The Biochemistry of Silage

Second Edition

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by

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Preface

Since the first edition of this book was published in 1981, silage has become even more important as a winter feed for ruminant animals and in many countries ensilage is now the major method of forage conservation. As a consequence, the level of research in this field during the past 10 years has increased considerably and, in this new edition of the Biochemistry of Silage, we have attempted to take into account the main findings of these studies. In the second edition we have followed a similar format to that of the first, but have found it more convenient to discuss the microbiology of silage in a single chapter.

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Chapter 1

Introduction

The main objective in the conservation of any crop is to preserve it at the optimum stage of growth for use during those seasons when the crop is unavailable. In countries such as the United Kingdom, where there is a restricted growing season, conserved forage has for many centuries played a major role in helping to meet the nutritional needs of ruminants for survival during the winter period.

Haymaking has long been the traditional technique of preserving forage, but the need to delay harvesting until a mature stage of growth of relatively high dry matter (DM) content was reached meant that the crop was of low digestibility before it was dried. This fact coupled with the vagaries of the weather, which frequently resulted in high losses during the drying process, led to a product of variable composition, and often of low nutritional value. Early interest by farmers in conserving crops by natural fermentation as silage stemmed from the realisation that this process was less dependent on both the weather and the need to harvest crops at an advanced stage of growth.

Today, although haymaking methods have been improved considerably by the introduction of new drying techniques, many farmers find these to be too specialised and time-consuming and prefer to preserve their crops instead as silage. In Western Europe, and in many other regions of the world, the quantity of forage preserved as silage now exceeds that preserved as hay[1,2] (Table 1.1).

Table 1.1. Estimated production of silage and hay in Western Europe, 1975 and 1985 (million tonnes DM)[1]

	1975			1985		
	Silage	Hay	Total	Silage	Hay	Total
European Community 12	44.6	58.3	102.9	70.9	45.8	116.7
Austria + Switzerland	1.7	5.8	7.5	3.2	4.7	7.9
Scandinavia	1.0	7.3	8.3	1.9	7.6	9.5
Total	47.3	71.4	118.7	76.0	58.1	134.1

HISTORICAL DEVELOPMENT OF SILAGE

Silage is the material produced by the controlled fermentation of a crop of high moisture content. Ensilage is the name given to the process and the container, if used, is called the silo. The storage of material in silos, either as grain or as a green crop, has been common practice for many centuries.[3]

The word silo is derived from the Greek *siros*, meaning a pit or hole sunk in the ground for storing corn.[4] Pliny, in his *Natural History*, mentions the preservation of corn in trenches, which were called *siri*, in Cappadocia and Thrace. In Spain and Africa special care was taken to select a dry site and to spread chaff or stubble on top of the soil. Among the Greeks who refer to silos are Euripides, Theophrastus, Hesychius and Suidas.[4] The Greek word *siromastes* referred to a type of instrument with an iron point or prong, for probing the silos to see if contraband or other improper goods were secreted in them. This instrument was also used as a serviceable weapon in time of war and is referred to in the Old Testament (2 Kings, xi, 10), where Jehoiada is represented as giving the centurions the siromastae of David.[4] Old paintings found in Egypt, dating from the period about 1000 to 1500 B.C., suggest that the ancient Egyptians were familiar with ensiling as a means of preserving crops.[5] According to Kirstein,[6] silos were found in the ruins of Carthage, which indicates that fodder was ensiled there in about 1200 B.C.

From ancient writings in the Mediterranean region it seems that air-tight sealing of the fodder was considered to be an important condition for successful conservation.[5] The Roman historian Cato (about A.D. 100) mentioned that the Teutons stored green fodder in the ground and then covered it with dung.[5] In Italy, ensiling of wilted grass has been practised for at least 700 years.[5] In Northern Europe, grass has been ensiled in Sweden and in the Russian Baltic provinces since the beginning of the eighteenth century, while beet tops and leaves were ensiled in Northern Germany at the beginning of the nineteenth century.[5]

In spite of this early knowledge of ensilage as a conservation technique in many parts of the world, it was not until the latter part of the nineteenth century that interest in this process became more widespread. The earliest noted description of silage making was that of Grieswald, who published his technique in the *Transactions of the Baltic Association for the Advancement of Agriculture* in 1842.[7] Grieswald recommended that the pits used should be filled with fresh grass as rapidly as possible by tramping, and even by ramming the grass into the silo. As soon as the silo was filled, the contents should be sealed off with a layer of boards or a well-fitting lid, on which should be placed a layer of earth about 45cm in height. In 1862, Reihleu of Stuttgart published an account of his ensiling procedure in the *Wurttemberg Wochenblatt*.[4] This was translated into French and published in the *Journal d'Agriculture Pratique* in 1870.

The chief credit for what may be termed the practical modernising of ensilage undoubtedly belongs to Goffart, a French farmer who, in 1877, published the first book on ensilage, which was based on his own experiences of ensiling green maize.[8] About a year later, an English translation of this book was published in the USA and the 'new' conservation technique was quickly taken up by American farmers. At the Fifth Ensilage Congress held in New York in January 1866, Smith of Vermont summarised the feeling of many farmers thus:[4]

'It is unnecessary to dwell on the advantages of a system of curing fodder which shall take place as independent of the weather, or which will enable us to keep two cows in the place of one on our farms. We all acknowledge its practical value, and we know we have found it in ensilage...Now, thanks to M. Goffart, to whom American farmers owe a livelier debt of gratitude than to any other living man, and to that amazing grass, our native Indian corn, our farms are capable of quadrupling their productions; the best and sweetest of butter can be made in the

season of the year when we have the most leisure to make it, and when it brings the best price in the market; our beeves can be kept growing the year round, and more of them to the acre than ever before, and for a less price.'

Not surprisingly, ensilage was enthusiastically adopted in the USA and France, but it was some time before it was taken up in the UK, and it was not until 1882 that the subject received general interest among British farmers. At the Reading Show of the Royal Agricultural Society, Vicomte de Chezelles, one of the most ardent supporters of ensilage in France, gave an impressive account of his system which included the filling of the 'largest silo in the world' of 1,000 tonnes capacity.[4] In 1883 it was reported that not more than half a dozen silos were in existence in the UK, but within the next few years interest increased dramatically, so that by the year 1886 there existed 1,605 silos in the country.[4]

In the UK, interest had concentrated on the use of grass rather than maize, as a silage crop, but it was realised that the procedures outlined by Goffart, such as rapid filling followed by sealing of the mass to make it airtight, applied equally to the grass crop as to maize. Unfortunately the principles outlined by Goffart received a set-back in this country on the publication, in 1885, of the book *Sweet Ensilage* by Fry.[9] The sweet ensilage process depended upon the development in the herbage of a temperature of at least 50°C, but frequently it rose to a higher level. To achieve this temperature it was necessary to allow air to penetrate the mass and it was usual to cut the crop late and to wilt before filling the silo. Sealing was therefore delayed until the herbage reached the desired temperature. Although silage produced by this method was usually well preserved, because of losses arising from oxidation and heating the product was often of poor nutritional value and of little better than maintenance quality.[10] This method of allowing the crop to heat up in the silo prior to sealing persisted as a technique in the UK until the middle of the present century, and may well have been a factor in delaying the advance of silage making in this country.[11]

Apart from short periods during the two world wars, general interest in ensilage was not revived until the 1950s as a result of improvements in mechanisation and the necessity for more intensive animal production, coupled with the high cost of concentrates. Today it is generally accepted that, provided certain basic principles of silage making are followed, the production of a high quality feed for ruminants is assured.

PRINCIPLES OF ENSILAGE

The first essential objective in preserving crops by natural fermentation is the achievement of anaerobic conditions. In practice anaerobiosis can be obtained by various methods.

The most efficient way is to store the material in a hermetically sealed container, and under these conditions the oxygen trapped in the herbage is rapidly removed by respiratory enzymes in the plant. In the open-type silo, the efficiency with which anaerobiosis can be obtained depends upon the degree of consolidation and the effectiveness of the final sealing. The main aim of sealing is to prevent re-entry and circulation of air during storage. Where oxygen is in contact with herbage for any period of time, aerobic microbial activity occurs and the material decays to a useless, inedible, and frequently toxic product.

The second main objective is to discourage the activities of undesirable microorganisms such as clostridia and enterobacteria. Clostridia are usually present on harvested forage in the form of spores,[12] but start to multiply as soon as conditions in the silo become anaerobic.[13] The growth of these organisms is undesirable, as they produce butyric acid and degrade amino acids to a variety of products which are of poor nutritional value. The enterobacteria are non-spore forming, facultative anaerobes (able to grow in both the presence and absence of oxygen), which ferment sugars to acetic acid and other products and also have the ability to degrade amino acids.[14] The commonest way of inhibiting the growth of these undesirable microorganisms is to promote lactic acid fermentation.[15,16]

The lactic acid bacteria are also normally present on harvested crops and, like the enterobacteria, are facultative anaerobes. These organisms ferment the naturally occurring sugars (mainly glucose and fructose) in the crop to a mixture of acids, but predominantly lactic. The lactic acid produced increases the hydrogen ion concentration to a level at which the undesirable bacteria are inhibited.[13,17,18] This inhibition is caused not only by the hydrogen ion concentration but also by the undissociated acids themselves.[13] It is difficult to state an exact critical pH value of the silage at which this inhibiting effect occurs, as the inhibition depends not only on pH but also on the moisture content and the temperature. The wetter the material, the lower will be the critical pH value. With unwilted grass crops of DM content of about 200 g kg^{-1}, it is normally accepted that the achievement of a pH value of about 4.0 will preserve the crop satisfactorily, provided the silo remains airtight and is free from penetration by rain.

An alternative method of inhibiting the growth of undesirable bacteria is to reduce the moisture content of the crop by wilting prior to ensiling. Lactic acid bacteria have a relatively high tolerance to low moisture conditions and are able to dominate the fermentation in high DM crops.[18] Clostridia are known to be particularly sensitive to water availability and they require very wet conditions for active development.[12] With very wet crops, i.e. those with a DM concentration of about 150 g kg^{-1}, even the achievement of a pH value as low as 4.0 may not inhibit clostridial growth.[19]

The rate of lactic acid production is an important factor in inhibiting the growth of undesirable bacteria and in reducing fermentation losses, and this depends upon the initial lactic acid bacterial population on the ensiled crop and upon the substrate availability. This in turn is influenced by the degree of physical damage (bruising, laceration, chopping and mincing).[20-23] Modern precision-chop forage harvesters are capable of chopping herbage into particle lengths <25mm. With such material, plant sap is rapidly liberated and lactic acid bacterial growth is stimulated.[24] Finely chopped silage of this type is more readily eaten by ruminant animals than long or coarsely chopped material.[25,26]

SILOS

Commercial silos

The types of silo in which the farmer may choose to ferment his crop are very varied and they have been reviewed by a number of workers.[3,10,11,27-30] For convenience commercial silos can be classified into seven main categories: stack

or clamp without retaining walls, tower, surface-walled clamp or bunker, flexible-walled, vacuum, plastic sausage and big bale.

Stack or clamp silo without retaining walls

The term stack, often used synonymously with the term clamp, is taken to comprise a heap of herbage built vertically to a height of about 2 to 3 metres on ground level, or on slightly excavated ground.[3,4,31] Originally the surfaces of stack silos were left exposed to the air and this resulted in very high oxidation losses.[3,32] Since the 1950s, when plastic sheeting was first introduced into agriculture, oxidation losses in stack silos have been markedly reduced.[33]

In the modern technique, the stack is built on a sheet of plastic placed on the ground and when sufficient herbage has been deposited on this, it is covered with a second sheet of plastic which is sealed to the lower one using a simple strip-seal or other suitable technique.[3] In the Netherlands, small clamps of herbage of about 1.5 metres in height are covered with two plastic sheets, weighted down at the top and sides with old rubber tyres and held in place with sandbags positioned round the periphery.

