.1	7.82	*
12:30	277.0	306.0	305.4	300.9	8.59	NS
13:30	288.2	288.9	292.0	266.1	7.65	NS
14:30	270.6	275.9	274.7	281.7	6.97	NS
15:30	257.9	267.6	269.1	272.4	6.11	NS
16:30	286.4	284.2	283.9	276.0	4.87	NS

Across rows supercripts indicate a significant difference from the control (a=$P<0.05$, b=$P<0.01$ and c=$P<0.001$).

Table A.9
The effect of intra-ruminal infusions of urea upon rumen fluid osmolality of hay or silage fed steers.

Time	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M	sig
		Osmolality (mOsm/kg)					
09:15	Hay	267.2	266.0	278.4	262.6	8.83	NS
	Silage	264.0	269.0	268.1	275.4		
09:30	Hay	270.2	261.0	278.3	254.4	4.75	NS
	Silage	257.8	272.7	258.4	283.3		
09:45	Hay	274.4	273.3	286.7	261.3	5.89	NS
	Silage	281.8	308.4	281.3	285.2		
10:00	Hay	260.8	291.0	298.1	268.1	7.29	NS
	Silage	293.3	347.2	308.1	298.4		
10:15	Hay	272.7	328.3	294.5	280.3	8.51	NS
	Silage	304.5	343.0	319.8	292.7		
10:30	Hay	248.6	312.4	289.2	303.9	9.39	NS
	Silage	304.0	345.7	335.9	298.4		
10:45	Hay	262.9	319.6	286.2	288.8	7.83	NS
	Silage	303.4	354.4	326.3	278.5		
11:00	Hay	261.0	321.8	290.0	310.0	10.12	NS
	Silage	309.0	341.7	334.8	263.0		
11:30	Hay	258.0	321.1	289.7	299.0	11.06	NS
	Silage	316.5	317.2	314.0	280.7		
12:30	Hay	277.6	290.6	292.1	321.1	12.16	NS
	Silage	276.4	321.4	318.6	280.6		
13:30	Hay	254.6	279.6	285.0	297.3	10.82	NS
	Silage	277.6	296.9	292.8	286.7		
14:30	Hay	268.0	277.5	273.3	271.5	9.87	NS
	Silage	273.3	274.2	276.2	292.0		
15:30	Hay	250.5	267.7	270.1	260.4	8.64	NS
	Silage	265.2	267.5	268.1	284.4		
16:30	Hay	281.8	283.0	277.9	276.1	6.89	NS
	Silage	291.8	285.5	289.9	275.9		

Table A.10
The effect of intra-ruminal infusions of lactic acid on the intake of hay and silage at various times post feeding

Hours post feeding	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
		Total Daily Intake (gDM/kgLW)					
1	Hay	4.1	4.0	2.9	3.4	0.32	NS
	Silage	4.2	3.5	3.1	3.3		
2	Hay	6.8	4.8	3.3	5.2	0.41	NS
	Silage	5.4	4.3	3.6	4.1		
3	Hay	7.3	5.7	3.2	6.1	0.46	NS
	Silage	6.0	4.5	4.6	5.0		
6	Hay	8.5	6.7	4.8	8.1	0.64	NS
	Silage	8.6	6.9	6.6	6.0		
12	Hay	10.6	15.8	6.3	15.9	1.07	NS
	Silage	16.6	10.2	15.7	8.0		
23.5	Hay	16.4	15.4	13.9	16.0	0.99	NS
	Silage	16.6	15.4	15.8	17.9		

Table A.11
The effect of intra-ruminal infusions of lactic acid on the size and length of the first meal of hay and silage fed steers.

End of meal pause	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
		Size (gDM/kgLW)					
8 mins	Hay	6.4	5.4	2.0	5.5	0.43	NS
	Silage	5.4	4.5	3.9	3.6		
16 mins	Hay	7.0	5.7	2.1	5.8	0.50	NS
	Silage	6.1	4.5	3.9	4.0		
24 mins	Hay	7.0	5.7	2.3	5.8	0.53	NS
	Silage	6.3	5.1	3.9	4.3		
		Length (minutes)					
8 mins	Hay	111.1	82.3	32.0	96.4	16.02	NS
	Silage	68.0	83.2	67.5	75.7		
16 mins	Hay	112.5	90.7	32.6	107.1	13.94	NS
	Silage	107.4	83.8	69.0	75.9		
24 mins	Hay	112.6	98.3	32.8	115.8	15.4	NS
	Silage	110.0	94.4	69.6	77.8		

Table A.12
The effect of intra-ruminal infusions of lactic acid upon ruminal lactic acid concentration of hay or silage fed steers.