Tower silo

Tall cylindrical towers, built of concrete, steel or wood, are probably the most effective way of storing herbage as silage, since surface exposure to the air is minimal. Pressures obtained in this type of silo are greater than those in other silos and, unless the crop is pre-wilted in the field, the outward pressures exerted on the bottom walls of the tower can cause a serious problem of effluent flow and may even damage the structure of the silo.[29]

Surface-walled clamp or bunker silo

This silo generally consists of three solid walls some 2 to 3 metres in height, and it is often built beneath a Dutch barn to protect the silage from the weather.[34-36] The crop is generally ensiled in a series of wedges, which are laid one upon the other progressively along the silo, and the top is sealed with plastic sheeting during the course of filling.[3,29] Finally, the surface is weighted with some suitable material, such as sandbags, tyres or bales of straw, to prevent re-entry of air. The walls of bunker silos must be strong enough to withstand the thrust of tractors used to consolidate the herbage, and of the silage itself.[37,38]

Flexible-walled silo

This type of silo was originally developed by the National Institute of Agricultural Engineering, England,[39] and resembles a bunker silo but with walls which can move outwards. With this arrangement, it is possible to fill the silo to a height of about 7 metres without exerting excessive pressure on the side walls. The silage eventually settles to a height of about 4 metres.

Vacuum silo

In the vacuum silo, the forage is placed on top of a sheet of plastic and built up to the required height. A similar sheet of material is then placed over the forage and

the edges of the sheeting are joined together using a strip-seal. The air inside the silo is then removed using a vacuum pump, which also helps to consolidate the forage. This method is only successful as long as the silo remains sealed. If the plastic is damaged during storage and air enters the silo, losses can be as high as in the unprotected stack silo.[3]

Plastic sausage silo

This type of silo was devised in Germany. A special machine fills a tube of plastic with chopped forage, which has previously passed through a shaping funnel. When filled, the sausage has a diameter of about 2.4 metres but can be of any length up to about 30 metres.[3]

Big bale

In recent years the amount of silage conserved as large bales has increased dramatically, and this system of making silage is now widely practised in the UK and in other countries.[40-48] There are two basic types of baling machines, the fixed chamber and the variable chamber. In the former, the perimeter of the bale-forming mechanism remains in a fixed position so that the crop is only subjected to a rolling action and consequent compression when it fills the chamber. The centre core of the bales is less dense than the outer layers which have been rolled during formation.[40,42] In the variable chamber machine, the bale forming mechanism engages the bale from the start and expands during bale formation. The bale is thus subjected to continuous rolling and consequently tends to be more densely uniform.[40]

Bales vary in size but usually weigh from 0.5 to 0.75 tonne. The bales may be stacked on a ground sheet and covered with plastic sheeting which is sealed at the base or, more commonly, placed into large plastic bags which are tied at the neck. These bales are then stacked in pyramid formation and the whole pyramid is covered with a weighted plastic sheet or net to protect it from damage.[3,40]

A recent innovation is the use of stretch film instead of plastic bags.[1] It is claimed that the plastic film wrapping is cheaper than bags and makes the system less labour intensive.[2,49] In a comparison of bags versus wrapping, Braithwaite and Jones[50] found that, providing the bales were well-sealed, there was no difference in silage quality between bagged and wrapped big bales. However, the risk of poor fermentation or complete loss of a bale due to aerobic deterioration following damage during storage was greater with bags. Aerobic deterioration in wrapped bales tended to be confined to the site of damage. Wrapping of low DM bales is not recommended because of possible problems with sealing and effluent.[51]

Experimental silos

Laboratory silo

Barnett[27] has reviewed the literature on early laboratory techniques and has described a range of different types of silo including test-tubes,[52] milk bottles,[53] glass jars,[54] and glass cylinders.[55] The most widely used type of laboratory silo is

the test-tube, with a capacity normally ranging from 50 g to 250 g. The tube is fitted with some kind of sealing device which permits the escape of gases but prevents the entry of air. Suitable sealing devices are rubber stoppers carrying either a valve of mercury supported on a sintered glass disc[55] or, preferably, a simple glass or plastic fermentation trap filled with water.[56] Virtanen[57] used porcelain containers holding about 15 to 20 kg of fresh crop which permitted DM loss, carbon dioxide (CO_2) production, and effluent flow to be monitored. Wieringa[58] used glass preserving jars of 1 to 2 litres capacity which could be hermetically sealed, while Ohyama and Masaki[59] used 1 litre glass bottles with mercury seals.

In the studies carried out by Jones,[60] thin gauge polyethylene bags (20 × 40 cm) were filled with herbage and sealed at the neck. These bags were then placed in a larger, thick-gauge polyethylene bag (40 × 80 cm) fitted with a simple connector for attaching to a suction pump. Polyethylene bags, placed in small PVC cylinders, have also been used for consolidation tests and friction measurements.[61]

Wilson and Wilkins[62] evaluated laboratory ensiling techniques by comparing 18 grasses and eight legumes in test-tube silos holding 100 g of fresh herbage with the same crops ensiled in PVC bags holding 1 tonne of fresh material. Measurements made on silages from the test-tube silos were closely correlated with those from the PVC bags. Our own results at Edinburgh in general confirm the above findings, although in some instances fermentations in laboratory silos may be atypical unless precautions are taken to ensure that the herbage contains normal levels of lactic acid bacteria or, alternatively, herbage is inoculated with these organisms prior to ensiling.

Pilot-scale silo

This type of silo is used in experiments when a sufficient quantity of silage is needed for feeding trials with ruminants, or when detailed measurements of nutrient losses are required. An early experimental silo of this type was that used by Watson and Ferguson[63] in 1937, which consisted of a scaled-down tower silo made of wood or concrete, holding about 1 tonne of material. Golf and Gneist[64] used a similar silo made of steel, which had a capacity of 0.75 tonne. Barnett and Miller[65] used a sewage pipe of height 1.8 metres and internal diameter 1.2 metres. This pipe rested on a solid concrete base through which passed an iron tube which drained off the effluent. Nash[66] used similar silos made of concrete, each being fitted with a roof, thermocouples, and effluent-collecting apparatus. The experimental tower silos used at the USDA research centre at Beltsville[67] in the 1950s were made of enamelled steel and possessed sampling ports. Each silo, which had a diameter of 1.2 metres and a height of 2.4 metres, was capable of being transported for weighing purposes.

An experimental silo unit designed specifically for measuring losses during ensilage was built by McDonald and Attwood.[68] The unit consisted of four silos, each constructed of steel and having a diameter of 1.2 metres and a height of 1.8 metres. Each silo had seven sampling ports and ten thermocouples, and was suspended from a weighing apparatus of the steelyard type, bolted to a steel beam. The weighing apparatus was designed to counterbalance the whole load and to measure the actual weight loss. The silo held about 1 tonne of forage and the equipment was sufficiently sensitive to record a change in weight of 0.1 kg.

In 1970 an experimental bunker silo of 40 tonne capacity was built at Edinburgh specifically to measure gaseous and effluent losses. This silo was constructed of steel and was mounted on load cells which were supported on concrete pillars sunk into the ground.[69]

Probably the most sophisticated experimental silo unit was that built at Braunschweig,[70] which consisted of six sealed tower silos, each of 2 to 3 tonnes capacity. The first two of these were constructed of glass-lined steel, while the remaining four were built of glass fibre reinforced polyester resin. All silos were capable of being weighed and were equipped with facilities for continuous automatic measurement and analysis of gases.

More recently, silos made of PVC have become available for pilot-scale studies. These can be manufactured to any size up to about 3 tonnes and are usually fitted with drainage taps for effluent collection.[62,71,72]

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Chapter 2

Crops for Silage

Silage can be made from a large variety of crops. Many are purposely grown to make silage, but others are ensiled because they are surplus to immediate requirements. The characteristics of an ideal crop for preservation as silage are that it should contain an adequate level of fermentable substrate in the form of water soluble carbohydrates (WSC), a relatively low buffering capacity and a DM content above 200 g kg^{-1}. Ideally it should also possess a physical structure which will allow it to compact readily in the silo after harvesting. Many crops do not fulfil these requirements and with such materials pretreatments, such as field wilting, fine chopping or the use of additives, may be necessary.

GRASS

Grass is one of the commonest crops to be conserved as silage. Although single species of grasses such as Italian ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*), timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) may be grown as silage crops, it is quite common to find mixed species of both grasses and legumes such as clovers (*Trifolium* spp.) being used.

In cold and temperate climates grass starts to grow in the spring when soil temperatures reach 4 to 5°C. From then on the pattern of growth is very much the same irrespective of species or cultivar. There is a rapid production of leaf followed by an increase in the growth of the stem, leading to the ultimate emergence of the flowering head and, finally, to the formation of the seed.[1] As the grass grows in the spring the amount of DM in the crop increases, at first slowly then more rapidly as the stems grow and the ears emerge and, finally, more slowly as the ears begin to ripen. During this period of growth, the moisture and crude protein (nitrogen × 6.25) contents decrease while the cell wall components increase (Table 2.1).[2-6]

Digestibility decreases with maturity, although the decrease is not linear since there is a spring 'plateau' period of up to a month during which the herbage digestibility remains almost constant. The end of this period is associated in some plant species with ear emergence, after which the digestibility of organic matter may decrease abruptly. Differences in digestibility of grasses are influenced by leaf:stem ratios. In very young grass the stem is more digestible than the leaf, but as plants mature this property is reversed. These changes are illustrated in Figure 2.1.[7]

Carbohydrate components

Carbohydrates in plants may be classified into two main categories, structural and nonstructural compounds.

Table 2.1. Changes in the composition (g kg^{-1} DM) of three grass species cut at four stages of growth[2]

Grass	Date cut	Average height (cm)	Leaf:stem ratio (dry weight)	Crude protein	Ether soluble	WSC	Cell wall components			Ash
							Hemi-celluloses	Cellulose	Lignin	
Ryegrass S23										
Cut										
1	22 April	10.5	10.0	209	80	158	113	170	30	101
2	14 June	23.3	1.1	61	36	221	127	217	33	59
3	19 July	52.3	0.1	34	27	177	183	284	72	42
4	13 Sept.	56.3	0.1	31	28	42	210	331	100	39
Timothy S48										
Cut										
1	25 April	11.8	4.0	164	72	199	107	173	36	83
2	30 May	27.0	2.0	86	33	197	140	219	46	64
3	12 July	75.0	0.3	46	35	142	206	295	92	32
4	14 Sept.	87.5	0.2	24	23	114	192	313	119	31
Cocksfoot S143										
Cut										
1	25 April	11.5	5.0	200	70	120	108	176	48	125
2	26 May	26.0	1.6	107	53	141	148	236	51	89
3	6 July	80.5	0.1	45	31	116	219	295	89	73
4	16 Sept.	85.5	0.1	25	24	5	252	389	146	49

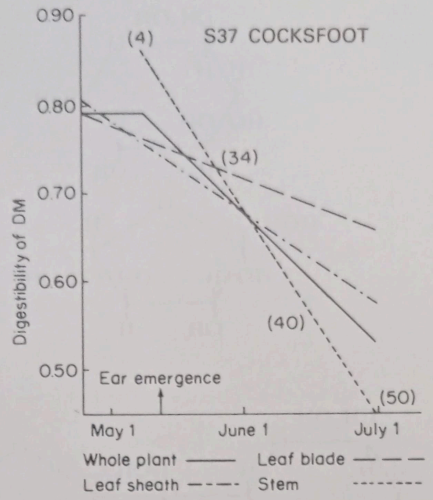


Figure 2.1. **The digestibility *in vitro* of the dry matter in the whole plant and various parts of S37 cocksfoot during first growth in the spring. Figures in parenthesis are the percentage of the stem in the whole plant[7]**

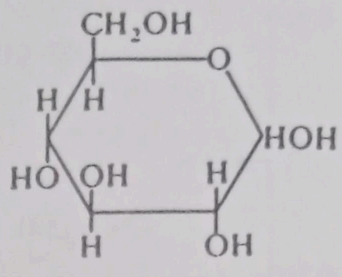
Structural carbohydrates

The structural carbohydrates may be further subdivided into matrix and fibre polysaccharides.[8] The main components of matrix polysaccharides are hemicelluloses and pectic substances. Structurally, hemicelluloses in grasses contain a main chain of xylan, made up of β-(1 → 4)-linked D-xylose units with side-chains containing methylglucuronic acid and frequently glucose, galactose and arabinose. Pectic substances are a group of closely related polysaccharides of which pectin is the main component. Pectin consists largely of unbranched chains of α-(1 → 4)-linked D-galacturonic acid units, with many of the carboxyls esterified with methyl groups or neutralised with calcium or magnesium.