Time	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M	sig
		Lactic acid concentration (mmol/ml)					
09:15	Hay	0.0	0.0	0.1	0.0	0.10	NS
	Silage	0.3	0.0	0.1	0.2		
09:30	Hay	0.0	0.0	0.0	0.0	0.03	NS
	Silage	0.1	0.0	0.1	0.0		
09:45	Hay	2.5	4.1	2.8	4.5	1.70	NS
	Silage	11.4	3.4	10.0	1.5		
10:00	Hay	0.3	4.2	2.0	4.7	2.81	NS
	Silage	12.6	17.9	16.4	4.4		
10:15	Hay	1.8	7.2	1.9	5.1	5.13	NS
	Silage	10.9	25.0	30.8	5.3		
10:30	Hay	3.9	6.8	1.4	6.3	6.82	NS
	Silage	8.6	28.6	30.0	11.2		
10:45	Hay	1.1	11.1	5.0	11.0	10.57	NS
	Silage	7.2	40.7	28.2	4.8		
11:00	Hay	1.5	15.2	5.9	14.2	6.20	NS
	Silage	8.73	26.6	23.5	0.6		
11:30	Hay	0.8	14.7	3.5	13.4	5.45	NS
	Silage	9.6	16.8	21.9	3.6		
12:30	Hay	1.9	7.2	2.5	14.0	3.85	NS
	Silage	6.4	2.9	14.5	7.1		
13:30	Hay	0.4	4.7	2.3	9.2	6.68	NS
	Silage	2.9	0.5	4.8	5.4		
14:30	Hay	0.4	2.0	1.8	4.2	0.84	NS
	Silage	0.6	0.8	1.4	1.9		
15:30	Hay	0.1	1.3	1.5	1.8	0.93	NS
	Silage	2.7	0.3	0.8	1.2		
16:30	Hay	0.3	1.9	0.2	2.8	0.84	NS
	Silage	2.4	1.0	4.1	4.0		

Table A.13
The effect of intra-ruminal infusions of lactic acid upon rumen fluid pH of hay or silage fed steers.

Time	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M	sig
			p H				
09:15	Hay	6.77	6.78	6.66	6.85	0.059	NS
	Silage	6.93	6.90	6.94	6.90		
09:30	Hay	6.67	6.73	6.57	6.82	0.048	NS
	Silage	6.92	6.82	6.86	6.82		
09:45	Hay	6.68	6.64	6.63	6.71	0.050	NS
	Silage	6.70	6.65	6.68	6.68		
10:00	Hay	6.65	6.74	6.53	6.75	0.068	NS
	Silage	6.50	6.36	6.51	6.45		
10:15	Hay	6.62	6.55	6.47	6.70	0.091	NS
	Silage	6.55	5.94	6.38	6.29		
10:30	Hay	6.50	6.35	6.38	6.79	0.098	NS
	Silage	6.60	5.85	6.39	6.19		
10:45	Hay	6.47	6.45	6.58	6.67	0.104	NS
	Silage	6.49	5.64	6.36	6.29		
11:00	Hay	6.42	6.59	6.28	6.67	0.253	NS
	Silage	6.63	5.89	6.33	6.27		
11:30	Hay	6.38	6.48	6.52	6.63	0.122	NS
	Silage	6.69	6.14	6.28	6.20		
12:30	Hay	6.46	6.46	6.49	6.47	0.099	NS
	Silage	6.62	6.54	6.47	6.30		
13:30	Hay	6.54	6.43	6.57	6.46	0.084	NS
	Silage	6.55	6.63	6.72	6.55		
14:30	Hay	6.52	6.58	6.57	6.55	0.071	NS
	Silage	6.67	6.59	6.77	6.64		
15:30	Hay	6.66	6.58	6.51	6.57	0.074	NS
	Silage	6.75	6.86	6.71	6.66		
16:30	Hay	6.53	6.60	6.61	6.61	0.078	NS
	Silage	6.49	6.54	6.66	6.49		

Table A.14
The effect of intra-ruminal infusions of lactic acid on the total VFA concentration of rumen fluid.

Time	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
	Total VFA conc. (mmol/l)					
09:15	80.0	76.1	82.5	73.5	4.05	NS
09:25	72.9	73.0	73.4	68.7	3.98	NS
09:45	72.8	74.8	76.8	72.1	3.98	NS
10:00	82.1	81.3	81.9	73.3	4.90	NS
10:15	81.2	87.3	84.6	83.3	6.31	NS
10:30	89.5	89.7	102.7	83.5	8.55	NS
10:45	94.3	101.5	85.8	79.6	10.02	NS
11:00	91.9	105.8	97.2	80.3	7.55	NS
11:30	97.8	89.4	81.4	85.2	6.72	NS
12:30	84.0	86.3	81.1	83.0	3.98	NS
13:30	88.2	84.6	78.3	84.1	6.33	NS
14:30	82.3	83.2	76.1	79.5	5.79	NS
15:30	85.5	83.2	78.9	76.6	5.91	NS
16:30	90.8	84.6	84.5	85.4	5.03	NS

Table A.15
The effect of intra-ruminal infusions of lactic acid on the ruminal concentration of acetic acid.

Time	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
	Acetic acid conc. (mmol/l)					
09:15	54.1	49.6	56.8	51.7	3.30	NS
09:25	48.2	51.2	55.0	52.7	1.71	NS
09:45	53.8	49.4	51.6	49.4	2.39	NS
10:00	50.5	48.3	50.0	48.1	2.79	NS
10:15	51.0	53.6	63.0	48.4	2.43	NS
10:30	55.4	54.8	53.6	51.1	2.95	NS
10:45	52.7	56.8	59.2	54.9	5.49	NS
11:00	58.6	61.1	58.8	48.9	5.20	NS
11:30	57.7	54.8	48.2	67.2	6.55	NS
12:30	54.4	56.5	54.3	51.4	2.21	NS
13:30	51.3	52.7	50.2	47.2	2.07	NS
14:30	57.3	51.2	47.3	49.9	2.15	NS
15:30	55.6	51.7	47.4	47.5	2.82	NS
16:30	52.8	49.4	47.3	49.5	2.45	NS

Table A.16
The effect of intra-ruminal infusions of lactic acid on the ruminal concentration of propionic acid.

Time	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
	Propionic acid conc. (mmol/l)					
09:15	17.8	18.1	20.7	18.5	1.32	NS
09:25	15.2	16.3	18.7	17.0	0.96	NS
09:45	16.7	15.0	16.6	14.2	1.20	NS
10:00	15.6	14.0	15.8	14.2	0.91	NS
10:15	16.1	17.1	17.5	15.5	1.23	NS
10:30	19.3	20.2	19.3	17.9	1.18	NS
10:45	19.1	23.0	23.8	20.6	1.33	NS
11:00	22.3	26.1	23.3	19.5	1.33	NS
11:30	25.9	26.0	22.0	22.3	1.42	NS
12:30	20.7	26.2	22.0	22.3	1.31	NS
13:30	18.7	24.1	21.1	21.3	1.14	NS
14:30	20.9	20.9	19.0	22.3	0.92	NS
15:30	19.5	20.2	18.0	19.4	1.17	NS
16:30	17.4	18.5	17.0	19.2	0.82	NS

Table A.17
The effect of intra-ruminal infusions of lactic acid on the ruminal concentration of butyric acids.

Time	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
	Butyric acids conc. (mmol/l)					
09:15	8.7	9.5	10.7	9.7	1.72	NS
09:25	7.3	8.0	9.4	8.1	0.66	NS
09:45	7.8	6.8	7.8	6.7	0.67	NS
10:00	7.3	6.5	7.3	6.8	0.45	NS
10:15	7.3	7.7	7.9	7.2	0.39	NS
10:30	8.7	8.6	8.6	8.0	0.54	NS
10:45	8.5	9.3	11.1	9.1	1.03	NS
11:00	10.1	10.6	10.6	8.7	0.81	NS
11:30	11.3	10.6	10.6	8.6	1.23	NS
12:30	9.5	11.0	10.3	10.3	0.42	NS
13:30	8.7	10.7	10.4	10.5	0.48	NS
14:30	10.6	10.9	10.3	11.6	0.57	NS
15:30	9.6	10.8	9.6	10.2	0.60	NS
16:30	8.4	9.6	10.3	8.4	0.60	NS

Table A.18
The effect of intra-ruminal infusions of lactic acid on the molar proportions of ruminal acetic acid of silage and hay fed steers.

Time	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
	Acetic acid molar proportion					
09:15	66.2	68.2	66.9	68.1	0.78	NS
09:25	67.8	68.5	67.5	68.5	0.74	NS
09:45	67.5	67.7	66.8	67.7	0.61	NS
10:00	65.5	65.5	64.8	66.1	0.47	NS
10:15	64.0	63.4	62.5[a]	64.4	0.37	*
10:30	63.5	61.8	62.0	63.2	0.45	NS
10:45	62.9	60.6	63.5	62.0	0.78	NS
11:00	62.4	59.8	60.8	60.9	0.73	NS
11:30	62.4	58.8[b]	60.3	59.9[a]	0.70	*
12:30	63.2	58.8[c]	59.7[c]	58.9[c]	0.57	**
13:30	63.8	60.4[c]	61.2[b]	58.5[c]	0.53	***
14:30	64.9	61.1[c]	61.6[c]	61.2[c]	0.51	***
15:30	66.2	62.4[c]	62.6[b]	61.2[c]	0.64	**
16:30	63.9	62.4	61.9	62.1	0.69	NS

Across rows supercripts indicate a significant difference from the control (a=P<0.05, b=P<0.01 and c=P<0.001)

Table A.19
The effect of intra-ruminal infusions of lactic acid upon the molar proportions of ruminal acetic acid of hay or silage fed steers.

Time	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M	sig
		Acetic acid molar proportion					
09:15	Hay	70.4	69.9	69.7	70.0	1.10	NS
	Silage	62.3	66.3	64.9	65.6		
09:30	Hay	71.0	71.2	70.5	70.3	1.04	NS
	Silage	64.9	66.6	65.4	66.7		
09:45	Hay	70.4	70.3	70.1	71.0	0.86	NS
	Silage	64.7	63.6	65.1	64.3		
10:00	Hay	70.4	69.6	69.4	69.4	0.66	NS
	Silage	61.2	61.2	60.7	62.4		
10:15	Hay	69.9	68.2	68.0	68.5	0.52	NS
	Silage	59.5	58.1	57.1	60.1		
10:30	Hay	68.6	67.2	67.2	67.9	0.64	NS
	Silage	58.4	56.6	56.7	58.4		
10:45	Hay	68.1	66.1	68.7	66.6	1.11	NS
	Silage	57.1	54.9	57.2	57.1		
11:00	Hay	67.5	65.3	66.4	66.3	1.03	NS
	Silage	57.6	54.1	55.3	55.6		
11:30	Hay	66.9	63.5	65.5	64.8	0.99	NS
	Silage	57.8	53.7	54.9	55.1		
12:30	Hay	66.8	62.5	64.0	63.3	0.81	NS
	Silage	59.0	55.2	55.2	54.9		
13:30	Hay	68.3	63.2	64.5	62.4	0.75	NS
	Silage	59.4	57.5	57.8	54.6		
14:30	Hay	64.5	64.2	65.0	63.6	0.84	NS
	Silage	61.8	57.8	58.4	58.6		
15:30	Hay	69.2	65.1	65.8	62.6	0.90	NS
	Silage	63.5	59.4	59.9	60.2		
16:30	Hay	68.4	65.8	66.2	64.7	0.97	NS
	Silage	60.4	58.6	58.5	58.5		

Table A.20
The effect of intra-ruminal infusions of lactic acid on the molar proportions of ruminal propionic acid of silage and hay fed steers.