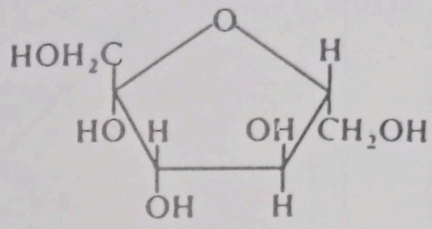
The fibre polysaccharides are largely crystalline, and are present as microfibrils which are held together by various bonds in a cement of largely amorphous matrix polysaccharides, lignin and some protein.[8] Cellulose, which is the main crystalline microfibril polysaccharide, is a linear, high molecular weight polymer made up of β-(1 → 4)-linked D-glucose units.

The sugar components (glucose, galactose, mannose, xylose and arabinose) of these structural carbohydrates are not immediately available as fermentable substrates for the lactic acid bacteria, but they may eventually become available as a result of hydrolysis brought about by the action of enzymes present in the plant itself (p. 56), enzymes added to the crop at the time of ensiling (p. 195) or by acid hydrolysis (p. 57).

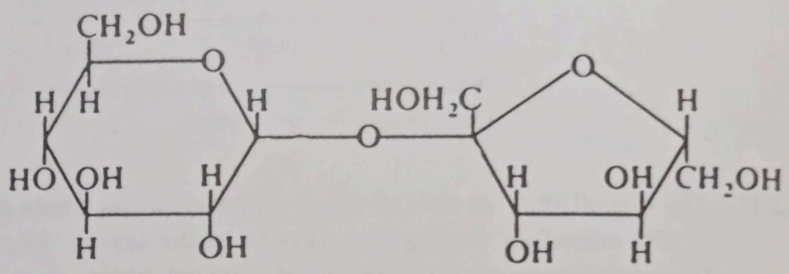
D-Glucose



D-Fructose



Sucrose



Fructans

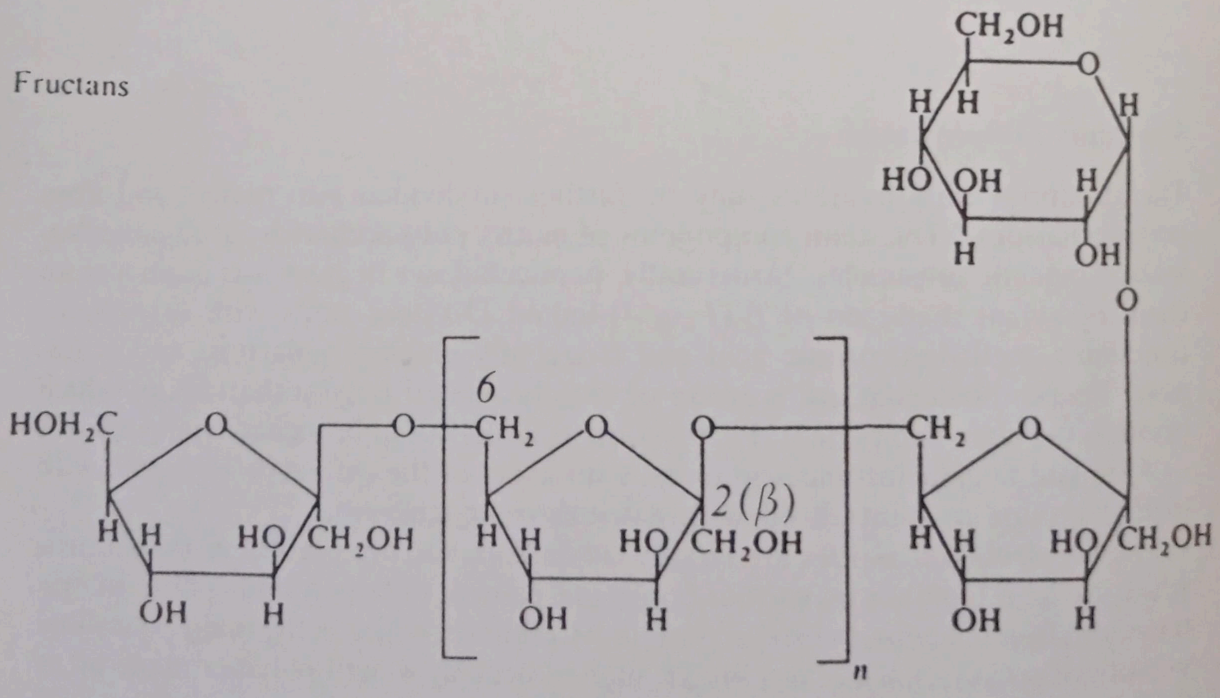


Figure 2.2. Major water soluble carbohydrates commonly found in the vegetative parts of grasses

Nonstructural carbohydrates

The main nonstructural carbohydrates occurring in temperate grasses are glucose,

fructose, sucrose and fructans (Figure 2.2). The disaccharide, melibiose, the trisaccharides, raffinose and kestose, and the tetrasaccharide, stachyose, have also been detected in certain grasses, but only in relatively small concentrations.[9-11] These carbohydrates are all soluble in cold water and are collectively known as the 'water soluble carbohydrates' (WSC).

Glucose and fructose are the two most important monosaccharides occurring as free sugars in temperate grasses, concentrations in the DM being of the order of 10 to 30 g kg^{-1}. The disaccharide sucrose normally occurs in larger amounts than the monomers, in the range 20 to 80 g kg^{-1} DM.[12] The fructans present in grasses are levans consisting of β-(2 → 6)-linked fructofuranose units terminating in sucrose residues (Figure 2.2).[13] Fructans have a low degree of polymerisation (DP), the longest chain compounds being found in stem base tissue, where they vary from a DP of about 26, as found in brome grass (*Bromus inermis*), to about 260, as found in timothy.[12] As in sucrose, the glycoside links in fructans involve the carbon 2 of the furanosyl units, and they are therefore very acid labile compared with other glycoside links.[12]

In temperate grasses fructans are the most abundant of the WSC, being present in concentrations of about 50 to 90 g kg^{-1} DM, although values as high as 120 g kg^{-1} DM have been reported for perennial ryegrass.[10] These polysaccharides appear to be concentrated in the stem. Mackenzie and Wylam,[11] in studies with perennial ryegrass, found that the fructan content of the leaf was never greater than 40 g kg^{-1} DM while the content in the stem frequently exceeded 150 g kg^{-1} DM. The fructan content of grasses grown in warmer climates is apparently lower than that of grasses grown in cooler regions.[14]

The WSC can be fermented rapidly by lactic acid bacteria provided they are readily available. It has been suggested that rates of diffusion of soluble carbohydrates from intact and ruptured cells into the aqueous phase may be more important than absolute amounts in the crop.[15]

Grasses of tropical and subtropical origin accumulate starches instead of fructans in their vegetative tissues.[12,16] Starches are made up of two structurally different components, amylose and amylopectin. Amylose is a linear molecule composed of D-glucose units joined through α-(1 → 4)-glycoside links. Amylopectin has a bush-like structure containing primarily α-(1 → 4)-linkages, but also an appreciable number of α-(1 → 6)-linkages. Whereas in temperate grasses fructans are stored mainly in the stems, in grasses of tropical origin starches are stored primarily in the leaves.[12] In contrast to fructans, starches are only sparingly soluble in cold water.[12]

It is generally considered that most naturally-occurring lactic acid bacteria do not have the ability to ferment starch directly,[17] although some workers have isolated lactic acid bacteria capable of breaking down this polysaccharide.[18-21] The main nonstructural carbohydrate components of some temperate and some tropical grasses are shown in Table 2.2.

Factors influencing the water soluble carbohydrate content of grasses

Species

Of the common temperate species, the ryegrasses have the highest WSC content

Table 2.2. Nonstructural carbohydrate components (g kg^{-1} DM) of some temperate and tropical grasses[12,22,23]

Grass	Leaf (L) or stem (S)	Reducing sugars	Other sugars	Fructans	Starch	Total
Temperate						
Perennial ryegrass	L	18	49	41	—	108
(*Lolium perenne*)	S	23	64	141	—	228
Timothy	L	14	54	6	—	76
(*Phleum pratense*)	S	23	36	49	—	108
Meadow fescue	L	12	42	13	—	67
(*Festuca pratensis*)	S	21	40	54	—	115
Cocksfoot	L	7	30	3	—	40
(*Dactylis glomerata*)	S	24	20	14	—	58
Tropical						
Pangola grass	L	20	21	—	45	86
(*Digitaria decumbens*)	S	26	96	—	1	123
Buffel grass	L	14	17	—	26	57
(*Cenchrus ciliaris*)	S	18	30	—	6	54
Golden Timothy grass	L	32	17	—	38	87
(*Setaria sphacelata*)	S	63	22	—	11	96

and cocksfoot has the lowest.[15,24-26] Henderson[24] examined the WSC contents of 373 samples of five different species of grasses grown in south-east Scotland; the results of this study are shown in Table 2.3.

Table 2.3. Water soluble carbohydrate contents (g kg^{-1} DM) of five grass species[24]

Species	Number of samples	Range	Mean	s.e.
Italian ryegrass	38	74–314	181	± 9.6
Perennial ryegrass	191	46–315	170	± 3.8
Timothy	20	53–199	110	±10.7
Meadow fescue	17	35–263	96	±14.4
Cocksfoot	107	5–191	79	± 4.1

The WSC contents of tropical species of grasses are generally considered to be lower than those of temperate species.[27] This difference may, in part, be due to a lack of fructans in the former. Catchpoole and Henzell[28] analysed a number of tropical species and found the following concentrations of WSC (g kg^{-1} DM): *Setaria sphacelata* cv.Nandi, 45-61; *Chloris gayana* cv. Samford, 30-35; *Paspalum dilatum*, 27-34; *Desmodium intortum* cv. Greenleaf, 25; and *Lotononis bainesii*, 99. In studies with *Chloris gayana* cut for silage, Uchida and Kitamura[29,30] recorded a WSC level of 85 g kg^{-1} DM for the original herbage.

Cultivar

Differences in WSC content among cultivars within a species are known to exist. McGinn[31] analysed five cultivars of Italian ryegrass and three cultivars of perennial ryegrass at weekly intervals from 10 May to 15 June (Table 2.4). The cultivar Tribune showed almost consistently higher WSC contents than the other cultivars examined. The tetraploid cultivars of perennial ryegrass, Reveille and Petra, were shown by Dent and Aldrich[32] to have higher WSC contents than diploid cultivars. The same workers showed that early maturing cultivars of cocksfoot had higher WSC contents than late maturing ones. They also found differences among cultivars of timothy and meadow fescue.

Stage of growth

The concentration of WSC in grasses of temperate origin is influenced greatly by the ratio of stem to leaf tissue. Concentration appears to increase with advancing maturity as the proportion of stem tissue increases.[12] This increase in WSC is due primarily to an increase in fructan content. Henderson[24] examined the WSC of perennial ryegrass grown in Scotland and harvested daily from different plots over a 24-day period from 11 May to 3 June. The results indicated that there was a positive correlation between WSC content and stage of growth, values increasing from 139 g kg^{-1} DM on day 1 to 245 g kg^{-1} DM on day 24. In a similar study over the late summer and autumn period (30 August to 22 September) using Italian ryegrass, the WSC values increased from 105 g kg^{-1} DM (day 1) to 211 g kg^{-1} DM (day 24).