	Length of infusion (h).					
Time	Water	One	Two	Three	S.E.M.	sig
	Propionic acid molar proportion					
09:15	21.8	19.9	20.8	19.8	0.47	NS
09:25	20.2	19.6	20.5	19.8	0.32	NS
09:45	20.5	20.5	21.0	20.4	0.35	NS
10:00	21.9	22.2	22.5	21.7	0.32	NS
10:15	22.9	24.1	23.9	23.0	0.40	NS
10:30	23.7	25.3	24.5	23.8	0.45	NS
10:45	24.1	26.0	24.6	24.7	0.63	NS
11:00	24.1	26.4	24.8	25.1	0.60	NS
11:30	24.0	26.8	25.2	25.8	0.58	NS
12:30	23.1	26.2^{c}	25.1^{b}	26.2^{c}	0.45	**
13:30	22.3	24.1^{b}	23.8^{a}	26.1^{c}	0.35	***
14:30	21.8	23.6^{c}	23.0^{a}	23.7^{c}	0.30	**
15:30	21.8	22.9	22.1	23.3^{c}	0.37	*
16:30	22.1	22.7	23.1	23.6	0.48	NS

Across rows supercripts indicate a significant difference from the control (a=P<0.05, b=P<0.01 and c=P<0.001)

Table A.21
The effect of intra-ruminal infusions of lactic acid upon the molar proportions of ruminal propionic acid of hay or silage fed steers.

Time	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M	sig
		Propionic acid molar proportion					
09:15	Hay	19.8	19.4	20.6	19.1	0.66	NS
	Silage	22.7	20.5	21.0	20.8		
09:30	Hay	19.0	18.8	20.2	19.1	0.46	NS
	Silage	21.2	20.3	20.8	20.4		
09:45	Hay	19.4	19.2	20.1	19.4	0.49	NS
	Silage	21.8	21.7	22.1	21.4		
10:00	Hay	19.9	20.4	20.7	20.3	0.45	NS
	Silage	24.0	24.0	24.3	23.1		
10:15	Hay	20.8	21.9	21.5	21.1	0.57	NS
	Silage	24.9	26.4	26.3	25.0		
10:30	Hay	21.0	22.9	22.1	21.8	0.64	NS
	Silage	25.7	27.6	26.9	25.8		
10:45	Hay	21.4	24.0	23.1	23.1	0.89	NS
	Silage	27.0	28.2	26.3	26.4		
11:00	Hay	21.8	24.5	22.8	23.3	0.85	NS
	Silage	26.3	28.5	26.9	26.9		
11:30	Hay	22.4	25.7	23.2	24.6	0.82	NS
	Silage	25.6	27.9	27.2	26.9		
12:30	Hay	22.2	26.4	23.9	25.7	0.64	NS
	Silage	24.0	26.1	26.4	26.6		
13:30	Hay	21.4	24.1	23.6	25.3	0.50	NS
	Silage	23.3	24.0	24.0	26.9		
14:30	Hay	21.0	23.1	22.8	23.8	0.42	NS
	Silage	22.5	24.1	23.3	23.5		
15:30	Hay	20.3	22.7	21.7	23.5	0.52	NS
	Silage	22.0	23.1	22.6	23.1		
16:30	Hay	21.2	21.8	22.2	23.0	0.68	NS
	Silage	23.0	23.7	24.0	24.1		

Table A.22
The effect of intra-ruminal infusions of lactic acid on the molar proportions of ruminal butyric acid of silage and hay fed steers.

Time	Length of infusion (h). Water	One	Two	Three	S.E.M.	sig
	Butyric acid molar proportion					
09:15	9.8	9.2	9.6	9.5	0.31	NS
09:25	9.5	9.1	9.4	9.3	0.34	NS
09:45	9.6	9.4	9.6	9.6	0.24	NS
10:00	9.9	9.8	10.1	9.8	0.21	NS
10:15	10.3	10.3	10.8	10.2	0.16	NS
10:30	10.5	10.6	10.8	10.6	0.18	NS
10:45	10.7	10.9	10.8	10.8	0.21	NS
11:00	10.8	11.2	11.4	11.3	0.24	NS
11:30	11.1	11.9	11.8	11.8	0.24	NS
12:30	11.3	12.8^{c}	12.8^{c}	12.8^{c}	0.24	*
13:30	11.7	12.9^{b}	12.4^{a}	13.5^{c}	0.22	***
14:30	10.7	12.8^{b}	12.7^{c}	12.5^{c}	0.27	***
15:30	10.2	12.1^{b}	12.5^{b}	12.3^{b}	0.45	*
16:30	11.2	12.3	12.3	11.9	0.25	NS

Across rows supercripts indicate significant differences from the control (a=P<0.05, b=P<0.01 and c=P<0.001

Table A.23
The effect of intra-ruminal infusions of lactic acid upon the molar proportions of ruminal butyric acid of hay or silage fed steers.

Time	Diet	Length of infusion (h). Water	One	Two	Three	S.E.M	sig
		Butyric acid molar proportion					
09:15	Hay	8.0	8.1	8.1	8.3	0.44	NS
	Silage	11.7	10.4	11.6	10.7		
09:30	Hay	8.0	7.7	8.2	8.1	0.48	NS
	Silage	10.9	10.3	10.7	10.3		
09:45	Hay	7.9	7.9	8.3	7.9	0.34	NS
	Silage	11.3	10.8	11.4	10.9		
10:00	Hay	7.9	8.0	8.0	8.3	0.29	NS
	Silage	11.9	11.6	12.2	11.4		
10:15	Hay	7.8	8.1	8.2	8.3	0.23	NS
	Silage	12.6	12.3	13.1	11.9		
10:30	Hay	7.8	8.4	8.2	8.4	0.26	NS
	Silage	13.0	12.7	13.3	12.5		
10:45	Hay	7.8	8.6	8.1	8.6	0.29	NS
	Silage	13.3	13.2	13.3	12.9		
11:00	Hay	8.1	8.4	8.5	8.5	0.34	NS
	Silage	13.3	13.9	14.2	13.9		
11:30	Hay	8.1	9.1	8.7	9.0	0.34	NS
	Silage	13.7	14.6	14.7	14.3		
12:30	Hay	8.2	9.9	9.5	10.1	0.34	NS
	Silage	13.7	15.1	14.9	13.7		
13:30	Hay	8.2	10.7	10.1	10.9	0.31	NS
	Silage	13.8	14.8	14.5	15.8		
14:30	Hay	8.4	10.6	10.5	10.2	0.38	NS
	Silage	12.9	14.9	14.8	14.6		
15:30	Hay	8.7	9.7	10.7	11.1	0.63	NS
	Silage	11.6	14.4	14.1	13.5		
16:30	Hay	8.8	9.8	10.2	9.9	0.35	NS
	Silage	13.4	14.6	14.1	13.7		

Table B.1
The effect of the dietary treatments on concentation of acetate (mmol/l) in the rumen fluid of dairy cows.

	DIET				
Time	S	F	L	S.E.M.	sig
	Acetic acid (mmol/l)				
08:45	62.3	57.6	59.8	2.52	NS
08:55	64.9	61.7	55.2	5.38	NS
09:15	61.3	54.9	64.3	5.19	NS
09:30	68.2	57.2	61.4	3.99	NS
09:45	70.0	60.0	64.5	5.25	NS
10:00	60.4	59.3	62.1	2.95	NS
10:15	63.3	60.9	63.7	3.32	NS
10:30	67.7	65.0	65.0	3.43	NS
11:00	63.8	61.2	68.6	4.77	NS
12:00	65.7	62.7	59.7	4.33	NS
13:00	63.6	66.2	65.2	4.07	NS
14:00	59.5	65.1	63.6	4.54	NS
15:00	57.7	65.8	61.4	4.62	NS
16:00	63.3	66.0	57.6	7.06	NS

(These results were unaffected by omission of data from #32)

Table B.2
The effect of the dietary treatments on concentation of propionate (mmol/l) in the rumen fluid of dairy cows.

	DIET				
Time	S	F	L	S.E.M.	sig
	Propionic acid (mmol/l)				
08:45	23.9	20.1	20.5	1.83	NS
08:55	26.7	21.0	20.5	1.92	NS
09:15	24.4	21.9	23.2	2.56	NS
09:30	26.7	21.3	22.4	1.43	NS
09:45	28.0	19.5	21.5^{b}	1.41	0.017
10:00	23.3	24.5	21.9	2.27	NS
10:15	27.9	22.7	24.4	1.34	NS
10:30	26.9	22.5^{c}	20.8^{c}	0.64	0.001
11:00	23.7	25.3	26.3	2.73	NS
12:00	27.4	24.4	23.7	4.33	NS
13:00	24.3	23.5	23.3	1.35	NS
14:00	20.4	26.8	25.1	3.06	NS
15:00	19.5	29.2	28.0	4.37	NS
16:00	22.5	25.5	21.8	3.26	NS

(These results were unaffected by omission of data from #32)
Across rows superscript indicate a significant difference from the control (b=$P<0.01$ and c=$P<0.001$).

Table B.3
The effect of the dietary treatments on concentation of butyrate in the rumen fluid of silage fed cows.

	DIET				
	Butyric acid (mmol/l)				
Time	S	F	L	S.E.M.	sig
08:45	13.8	11.9	13.9	0.71	NS
08:55	13.1	12.5	11.8	1.40	NS
09:15	13.5	12.3	13.9	1.40	NS
09:30	16.0	11.4	14.5	1.29	NS
09:45	14.1	11.9	14.1	0.96	NS
10:00	13.2	13.1	13.3	1.10	NS
10:15	15.5	11.8	14.9	0.92	NS
10:30	14.1	13.2	13.9	0.91	NS
11:00	14.5	13.2	14.6	1.53	NS
12:00	16.8	13.1	13.7	1.31	NS
13:00	12.9	15.1	13.7	0.74	NS
14:00	12.5	15.4	13.9	1.41	NS
15:00	12.8	15.2	13.9	1.03	NS
16:00	14.6	14.8	12.0	1.76	NS

(These results were unaffected by omission of data from #32)

Table B.4
The effect of the dietary treatments on rumen fluid pH of dairy cows.