Table 2.4. **The water soluble carbohydrate contents (g kg^{-1} DM) of some cultivars of Italian and perennial ryegrasses grown in south-east Scotland[31]**

Species	Cultivar	Cut 1 10 May	Cut 2 18 May	Cut 3 25 May	Cut 4 31 May	Cut 5 7 June	Cut 6 15 June	Mean
Italian ryegrass	Bartolini	169	285	246	298	288	324	268
	Tribune	235	312	292	330	315	462	324
	Barcolte	210	278	249	356	313	329	289
	Polly	191	327	234	263	252	264	255
	Dalita	190	214	252	303	216	282	243
	Mean	199	283	255	310	277	332	—
Perennial ryegrass	Peramo	210	272	251	237	213	—	237
	Cropper	225	256	201	278	247	—	241
	Merlinda	203	262	263	262	237	—	245
	Mean	213	263	238	259	232	—	—

Waite and Boyd[25] examined the individual sugar and fructan contents of perennial ryegrass, cocksfoot, meadow fescue and timothy grown in Scotland from April to October and found that the concentration of sucrose varied with the stage of growth, rising to a peak in May/June. The curves for the fructan contents of fescue, cocksfoot and timothy showed two peaks, the first in May and the second in July/August; ryegrass exhibited a single peak in June.

Diurnal variations

Concentrations of total sugars and WSC in temperate grasses appear to increase during the morning hours until sometime in the afternoon, and then to decrease until daylight the next day.[12] Most of the diurnal variation appears to be due to changes in sucrose concentration, and this is well illustrated from the studies by Waite and Boyd[25] with perennial ryegrass which are summarised in Table 2.5. In experiments with Kentucky bluegrass (*Poa pratensis*), Holt and Hilst[33] also showed that WSC contents increased during the day.

Table 2.5. Diurnal variation of water soluble carbohydrates (g kg^{-1} DM) in the aerial parts of perennial ryegrass[25]

Time (hours)	Hexoses	Sucrose	Fructan	Total
9.00	29	53	164	246
12.00	25	67	174	266
15.00	24	70	161	255
18.00	28	70	158	259
21.00	27	66	159	252
24.00	26	66	166	258
3.00	28	54	169	251
6.00	29	53	169	251

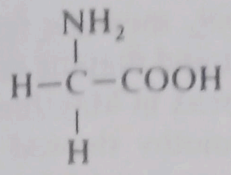
6-7 June. Total sunshine 9.8 hours. Temperature: maximum 22°C, minimum 8°C

Climate

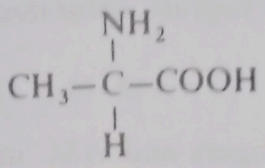
Reduction in light intensity reduces the WSC concentration in grasses.[12,34] Deinum[35] showed that the WSC content of ryegrass grown at a day temperature of 15°C and a night temperature of 10°C was only 90 g kg^{-1} DM at a low light intensity compared with nearly four times this value when grown under high light intensity conditions. Deinum's studies also suggested that WSC levels are highest in plants grown at high light intensities and low temperature and lowest in shade and high temperature, a conclusion also reached by Bathurst and Mitchell.[14] The effect of shading perennial ryegrass under field conditions was studied by Mackenzie and Wylam,[11] who examined the individual carbohydrate components of the WSC fraction. The sucrose content of ryegrass shaded with hessian fell sharply within 24 hours, while the fructan content remained the same in both plots after 24 hours but decreased slightly after 48 hours. Pettersson and Lindgren[36] found marked reductions in glucose, fructose, sucrose and fructan concentrations in grass and clover swards shaded by tarpaulins for three days before harvest. Environmental temperature appears not only to influence the concentration of fructans in temperate grasses, but also their molecular size distribution.[12]

1. Aliphatic amino acids

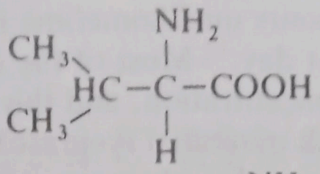
Glycine



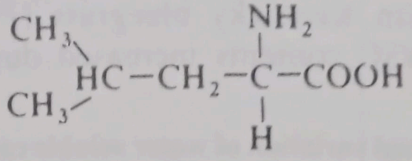
Alanine



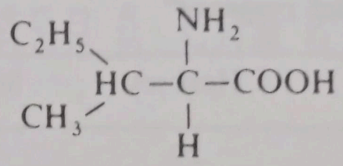
Valine



Leucine

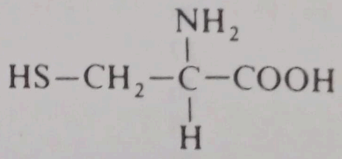


Isoleucine

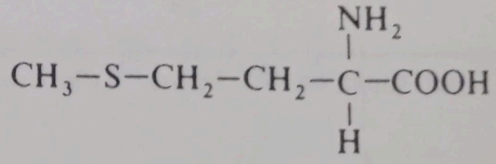


2. Sulphur-containing amino acids

Cysteine

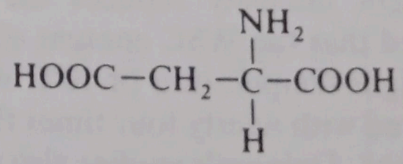


Methionine

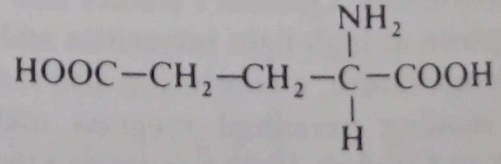


3. Dicarboxylic acids

Aspartic acid

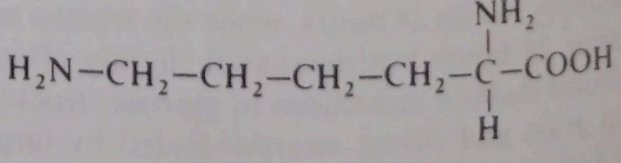


Glutamic acid

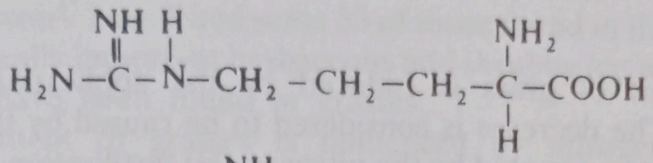


4. Basic amino acids

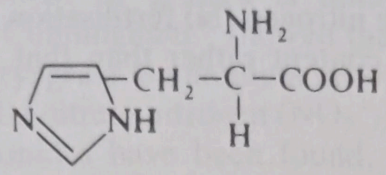
Lysine



Arginine

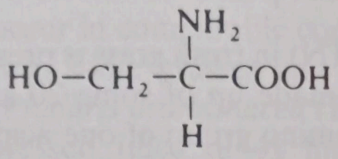


Histidine

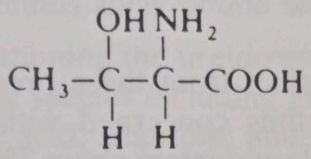


5. **Hydroxy amino acids**

Serine

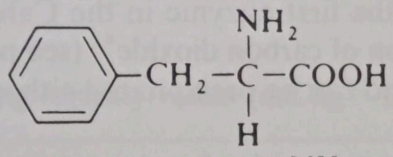


Threonine

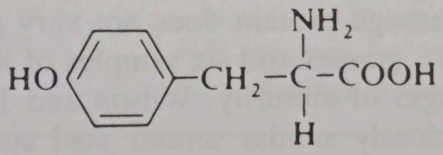


6. **Aromatic amino acids**

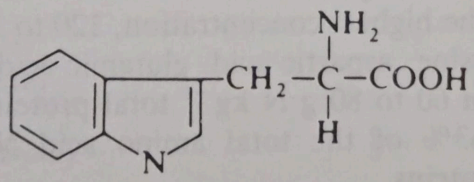
Phenylalanine



Tyrosine

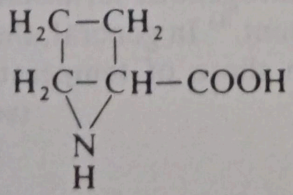


Tryptophan



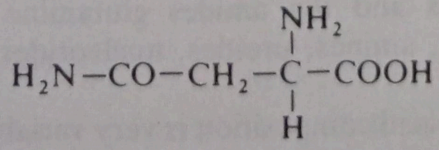
7. **Imino acids**

Proline



8. **Amides**

Asparagine



Glutamine

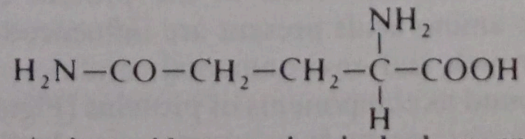


Figure 2.3. Amino acids occurring in herbage proteins

Fertiliser levels

The WSC content of grass is reduced by the application of nitrogenous fertilisers.[12,37-39] The decrease is considered to be caused by the acceleration of herbage growth rate promoted by the nitrogen (N) fertilisation and appears to be the result of a decrease in fructan content rather than that of total sugars.[12]

Nitrogenous components

Proteins

About 75 to 90% of the total nitrogen (TN) in fresh grass is present as protein. As in all other living cells, this protein is made up of some 20 amino acids linked through peptide bonds between the α-amino group of one acid and the carboxyl group of the next. Figure 2.3 shows those amino acids commonly occurring in herbage proteins.

The proteins present in forage, as distinct from those present in seeds, are almost all enzymic by nature and are thus concerned with the growth and biochemical function of the cells. Chloroplasts contain about 75% of the total leaf protein.[40] As much as 50% of this is one enzyme, ribulose bisphosphate carboxylase (RuBPCase), which is the first enzyme in the Calvin photosynthetic cycle and is responsible for the fixation of carbon dioxide[41] (see p. 50). Much of the plant protein is cold water soluble and can be precipitated either by heat or by the use of acids.[42]

The amino acid content of herbage protein does not vary greatly with plant species. In an examination of five grasses and six samples of lucerne (*Medicago sativa*) harvested at different stages of maturity, Wilson and Tilley[43] found that protein preparations were of closely similar amino acid composition. In all samples arginine occurred in the highest concentration, 120 to 140 g N kg^{-1} total protein N, with amounts of lysine, aspartic acid, glutamic acid, alanine, leucine and glycine each ranging from 60 to 80 g N kg^{-1} total protein N. These seven amino acids accounted for 63% of the total amino acid N recovered from hydrolysis of the herbage proteins.

The main factor influencing the protein content of herbage is stage of growth (Table 2.1), although the application of nitrogenous fertilisers can also have a marked effect in increasing the protein content.[44] In general, the protein contents of tropical grasses tend to be lower than those of temperate species.[44]

Non-protein nitrogenous compounds

Some 10 to 25% of the TN in herbage is made up of non-protein components. These include free amino acids and the amides glutamine and asparagine, peptides of varying chain length, amines, ureides, nucleotides, chlorophyll and nitrates.

In forage crops the free amino acid composition is very variable and bears little relationship to the amino acid composition of the protein fraction, which is relatively stable.[45] The free amino acids present are influenced by many factors, such as species, stage of growth and environmental conditions.[46] In addition to the amino acids normally found as components of proteins (Figure 2.3), a number of non-protein amino acids also occur free in grasses and other forage crops.

Hegarty and Peterson[47] have listed some 53 of those found in the seeds and leaves of some agronomically important herbaceous and shrubby legumes. Only a few of these, however, have been found in grasses.

The amide content of grasses is influenced by nitrogenous fertilisation. Nowakowski and Cunningham[48] showed that when 100 mg kg^{-1} of nitrogen was applied to Italian ryegrass, ammonium nitrogen (NH_4^+-N) produced more amide in the grass than did nitrate nitrogen (NO_3^--N). Asparagine was the predominant amide present. Amides have been found, usually in trace amounts, in higher plants but they may accumulate to high levels in some species.