	DIET				
Time	S	F	L	S.E.M.	sig
		pH			
08:45	6.94	6.91	6.84	0.105	NS
08:55	6.83	6.77	6.84	0.103	NS
09:15	6.56	6.45	6.53	0.143	NS
09:30	6.48	6.52	6.64	0.147	NS
09:45	6.45	6.49	6.59	0.071	NS
10:00	6.30	6.50	6.54	0.103	NS
10:15	6.31	6.40	6.47	0.080	NS
10:30	6.31	6.41	6.51	0.132	NS
11:00	6.35	6.47	6.34	0.157	NS
12:00	6.29	6.40	6.37	0.154	NS
13:00	6.30	6.42	6.40	0.123	NS
14:00	6.28	6.36	6.30	0.160	NS
15:00	6.62	6.42	6.30	0.165	NS
16:00	6.53	6.35	6.26	0.177	NS

(These results were unaffected by omission of data from #32)

Table B.5
The effect of infusions of lactic acid or a combination of acetic and propionic acids on rumen fluid osmolality (mOsm/kg) of silage fed cows.

	INFUSION				
Time	W	LC	AP	S.E.M.	sig
	Osmolality (mOsm/kg)				
08:45	258.2	268.4	265.6	7.25	NS
08:55	260.7	266.9	274.6	7.30	NS
09:15	273.7	285.7	292.9	9.62	NS
09:30	290.8	293.9	308.9	7.74	NS
09:45	285.6	301.1	319.8	14.51	NS
10:00	283.8	284.9	304.9	12.60	NS
10:15	287.1	298.0	307.1	7.94	NS
10:30	285.4	305.8	318.0	11.78	NS
11:00	270.1	300.7[b]	295.4[b]	5.73	0.038
12:00	286.1	288.2	266.5	8.05	NS
13:00	273.2	266.1	281.4	6.93	NS
14:00	275.9	258.1	268.9	2.29	NS
15:00	263.1	258.6	269.4	8.81	NS
16:00	271.5	266.7	269.2	6.53	NS

(#32 has been omitted from the analysis of data, n=15.)
Across rows superscript b is significantly different from the control, $p < 0.01$

Table B.6
The effect of infusions of lactic acid or a combination of acetic and propionic acids on the concentration of ammonia (mg/l) in the rumen fluid of silage fed cows.

	INFUSION				
Time	W	LC	AP	S.E.M.	sig
	Ammonia (mg/l)				
08:45	47.4	47.4	43.9	5.18	NS
08:55	43.7	46.3	43.2	4.15	NS
09:15	70.9	76.8	76.9	6.00	NS
09:30	88.6	97.3	99.8	6.15	NS
09:45	111.6	115.5	109.0	8.00	NS
10:00	126.0	109.6	125.3	7.24	NS
10:15	141.5	131.0	128.7	8.34	NS
10:30	158.0	144.9	172.5	8.79	NS
11:00	151.4	154.9	157.6	9.00	NS
12:00	139.7	134.9	168.1	9.27	NS
13:00	115.9	130.8	156.2	11.65	NS
14:00	67.9	84.4	97.5	8.38	NS
15:00	57.2	69.8	76.8	7.64	NS
16:00	50.0	62.3	77.5	7.19	NS

(#32 has been omitted from the analysis of data, n=15.)

Table B.7
The effect of infusions of lactic acid or a combination of acetic and propionic acids on the concentration of acetic acid in the rumen fluid of silage fed cows.

Time	INFUSION W	LC	AP	S.E.M.	sig
	Acetic acid (mmol/l)				
08:45	59.8	54.9	54.7	2.15	NS
08:55	58.0	52.8	53.8	2.06	NS
09:15	57.8	56.9	71.5	9.38	0.02
09:30	59.8	59.7	77.5	7.46	<0.001
09:45	69.9	61.8	79.0	6.01	<0.001
10:00	62.8	58.1	77.8	8.43	<0.001
10:15	60.9	58.9	80.3	8.77	<0.001
10:30	69.2	59.2	86.2	11.92	<0.001
11:00	59.3	59.3	82.9	8.36	<0.001
12:00	64.1	57.8	69.9	4.78	<0.001
13:00	65.7	63.6	68.2	4.97	NS
14:00	65.0	60.9	66.5	4.02	NS
15:00	64.7	62.6	65.7	4.44	NS
16:00	64.5	63.6	65.3	3.83	NS

(Results unaffected by omission of data from #32, n=18)

Table B.8
The effect of infusions of lactic acid or a combination of acetic and propionic acids on the concentration of propionic acid in the rumen fluid of silage fed cows.