Plant amines are almost as widespread and chemically diverse as the amino acids, but do not occur in comparable concentrations in most plants.[45] Histamine has been detected in some grasses and clovers,[49] while tryptamine derivatives occur in the grass *Phalaris arundinacea* (Reed canary grass).[47] Ethanolamine was found in the expressed juice of 41 higher plants, including several herbage species.[50]

Compounds containing the ureido group, NH_2-CO-NH-, have been found in a number of herbage species including grasses. It is thought that these substances, which include uric acid, allantoin, and allantoic acid, may play a role as nitrogen storage compounds and in nitrogen translocation.[47]

The nitrate content of grass is markedly influenced by the level of nitrogenous

Table 2.6. Buffering capacity values (mE kg^{-1} DM) of a number of herbage species[54-62]

Species	Number of samples	Buffering capacity	
		Range	Mean
Grasses			
Timothy (*Phleum pratense*)	2	188-342	265
Cocksfoot (*Dactylis glomerata*)	5	247-424	335
Italian ryegrass (*Lolium multiflorum*)	11	265-589	366
Perennial ryegrass (*Lolium perenne*)	13	257-558	380
Rhodes grass (*Chloris gayana*)	1	—	435
Legumes			
Red clover (*Trifolium pratense*)	1	—	350
White clover (*Trifolium repens*)	1	—	512
Lucerne (*Medicago sativa*)	9	390-570	472
Stylo (*Stylosanthes guianensis*)	1	—	469
Siratro (*Macroptilium atropurpureum*)	1	—	621

fertiliser applied. It is not uncommon to find levels of nitrate-N exceeding 100 g kg^{-1} TN in commercially grown samples harvested within a few weeks of fertiliser application.[51]

Buffering capacity

The buffering capacity of plants, or their ability to resist pH change, is an important factor in ensilage. Virtanen,[52] in developing the AIV process (see p. 196), was aware of this fact and he devised a rapid method for determining the amount of hydrochloric acid which must be added to a fresh crop in order to obtain the desired pH value. Measurements of the buffering capacity of plants are often made in this way, either with mineral acids or organic acids such as lactic acid.[53] Interest is usually concentrated within the pH range 4 to 6 since most plant materials, after maceration, have pH values of about 6 or slightly above and the pH of well-preserved silage is about 4. A method frequently used is that suggested by Playne and McDonald,[54] in which herbage macerates are first titrated to pH 3 with 0.1 M hydrochloric acid in order to release bicarbonate as carbon dioxide, and then titrated to pH 6 with 0.1 M sodium hydroxide.

Buffering capacity is expressed as milliequivalents (mE) of alkali required to change the pH of 1 kg DM from 4 to 6. Very few buffering capacities of plants have been determined using the technique of Playne and McDonald as described above, but Table 2.6 lists a few values for a number of grasses and legumes.[54-62] In general these results indicate that legumes are more highly buffered than are grasses, and this conclusion has been confirmed in other studies.[53]

Most of the buffering properties of herbage can be attributed to the anions present (organic acid salts, orthophosphates, sulphates, nitrates and chlorides), with only about 10 to 20% resulting from the action of plant proteins.[54] A number of organic acids have been identified in grasses, and those occurring in largest concentration in a number of species are shown in Table 2.7.[63] Quantitatively, the most important acids occurring in grasses are malic, citric and quinic acids. Oxalic acid may also be present, but in temperate grasses the level is usually regarded as being low.[64] However, in some tropical species, e.g. *Setaria sphacelata*, levels as high as 70 g kg^{-1} DM have been reported.[65]

CEREALS

Maize

Maize (*Zea mays*) is the most popular cereal crop conserved as silage, and large areas are grown for this purpose in many parts of the world.[66-75] Maize is, however, essentially a tropical crop; germination will not occur and little or no growth is made if the temperature is below 10°C.[66] In the UK, maize can only be grown satisfactorily in the south of the country.[76]

Like grass, the DM content of the whole plant increases with maturity but, unlike grass, the digestibility value of maize remains relatively constant. This occurs as the declining quality of leaf and stem is balanced by an increasing proportion of highly digestible ear and grain.[66,77-79] During the vegetative stage of growth soluble sugars are the main nonstructural carbohydrates present in maize, but these decline after fertilisation and starch content rises during subsequent

Table 2.7. Organic acids of some grass species grown under similar conditions[63]

Species	Organic acids (g kg^{-1} DM)						
	Citric	Fumaric	Malic	Quinic	Shikimic	Succinic	Total
Cocksfoot S143	3.2	—	8.1	4.4	1.4	2.8	19.9
Cocksfoot S37	2.7	0.2	9.4	6.6	3.7	3.2	25.8
Meadow fescue	3.7	—	11.0	6.2	1.2	2.5	24.6
N.Z. Agrostis*	2.4	0.1	5.6	1.4	0.5	3.0	13.0
Perennial ryegrass	4.5	0.2	8.8	3.0	1.2	2.4	19.9
Red fescue†	2.8	—	13.0	2.4	2.2	2.8	23.2
Timothy	4.2	0.2	8.2	5.6	1.8	2.2	22.2

* *Agrostis tenuis*
† *Festuca rubra*

grain development.[80] Phipps and co-workers[81-85] have made a comprehensive study of the composition of whole crop maize grown in the south of England. In this study maize was harvested on three occasions from three cultivars (Intra 200, LG 11 and Anjou 210) grown at different plant densities. Differences between the three cultivars in DM, ash, TN, D value (% digestible organic matter in DM), and fibre components were small, although the amounts of WSC and starch differed significantly. Increasing plant density was reflected in a decrease in the contents of DM, starch and water soluble nitrogen. The effects of time of harvesting can be seen in the results summarised in Table 2.8.

Table 2.8. Composition and *in vitro* digestibility of whole crop maize harvested on three different dates[85]

	27 August	18 September	8 October
Days from ear emergence	96	118	138
DM (g kg^{-1})	236	269	335
Buffering capacity (mE kg^{-1} DM)	225	180	149
Neutral detergent fibre (g kg^{-1} DM)	413	426	432
Acid detergent fibre (g kg^{-1} DM)	217	227	223
Acid detergent lignin (g kg^{-1} DM)	16.6	15.2	18.5
WSC (g kg^{-1} DM)	307	136	80.1
Starch (g kg^{-1} DM)	68.2	190	278
Ash (g kg^{-1} DM)	46.3	53.2	54.0
TN (g kg^{-1} DM)	13.1	14.0	13.8
Water insoluble-N (g kg^{-1} TN)	674	669	662
D-value*	77.2	72.1	69.2

* Digestible organic matter in DM (%)

In a separate study, Phipps and co-workers[86] compared the composition of isogenic fertile and sterile forage maize hybrid (cv. LG 11) grown over a period of 79 days from 16 July until 3 October, when they were harvested and ensiled. A summary of the main carbohydrate components of both crops, cut at regular intervals over the growing period, is shown in Table 2.9. The main differences in composition between the two plants occurred in October at harvesting, when the concentration of soluble sugars in the sterile plants was more than three times as much as in fertile plants. In both cases sucrose was found to be the predominant sugar, contributing approximately 65 to 75% to the totals. The starch concentration of the sterile plants was low and did not change much during growth, whereas the concentration of this polysaccharide in the fertile plants was low in the early part of the growing season, but increased markedly from mid-September until harvest. Xylose was found to be the main sugar component of the hemicelluloses in both plants.

In terms of ensiling characteristics, maize can be regarded as an ideal crop since it is relatively high in DM content, is of low buffering capacity and contains adequate levels of WSC for satisfactory fermentation to lactic acid. The main components of the WSC fraction are sucrose, glucose and fructose.[83]

A major disadvantage of maize as a silage crop is its low crude protein content, which is usually less than 100 g kg^{-1} DM.[87] This can be increased by the addition

Table 2.9. **Carbohydrate components (g kg^{-1} DM) of isogenic fertile (F) and sterile (S) maize plants[86]**

Sample date	Soluble sugars		Starch		Hemicelluloses		Cellulose	
	F	S	F	S	F	S	F	S
16 July	132	61	29	38	113	115	231	217
30 July	197	101	29	22	120	116	235	227
13 August	170	195	34	39	141	155	225	235
26 August	271	277	30	26	186	121	231	245
10 September	289	393	53	26	149	125	221	227
24 September	189	377	204	37	180	119	217	221
30 October	101	343	369	54	110	169	214	218

of urea at the time of ensiling.[88,89] Silages treated in this way contain higher true protein contents than untreated silages (see p. 218).[90,91] Alternatively, the crude protein content can be increased by mixing maize with a forage legume prior to ensiling.[87]

Barley

In parts of the world, such as Northern Europe, where the climate is unsuitable for growing maize, barley (*Hordeum sativa*) is often grown as an alternative cereal crop for silage. Several studies have been made on the composition of the whole crop at different stages of growth,[92-101] but probably the most comprehensive analytical study was that made by Edwards and co-workers[94,95] who examined eight cultivars of barley at seven stages of growth. A summary of these results is shown in Table 2.10. The DM content of the crop increased from about 190 g kg^{-1} to about 430 g kg^{-1} as the plants matured. The D-value showed only small variations (54 to 63) but was markedly lower than that for whole crop maize (Table 2.8).

The WSC contents of barley are higher than those found in maize, reaching peak values at the mealy ripe stage of growth. Initially glucose and fructose are present in the highest concentration but as barley matures the relative amounts of these decrease, and fructose-containing oligosaccharides and fructans increase in concentration until the milky kernel stage is reached. Thereafter, the concentrations of these carbohydrates also decrease.

The main organic acid present appears to be malic. Edwards *et al.*[94] suggested that from the point of view of preservation and nutritive value, barley intended for silage should be cut between the milky and late mealy ripe stages of growth and ideally the silage should be made in tower silos.

The main disadvantage of whole crop barley, as with all cereals, is the low crude protein content,[102] although this can be improved by the addition of urea.[101]

Sorghum

Sorghum (*Sorghum bicolor*), grown as a crop for silage, has received considerable attention in recent years,[103-111] particularly in the USA, where its drought

Table 2.10. The composition of whole barley plants at different stages of growth*[94, 95]

	Heading completed (58)	Flowering (68)	Watery ripe (71)	Milky ripe (75)	Mealy ripe early (84)	Mealy ripe late (86)	Ripe for cutting (92)
A. **Proximate constituents** (mean of 8 cultivars)							
DM (g kg^{-1})	191	200	206	293	353	387	425
Crude protein (g kg^{-1} DM)	103	87	72	67	56	60	66
Ether extract (g kg^{-1} DM)	19	14	13	13	14	16	13
Crude fibre (g kg^{-1} DM)	313	321	286	253	204	195	254
Ash (g kg^{-1} DM)	75	65	56	47	38	35	35
WSC (g kg^{-1} DM)	169	180	249	318	242	147	46
D-value†	62	54	58	61	63	61	55
B. **Carbohydrate and organic acid components** (c.v. Zephyr)							
Fructose (g kg^{-1} DM)	60	50	41	31	29	31	22
Glucose (g kg^{-1} DM)	60	60	42	29	28	20	11
Galactose (g kg^{-1} DM)	0	0	0	1.6	4.4	4.6	5.8
Sucrose (g kg^{-1} DM)	19.0	14.6	22.8	33.0	21.3	19.7	4.4
Oligosaccharides†† (g kg^{-1} DM)	16.1	33.5	68.8	76.0	32.1	34.5	15.1
Fructans (g kg^{-1} DM)	30.8	32.5	72.0	128	122	66.0	23.0
Starch (g kg^{-1} DM)	2.8	2.5	3.8	10.2	18.5	34.8	41.3
Acetic acid (g kg^{-1} DM)	4.3	7.1	3.3	6.5	6.4	6.4	5.1
Malic acid (g kg^{-1} DM)	56.3	28.2	17.3	13.1	7.1	3.2	1.5
Citric acid (g kg^{-1} DM)	4.8	4.0	5.4	4.3	2.4	0.9	0.6
pH	5.9	5.9	5.9	5.9	5.9	6.0	6.0

* Numbers in parenthesis refer to Zadoks 2 digit code classification
† Digestible organic matter in DM (%)
†† Excluding sucrose but including short-chain fructans

tolerance and extended planting period make it a useful crop in areas of low or uncertain rainfall.[104,105] Black and co-workers[104] examined the composition of sorghum harvested at six stages of growth, from early bloom to hard dough, and found that the DM increased from 232 to 308 g kg^{-1} and the crude protein decreased from 84 to 59 g kg^{-1} DM with increasing maturity of the crop. In a comparison of forage sorghum and maize harvested at two locations, Fisher and Burns[110,111] found that at location 1 (crops harvested at the medium-dough stage) total sugar levels (g kg^{-1} DM) for sorghum and maize were 131 and 92 and starch contents (g kg^{-1} DM) were 116 and 224 respectively. Corresponding values (g kg^{-1} DM) at location 2 (crops harvested at the early milky-dough stage) were total sugars 176 and 165, and starch 53 and 72. In this study sorghum was found to have a higher buffering capacity than maize.

McCullough and co-workers[105] stated that, at its best, sorghum silage had only about 80 to 85% of the digestibility of maize silage. As sorghum matured, dry matter digestibility declined, and at the mature seed stage it had a percentage digestibility in the low 50 range, compared to 65 to 68 for maize. These workers concluded that harvesting the crop for silage should begin when the heads are out and bloom has begun, and should be completed by the time the seeds are in the milky stage.

Other cereals

Rye (*Secale cereale*),[1,112,113] wheat (*Triticum aestivum*),[1,114-117] oats (*Avena sativa*),[1,118-122] and rice (*Oryza sativa*)[123] have all been ensiled successfully. Witt[117] harvested green rye at nine different stages of growth and three nitrogen fertiliser treatments. The crude protein content of the DM declined with maturity in the low fertiliser treatment (50 kg N ha^{-1}) from 186 to 38 g kg^{-1} DM and in the high fertiliser treatment (150 kg N ha^{-1}) from 307 to 68 g kg^{-1} DM. WSC levels ranged from 97 to 270 g kg^{-1} DM, the lowest values occurring in the high fertiliser treated samples harvested at the mature stage of growth.

Ashbell and co-workers[124] studied the amino acid components of whole crop wheat during ensiling. They found that there was a close relationship between the amino acid content of the grain and the fresh plant. As in grasses (see p. 30), arginine occurred in the highest concentration.

Singh and co-workers[119] ensiled oats grown in India at the dough stage. The original crop had a DM content of 418 g kg^{-1}. The resulting silage was well-preserved at pH 4.6 and lactic acid formed 66% of the total acids present. The concentration of ammonia-N in the TN was 66 g kg^{-1}. Přikryl *et al.*[120] ensiled oats grown in Czechoslovakia, also at the dough stage, but found the DM content to be only 280 g kg^{-1}. The resultant silages were well-preserved.

As a potential crop for silage, Yahara *et al.*[123] investigated the composition of whole rice at four stages of maturity. In common with other cereal crops, DM content increased with increasing stage of growth. Crude protein and WSC contents decreased with maturity. The dough stage of growth was considered to be the most desirable stage at which to harvest for silage.

LEGUMES

Legumes have always been regarded as an important group of crops grown for

Table 2.11. Some carbohydrate components (g kg^{-1} DM) of lucerne and red clover harvested at different stages of growth[12,129]

Species	Carbohydrate	Growth stage					
		Vegetative	Pre-bud	Mid-bud	Early-bloom	Full-bloom	Green seed pod
Lucerne	Fructose	25	27	20	14	23	21
	Glucose	25	26	21	13	21	17
	Sucrose	42	56	27	24	27	16
	WSC	92	109	68	51	71	54
	Starch	87	48	68	60	62	45
Red clover	Fructose	23	24	27	24	25	20
	Glucose	24	30	38	45	38	34
	Sucrose	48	41	21	20	29	21
	WSC	95	95	86	89	92	75
	Starch	86	52	72	77	67	67

feeding farm animals because of their nitrogen-fixing properties and because they are a relatively rich source of protein. Lucerne, red clover (*Trifolium pratense*), white clover (*Trifolium repens*), sainfoin (*Onobrychis viciifolia*), field bean (*Vicia faba*), common vetch (*Vicia sativa*) and lupin (*Lupinus luteus*) are among the most common legumes grown for forage.

Until recently, legumes were regarded as being unsuitable for ensiling as the fermentation was invariably dominated by clostridia, leading to a butyrate-type silage. This has been attributed to three factors; firstly they are highly buffered, secondly they tend to have low WSC contents, and thirdly they are often of low DM content.

The high buffering capacity of legumes compared with grasses has already been mentioned (p. 32). This is mainly due to the higher organic acid content of legumes,[55,64,125] which may in some crops, for example lucerne, be as high as 100 g kg^{-1} DM.[126] The main acids in legumes are malic, citric, quinic, malonic and glyceric.[54,64] Glyceric acid appears to be particularly important in red clover, and in this species it may be the major plant organic acid present.[54,127] Although the plant organic acids and their salts are considered to be the main cause of the greater buffering properties of legumes compared with grasses, the high content of proteins in these crops also plays a contributory role.

Whereas the principal storage polysaccharide in grasses of temperate origin is fructan, the main reserve polysaccharide in legumes is starch.[12] Since starch is insoluble in cold water it is not a component of the WSC fraction and, furthermore, unhydrolysed starch is not available as a fermentable substrate to most lactic acid bacteria.[17,128]

The main sugars present in the WSC fraction of legumes are fructose, glucose and sucrose,[129] although raffinose and stachyose have also been detected.[130] Some workers have found no apparent pattern in the soluble carbohydrate components of legumes with increasing maturity,[130,131] while others have found decreases in certain sugars. Raguse and Smith[129] compared the fructose, glucose, sucrose and starch contents of a number of legumes at six stages of growth. Their results, obtained for lucerne and red clover, are shown in Table 2.11. In both species sucrose contents were markedly lower in the more mature samples than in those harvested at the beginning of the season. After the pre-bud stage, the WSC appeared to decline more rapidly in lucerne than in red clover.

Table 2.12. Dry matter and water soluble carbohydrate contents of six clovers harvested on 10 June (cut 1) and 27 August (cut 2) in south-east Scotland[24]

Species and cultivar	Cut 1		Cut 2	
	DM (g kg^{-1})	WSC (g kg^{-1} DM)	DM (g kg^{-1})	WSC (g kg^{-1} DM)
Late flowering red, Altaswede	99	108	166	53
Late flowering red, Montgomery	109	96	166	56
Broad red, English	116	87	184	65
Broad red, Essex	105	104	174	53
White, S100	127	71	176	51
White, N.Z.	108	91	156	56

Henderson[24] compared the WSC values of six clovers harvested in June (first cut) and in August (second cut). Of particular significance in this study were the low DM contents of the forage cut in June and the low WSC values obtained for the August sampled materials (Table 2.12).

More recently the disadvantages of legumes in terms of ensiling characteristics have been overcome by pretreatments such as wilting[132-137] and the use of additives.[57,60,136,138-141] The specific effects of these on the fermentation process are discussed in Chapters 7 and 9.

OTHER CROPS AND CROP BY-PRODUCTS

Sunflowers

Sunflowers (*Helianthus annus*) have been grown successfully as a silage crop in many parts of the world,[142] particularly in the USA[143-145] and the USSR, where low fibre types have been bred specifically for the purpose of conservation.[1] Sunflowers are also of particular interest in Western Europe because they can grow at lower temperatures than maize, and they also grow extremely rapidly following late sowings.[1] In general, sunflowers contain more fibre than maize[144,146,147] but are usually of higher crude protein content.[144,147]

Several detailed studies of the ensiling characteristics of sunflowers have been made.[148-150] In one such study in Scotland,[148] using the cultivar Armavirec, the WSC values were in the range 103 to 213 g kg^{-1} DM over a period of harvesting from the beginning of June to the end of September, covering a period of growth from flower bud to dough seed stage (Table 2.13). In a crop ensiled just after flowering the DM content was 117 g kg^{-1}, WSC 189 g kg^{-1} DM, and the buffering capacity 449 mE kg^{-1} DM. In a later study by the same workers,[150] the cultivar Fransol was harvested at regular intervals between early July and the end of September. The crop exhibited a similar growth pattern to the variety Armavirec, used in the earlier experiment, with WSC values ranging from 211 to 251 g kg^{-1} DM. The results of these studies indicated that the optimum time of cutting sunflowers for yield of nutrients is at the milky ripe stage of growth.

Although sunflowers have adequate levels of fermentable carbohydrates for satisfactory preservation and produce silages that are readily eaten by ruminants,[148,150,151] they have the disadvantage of being of relatively low digestibility and of low DM content. While wilting is beneficial, this is difficult because of the physical structure of the plant, since most of the moisture is held in the thick stems.[1]

Potatoes

Potatoes may, or may not, be cooked prior to ensiling.[1,152] In Germany, cooked potato silage has been produced for many years and portable automatic machines have been developed which carry out the process of washing, steaming and mashing in one continuous operation.[1] The DM content of potatoes is about 230 g kg^{-1} and the crude protein content about 100 g kg^{-1} DM. Although the main carbohydrate present is starch, fermentable sugars are always present, usually in concentrations of about 50 g kg^{-1} DM, but these can increase after harvesting and values as high as 300 g kg^{-1} DM have been reported in potatoes stored at −1°C.[153]

Table 2.13. Composition of sunflower plants grown in south-east Scotland and harvested at different stages of growth[148]

Stage of growth*	DM (g kg^{-1})	g kg^{-1} DM					D-value††
		Crude protein	Ether extract	MAD† fibre	Ash	WSC	
6	84	287	20	282	196	103	56
7	84	260	16	280	185	106	58
8	89	191	18	272	172	169	61
9	110	176	19	234	140	212	66
10	126	168	19	239	122	167	65
11	137	113	21	291	127	137	59
12	146	113	22	294	120	193	60
13	143	88	38	317	104	200	57
14	173	92	70	313	91	213	60
15	164	140	82	299	114	175	60
16	171	133	89	333	112	134	57
17	183	92	100	365	111	107	51

*Growth stages: 6–8 Flower bud development and completion of leaf expansion
9 Onset of flowering and achievement of maximum height
10–11 Flowering
12–13 Onset of seed development and leaf senescence
14–15 Seeds milky ripe
16–17 Dough seed stage

† Modified acid detergent

†† Digestible organic matter in DM (%)

It has been suggested[17] that an inoculum may benefit silage materials which have been subjected to heat treatment, or processed in such a way that they are likely to be devoid of lactic acid bacteria (see p. 184).

Sugar beet tops

Until recently, the ensiling of the tops of sugar beet (*Beta vulgaris*) was unpopular because attempts to make good quality silage were limited by the difficulty of collecting clean, soil-free material. The introduction of multi-row harvesting machines has made possible the rapid collection of tops with a minimum level of soil contamination.[154]

The chemical composition of the harvested tops depends upon how much of the crown is removed with the leaves and also upon the level of soil contamination. In a comparison of two harvesting techniques, Haigh[154] found that the DM of the leaves contained 142 g crude protein kg^{-1} and 141 g WSC kg^{-1}, while the DM of the leaves + crown contained 133 g crude protein kg^{-1} and 262 g WSC kg^{-1}. The ash contents of both products were high, 191 and 345 g kg^{-1} DM respectively, which suggested that in this experiment some soil contamination had occurred. In spite of this, silage made from these products was well preserved. The DM content of sugar beet tops is low (110 to 160 g kg^{-1})[154-156] and effluent losses can be high in ensiled material.[157]

Fibrous residues

Fibrous residues, after the commercial extraction of sugar from sugar beet and sugar cane (*Saccharum officinarum*), are often preserved as silage.[1,158-162] After sugar extraction from the beet, the pulp is extremely wet, having a DM content of about 80 g kg^{-1}. This by-product is usually pressed at the factory to leave material with a DM content of 180 to 250 g kg^{-1}, which is more suitable for ensiling.

Wet residues left after the expression of juice from fruits such as apples, pears, grapefruit and oranges have all been ensiled successfully.[1] Most of these materials are rich in WSC and low in crude protein.

Recently, attention has focused on the ensiling of fibrous residues after protein extraction from a range of green crops. These have included residues from grasses,[163,164] lucerne,[165] clover,[163,166,167] broad beans[168,169] and sorghum.[170]

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Chapter 3

Plant Enzymes

Immediately after cutting and during the early stages of ensiling changes occur, resulting from the continuing metabolism of plant cells and from the activity of the enzymes of dead tissue. The processes of respiration and proteolysis are of particular importance.