Time	INFUSION W	LC	AP	S.E.M.	sig
	Propionic acid (mmol/l)				
08:45	20.1	17.7	18.1	1.88	NS
08:55	18.6	17.8	17.1	1.64	NS
09:15	21.0	20.2	27.5	8.84	0.043
09:30	23.6	24.6	31.7	4.09	0.003
09:45	24.5	26.7	33.1	3.34	<0.001
10:00	26.5	26.6	32.8	3.66	0.010
10:15	25.9	26.9	35.1	2.97	<0.001
10:30	26.3	28.3	38.3	6.20	0.006
11:00	25.8	29.9	26.1	3.38	<0.001
12:00	25.7	26.9	27.9	2.67	NS
13:00	25.1	27.0	26.1	2.66	NS
14:00	23.8	24.5	24.3	2.24	NS
15:00	24.0	23.9	23.9	2.67	NS
16:00	22.7	23.4	23.2	2.31	NS

(Results unaffected by omission of data from #32, n=18)

Table B.9
The effect of infusions of lactic acid or a combination of acetic and propionic acids on the concentration of butyric acid in the rumen fluid of silage fed cows.

	INFUSION				
Time	W	LC	AP	S.E.M.	sig
	Butyric acid (mmol/l)				
08:45	13.0	11.6	11.8	1.23	NS
08:55	12.3	11.3	11.2	1.05	NS
09:15	12.5	11.6	11.8	1.05	NS
09:30	13.0	12.2	11.9	1.07	NS
09:45	13.1	13.0	12.0	1.13	NS
10:00	13.0	13.0	12.7	1.58	NS
10:15	13.6	13.3	12.7	1.60	NS
10:30	14.0	14.2	13.2	1.78	NS
11:00	13.8	14.9	12.8	1.49	0.039
12:00	15.0	15.5	13.3	1.47	0.040
13:00	15.6	17.3	14.2	0.38	0.006
14:00	15.8	16.8	14.4	1.21	0.027
15:00	15.7	15.4	16.0	1.13	0.035
16:00	15.6	16.3	14.9	1.12	NS

(Results unaffected by omission of data from #32, n=18)

Table B.10
The effect of infusions of lactic acid or a combination of acetic and propionic acids on the molar proportions of acetic acid in the rumen fluid of silage fed cows.

	INFUSION				
Time	W	LC	AP	S.E.M.	sig
	Molar proportions of acetic acid				
08:45	61.8	62.9	62.7	0.51	NS
08:55	60.9	61.3	62.8	0.62	NS
09:15	60.7	62.1	62.7	0.41	0.011
09:30	59.8	59.9	62.0	0.39	<0.001
09:45	59.1	58.9	61.9	0.52	<0.001
10:00	59.0	57.4	61.2	0.55	<0.001
10:15	58.5	57.2	60.6	0.65	0.002
10:30	58.1	56.4	60.7	0.60	<0.001
11:00	57.6	55.1	60.9	0.72	<0.001
12:00	58.7	55.8	60.8	0.63	<0.001
13:00	59.2	56.7	60.6	0.40	<0.001
14:00	59.8	57.0	61.0	0.75	0.002
15:00	59.6	58.4	60.5	0.58	0.024
16:00	60.4	59.2	60.7	0.47	NS

(Results unaffected by omission of data from #32, n=18)

Table B.11
The effect of infusions of lactic acid or a combination of acetic and propionic acids on the molar proportions of propionic acid in the rumen fluid of silage fed cows.

	INFUSION				
Time	W	LC	AP	S.E.M.	sig
	Pr. molar proportions				
08:45	20.6	20.3	20.6	0.36	NS
08:55	20.4	20.8	20.0	0.31	NS
09:15	22.1	22.0	23.3	0.62	NS
09:30	24.2	24.1	24.8	0.49	NS
09:45	24.2	25.3	25.7	0.46	NS
10:00	24.9	26.3	25.7	0.40	NS
10:15	24.8	26.0	26.5	0.52	NS
10:30	24.5	26.7	26.5	0.40	NS
11:00	25.0	27.5	26.3	0.59	NS
12:00	23.4	25.6	24.2	0.48	NS
13:00	22.5	23.9	23.1	0.35	NS
14:00	21.8	23.1	22.1	0.56	NS
15:00	22.0	22.1	21.9	0.51	NS
16:00	21.1	21.7	21.6	0.55	NS

(Results unaffected by omission of data from #32, n=18)

Table B.12
The effect of infusions of lactic acid or a combination of acetic and propionic acids on the molar proportions of butyric acid in the rumen fluid of silage fed cows.

	INFUSION				
Time	W	LC	AP	S.E.M.	sig
	Bu molar proportions				
08:45	13.4	13.3	13.9	0.24	NS
08:55	13.4	13.2	13.0	0.19	NS
09:15	13.2	12.7	11.0	0.40	0.001
09:30	12.9	12.3	9.9	0.29	<0.001
09:45	12.5	12.8	9.6	0.28	<0.001
10:00	13.0	12.8	10.3	0.10	<0.001
10.15	12.9	12.9	9.8	0.43	<0.001
10:30	13.1	13.4	9.9	0.57	<0.001
11:00	13.4	13.7	9.8	0.48	<0.001
12:00	13.7	14.8	11.6	0.34	<0.001
13:00	14.1	15.4	12.6	0.24	<0.001
14:00	14.5	15.8	13.2	0.34	<0.001
15:00	14.4	15.5	13.6	0.39	0.004
16:00	14.5	15.2	14.0	0.35	NS

(Results unaffected by omission of data from #32, n=18)

Table C.1
The effect of lactic acid infusions on the total ruminal VFA concentration (mmol/l).