RESPIRATION AND CARBOHYDRATE METABOLISM

Respiration can be defined as the oxidative degradation of organic compounds to yield usable energy. In higher plants oxygen (O_2) is the terminal electron acceptor. Although plants can and do respire proteins and lipids, carbohydrates are the major respiratory source. The substrate for oxidation is usually a hexose sugar which undergoes glycolysis (Figure 3.1) before entering the aerobic phase.

If oxygen is present, pyruvate from the glycolytic pathway is converted to acetyl coenzyme A by oxidative decarboxylation (Figure 3.2) and this is oxidised via the tricarboxylic acid (TCA) cycle to carbon dioxide, with the net synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and orthophosphate (Pi) (Figure 3.3). The NADH (reduced nicotinamide adenine dinucleotide) and FADH (reduced flavin adenine dinucleotide) formed during these reactions are energy-rich molecules because each contains a pair of electrons with a high transfer potential. These are transferred to molecular oxygen via an electron transport chain involving several cytochromes (CYT), as shown in Figure 3.4. During this, a large amount of energy is released and is used to generate ATP via the process of oxidative phosphorylation. Each molecule of NADH gives rise to three molecules of ATP.

$$NADH + 4H^+ + 3ADP + 3Pi + \tfrac{1}{2}O_2 \longrightarrow NAD^+ + 4H_2O + 3ATP$$

In higher plants, as in most aerobic organisms, the process of oxidative phosphorylation and the reactions of the cell with oxygen reside in the mitochondria.

The overall reaction for the complete oxidation of a molecule of glucose can be summarised as follows:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 2870\ kJ$$

A total of 38 molecules of ATP are produced from one molecule of glucose and these energy-rich molecules are used in the biosynthesis of many other plant components. The remaining energy is liberated as heat.

In the harvested plant, biosynthetic reactions are limited and it is assumed that virtually all the energy in the oxidised glucose is converted into heat. In the isolated plant this heat energy would be dissipated into the atmosphere, but in the

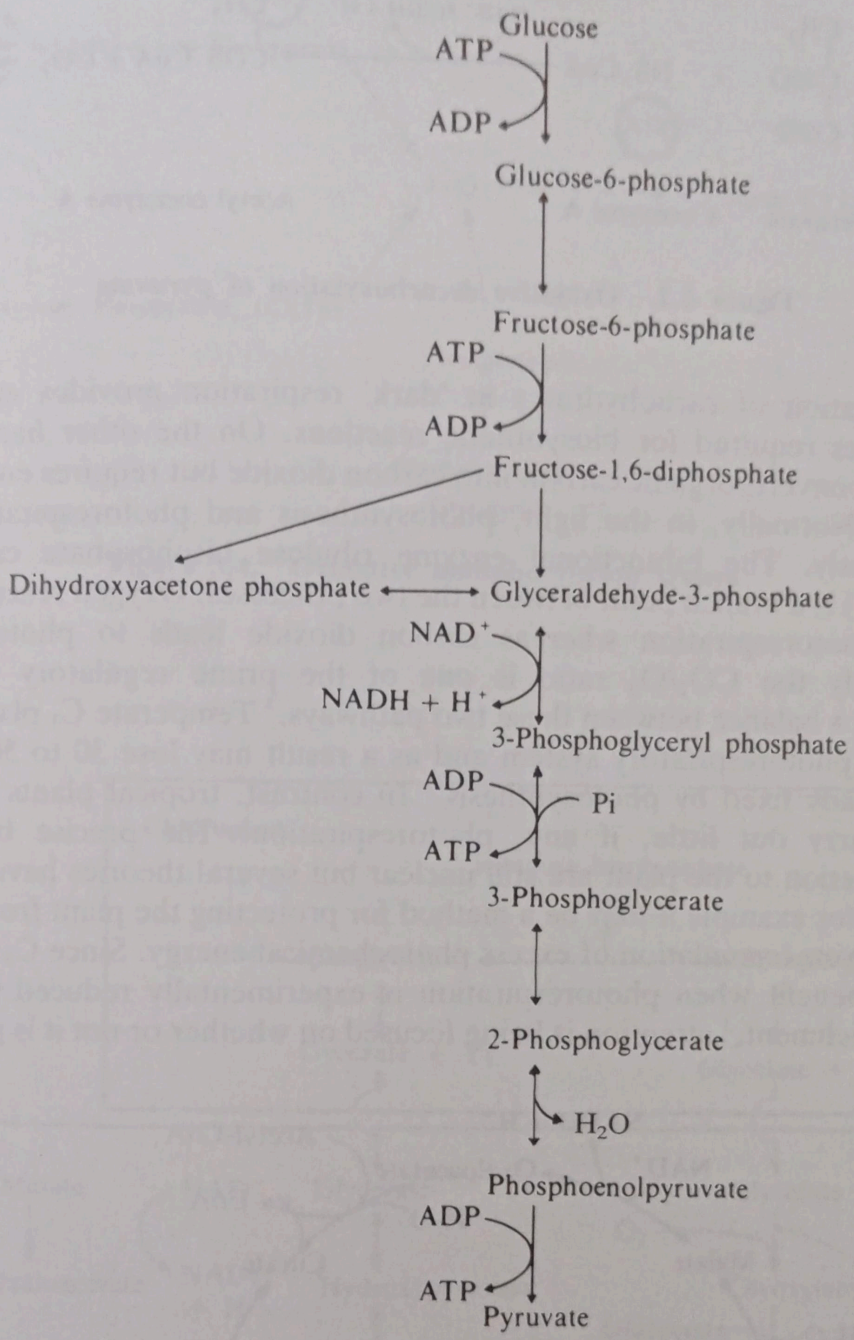


Figure 3.1. The glycolytic pathway (Embden–Meyerhof–Parnas pathway)

silo most of the heat arising from the action of respiratory enzymes is retained in the mass of herbage, causing an increase in temperature. Plant respiration continues as long as conditions in the silo are favourable, and both oxygen and a supply of substrate are available.

Mitochondrial or 'dark' respiration, as described above, is known to occur in the dark but it is also known that plants respire in the light while carrying out photosynthesis. This type of respiration is not mitochondrial, since it has been shown that it is not sensitive to characteristic inhibitors of mitochondrial electron transport.[1] It is usually referred to as 'light' respiration or photorespiration (Figure 3.5).

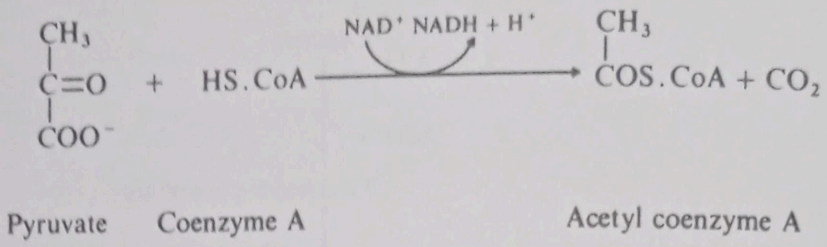


Figure 3.2. Oxidative decarboxylation of pyruvate

The oxidation of carbohydrates in 'dark' respiration provides energy and intermediates required for biosynthetic reactions. On the other hand, photorespiration converts organic carbon into carbon dioxide but requires energy input to do so.[2] Normally, in the light, photosynthesis and photorespiration occur simultaneously. The bifunctional enzyme ribulose bisphosphate carboxylase (RuBPCase) is a branch point between the two processes. Oxygen reacting with it leads to photorespiration whereas carbon dioxide leads to photosynthesis. Consequently the CO_2/O_2 ratio is one of the prime regulatory factors in maintaining a balance between these two pathways.[3] Temperate C_3 plants have a very active photorespiratory system and as a result may lose 30 to 50% of the carbon already fixed by photosynthesis.[2] In contrast, tropical plants with a C_4 pathway carry out little, if any, photorespiration. The precise benefits of photorespiration to the plant are still unclear but several theories have been put forward,[3,4] for example it may be a method for protecting the plant from damage due to an overaccumulation of excess photochemical energy. Since C_3 plants are known to benefit when photorespiration is experimentally reduced by carbon dioxide enrichment,[3] attention is being focused on whether or not it is possible to