	INFUSION					
Time	W	L	M	H	S.E.M.	sig
	Total VFA concentration (mmol/l)					
08:55	77.2	70.2	68.4	74.5	8.54	NS
09:15	77.2	74.9	74.6	76.0	8.52	NS
09:30	76.1	78.5	77.7	76.1	6.87	NS
09:45	80.6	80.9	79.6	80.2	2.92	NS
10:00	85.9	84.8	87.4	86.6	3.50	NS
10:15	87.0	91.0	90.8	84.2	4.59	NS
10:30	89.0	89.9	88.5	92.0	5.19	NS
11:00	83.6	86.3	83.9	84.2	3.39	NS
12:00	77.6	77.7	76.4	75.6	1.92	NS
13:00	78.9	79.0	78.4	73.4	1.84	NS
14:00	79.0	75.0	70.1	80.1	1.76	NS
15:00	74.6	74.1	72.8	74.6	3.01	NS
16:00	71.1	68.2	66.0	69.6	3.18	NS

Table C.2
The effect of lactic acid infusions on the ruminal concentration of acetic acid (mmol/l).

	INFUSION					
Time	W	L	M	H	S.E.M.	sig
	Acetic acid concentration (mmol/l)					
08:55	52.5	49.1	48.8	51.2	4.72	NS
09:15	52.0	51.0	52.4	51.7	7.07	NS
09:30	53.2	54.2	53.8	53.3	6.28	NS
09:45	55.7	55.6	53.8	55.1	2.89	NS
10:00	56.9	55.5	57.4	57.9	2.01	NS
10:15	57.1	54.3	57.3	51.2	4.48	NS
10:30	58.9	57.3	59.1	60.6	5.03	NS
11:00	55.3	55.8	53.7	54.6	3.70	NS
12:00	54.5	54.4	53.1	52.9	1.44	NS
13:00	54.5	55.1	53.5	51.5	1.09	NS
14:00	52.0	49.6	48.5	52.7	1.74	NS
15:00	51.5	49.8	48.9	50.3	3.44	NS
16:00	47.7	45.4	49.7	49.3	1.94	NS

Table C.3
The effect of lactic acid infusions on the ruminal concentration of propionic acid (mmol/l).

	INFUSION					
Time	W	L	M	H	S.E.M.	sig
	Propionic acid concentration (mmol/l)					
08:55	16.2	14.3	13.0	16.2	2.89	NS
09:15	16.7	16.8	16.2	16.5	2.58	NS
09:30	15.5	16.2	16.1	16.2	1.74	NS
09:45	16.9	17.7	17.8	17.9	1.18	NS
10:00	19.2	20.6	19.3	19.4	2.08	NS
10:15	21.9	24.5	22.0	20.5	2.87	NS
10:30	20.1	22.5	19.7	22.1	3.61	NS
11:00	19.2	21.6	20.7	20.0	2.54	NS
12:00	15.7	16.4	15.9	15.2	0.77	NS
13:00	14.6	14.8	15.0	12.9	1.06	NS
14:00	16.9	16.2	14.4	17.5	0.94	NS
15:00	14.2	14.6	15.4	14.6	1.17	NS
16:00	14.5	13.6	12.3	13.2	0.78	NS

Table C.4
The effect of lactic acid infusions on the ruminal concentration of butyric acid (mmol/l).

	INFUSION					
Time	W	L	M	H	S.E.M.	sig
	Butyric acid concentration (mmol/l)					
08:55	8.5	7.1	6.4	7.0	1.80	NS
09:15	8.4	7.9	5.9	7.8	1.76	NS
09:30	7.2	8.1	6.6	7.7	1.58	NS
09:45	8.1	7.6	8.0	7.2	1.74	NS
10:00	9.9	8.7	10.6	9.3	1.70	NS
10:15	11.9	12.2	11.4	12.4	1.88	NS
10:30	9.9	10.1	9.6	9.3	1.04	NS
11:00	9.0	8.9	9.6	9.6	0.83	NS
12:00	7.3	6.9	7.1	7.5	0.58	NS
13:00	10.4	9.0	10.9	9.1	0.97	NS
14:00	10.1	9.2	7.4	9.9	0.33	NS
15:00	8.8	8.5	9.7	8.9	0.78	NS
16:00	7.4	6.9	6.2	6.6	0.58	NS

Table C.5
The effect of intra-ruminal infusions of lactic acid on rumen fluid osmolality (mOsm/Kg).

	INFUSION					
Time	W	L	M	H	S.E.M.	sig
		Osmolality (mOsm/kg)				
08:45	303	292	293	299	12.6	NS
08:55	294	272	282	274	11.0	NS
09:15	286	293	287	318	7.1	NS
09:30	299	330	294	296	13.0	NS
09:45	304	340	323	316	14.4	NS
10:00	317	340	323	348	14.3	NS
10:15	309	312	319	319	11.6	NS
10:30	283	318	310	305	9.9	NS
10:45	269	304	310	281	14.0	NS
11:00	274	263	312	287	10.4	NS
11:30	303	243	291	307	18.9	NS
12:00	273	285	279	294	15.9	NS
13:00	304	275	293	294	14.5	NS
14:00	291	287	292	301	12.1	NS
15:00	306	294	294	295	16.0	NS
16:00	309	287	299	312	8.2	NS