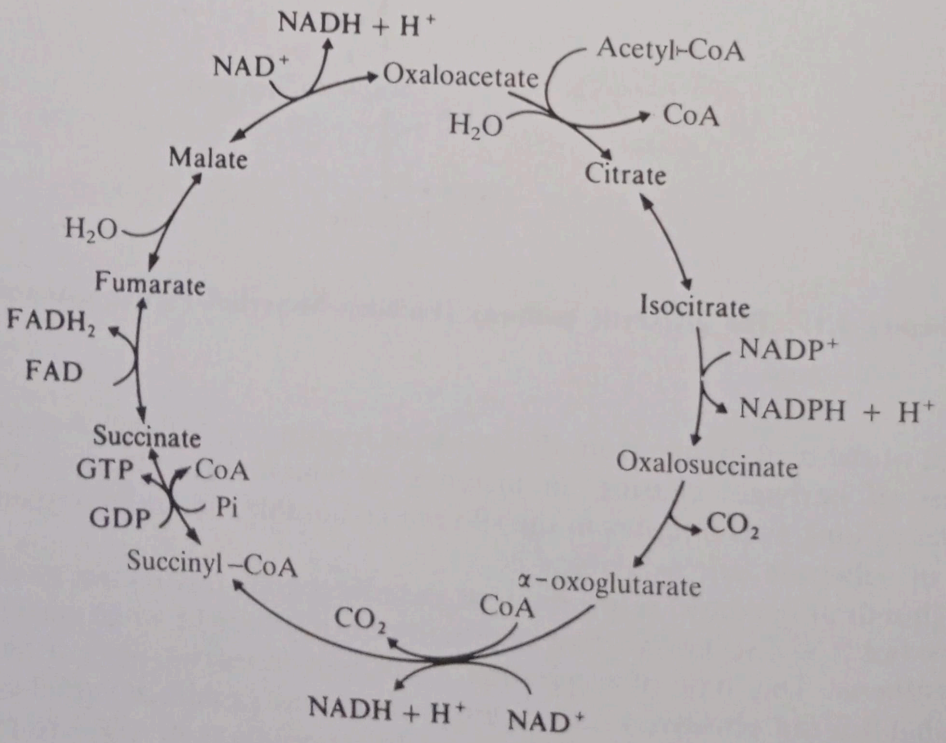


Figure 3.3. The tricarboxylic acid cycle

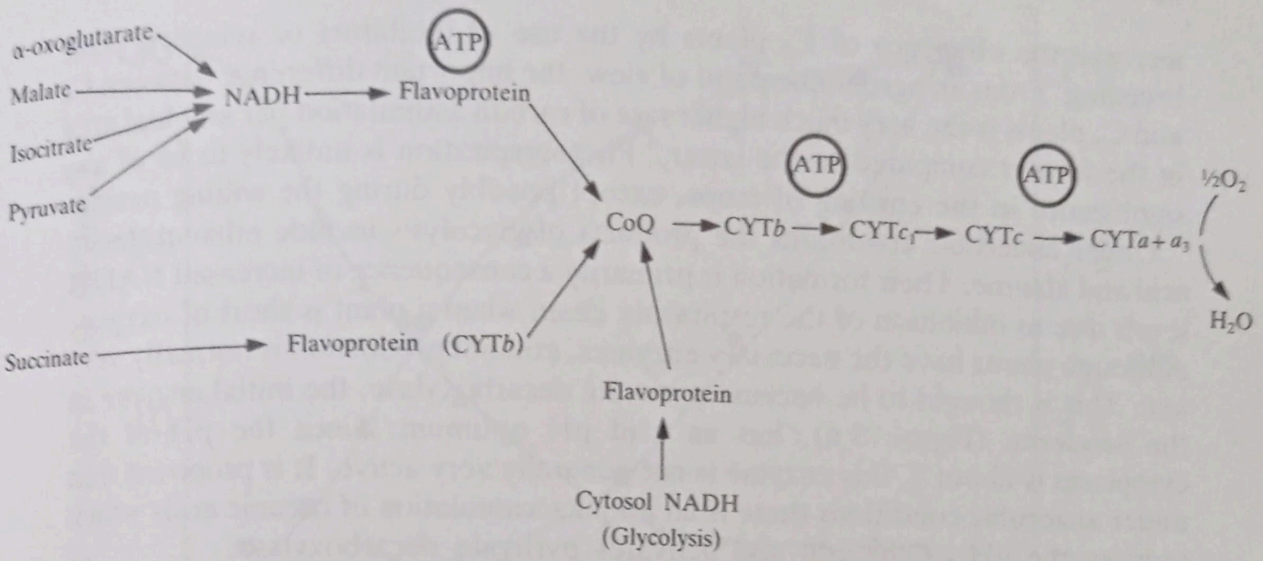


Figure 3.4. Oxidative phosphorylation system

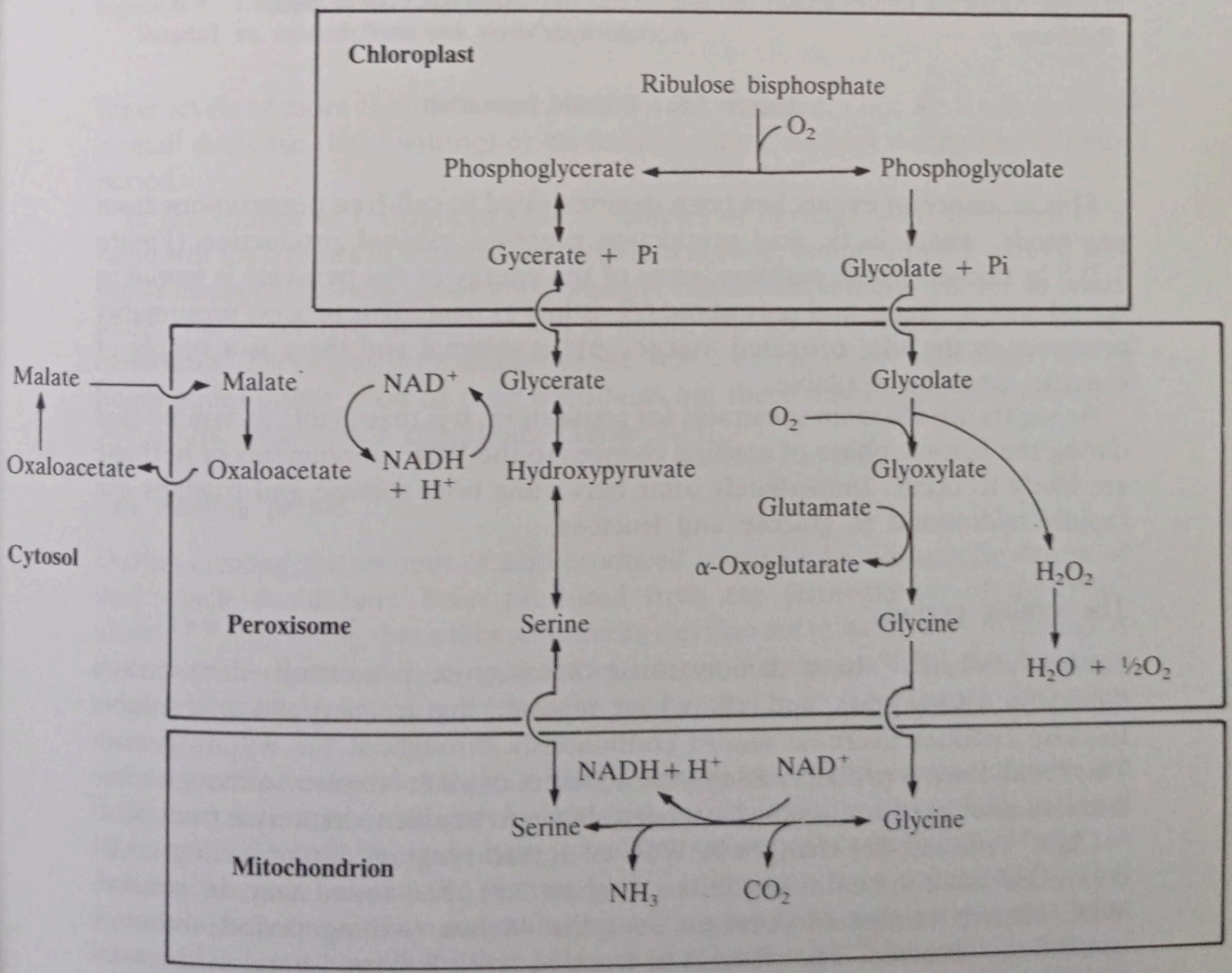


Figure 3.5. The metabolic pathway of photorespiration in green plants

increase the efficiency of C_3 plants by the use of inhibitors or selective plant breeding. From an agronomic point of view, the important difference between C_4 and C_3 plants is the very much higher rate of carbon assimilation per unit leaf area in the former compared to the latter.[5] Photorespiration is unlikely to be of any significance in the ensiling of crops, except possibly during the wilting period.

Under anaerobic conditions the products of glycolysis include ethanol, lactic acid and alanine. Their formation is primarily a consequence of increased NADH levels due to inhibition of the respiratory chain when a plant is short of oxygen. Although plants have the necessary enzymes, ethanol production is normally very low. This is thought to be because pyruvate decarboxylase, the initial enzyme in the sequence (Figure 3.6), has an acid pH optimum. Since the pH of the cytoplasm is about 7, this enzyme is not generally very active. It is proposed that under anaerobic conditions there is an initial accumulation of organic acids which reduces the pH of the cell and activates pyruvate decarboxylase.

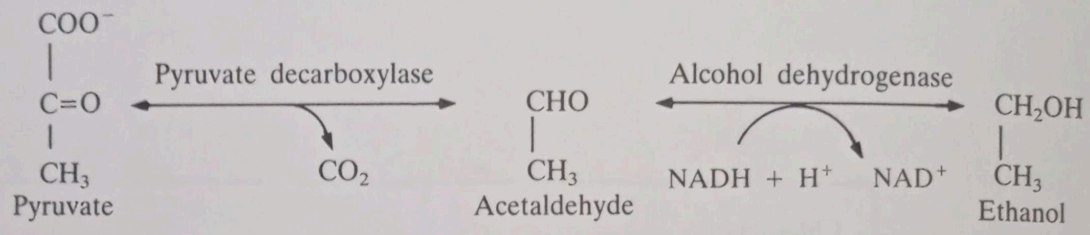


Figure 3.6. Ethanol formation

This sequence of events has been demonstrated in cell-free preparations from pea seeds, where lactic acid production precedes ethanol production (Figure 3.7).[6] In this anaerobic pathway, most of the energy of the pyruvate is stored in the ethanol molecule and only about 6% is lost as heat. This process presumably continues in the silo, provided that the pH is optimal and there is a supply of suitable substrate available.

As sugars are the main substrates for respiration, it is reasonable to assume that during the aerobic phase of ensiling changes to the WSC components of herbage are likely to occur. Immediately after harvesting both sucrose and fructans are rapidly hydrolysed to glucose and fructose.

The wilting period

Several workers[7-10] have demonstrated that sucrose is catabolised to carbon dioxide in wilting grass, and others have reported that fructans and total soluble fructose residues decrease almost continuously throughout the wilting period. This breakdown is probably due to the activities of plant enzymes, although some bacteria associated with silage have been shown to be able to hydrolyse fructans.[10]

Clark[11] studied the changes in WSC of Italian ryegrass during wilting under controlled environmental conditions (Figure 3.8). She found that, in general, WSC content tended to decrease over the 48 hour wilting period, although oscillations occurred. The changes in fructose levels followed those of the total WSC, but the glucose levels varied independently and with less magnitude. Oscillations were particularly great during dark wilting in air, when changes in

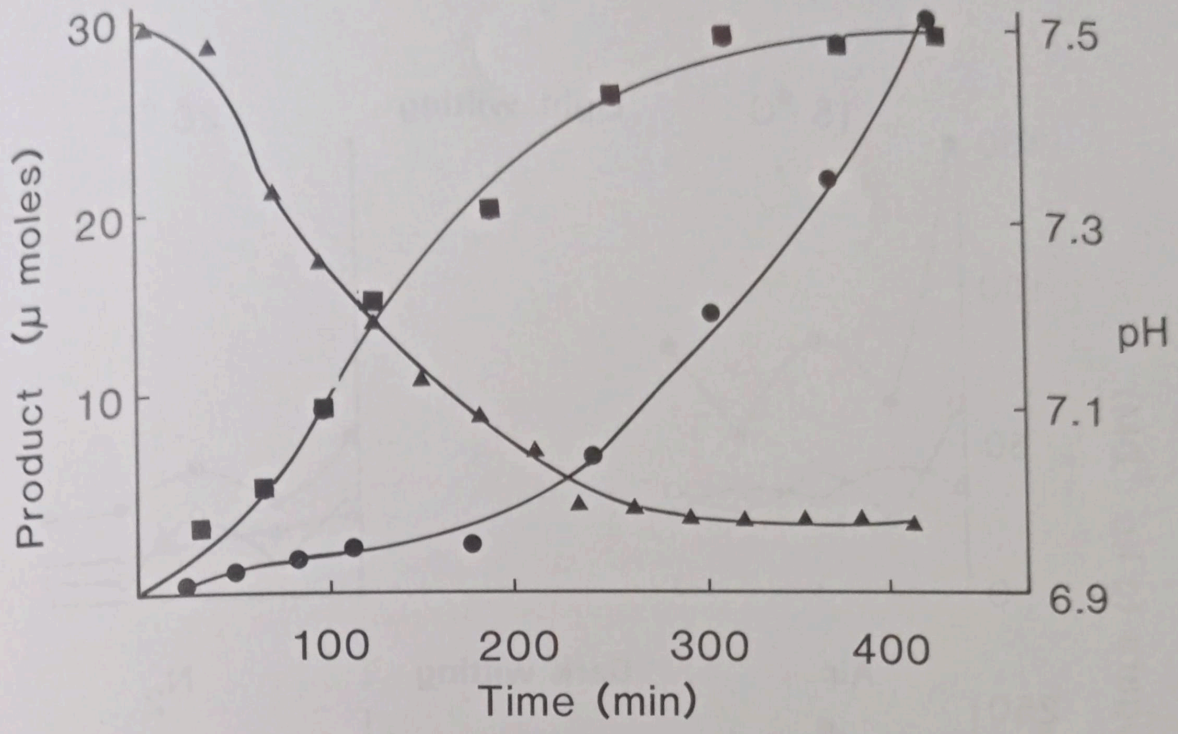


Figure 3.7. Changes in pH (▲), lactate (■) and ethanol (●) concentrations during glycolysis in an extract from pea seeds[6]

sugar levels of more than 50% in 12 hours were recorded. Glucose levels showed a small decrease (light wilting) or an increase (dark wilting) during the 48 hour period.

In the studies of Carpintero *et al.*[12] samples of herbage were subjected to a rapid wilt for 6 hours in a forced-air cupboard at room temperature, a natural wilt for 48 hours in the field under good weather conditions, a moist wilt for 48 hours under 100% relative humidity, and a moist wilt for 144 hours under similar conditions. There was little change in the WSC content of herbage wilted for 48 hours under either good or poor conditions but there was a significant fall after 144 hours under poor conditions (Table 3.1).

The ensiling period

During ensiling the amount of acid produced is usually found to be in excess of that which could have been produced from the fermentation of the WSC alone,[13-19] suggesting that other substances can also act as substrates. Although it is known that proteins, amino acids and organic acids can fulfil this function, structural carbohydrates are thought to be the main sources of this extra substrate.

Exact measurements of losses of WSC arising from plant enzyme activity are difficult to determine, as sugars lost through respiration may be partially replaced by sugar released by hydrolysis of plant structural carbohydrates such as cellulose, hemicelluloses and pectins.[11,20-23] It has also been suggested[24,25] that some photosynthesis may occur during wilting but, although activity has been observed in leaves during the first 24 hours after harvesting,[26] it is unlikely to be of much significance under practical wilting conditions, owing to insufficient penetration of light into the swath and the closure of the stomata.[11,27] Proof that

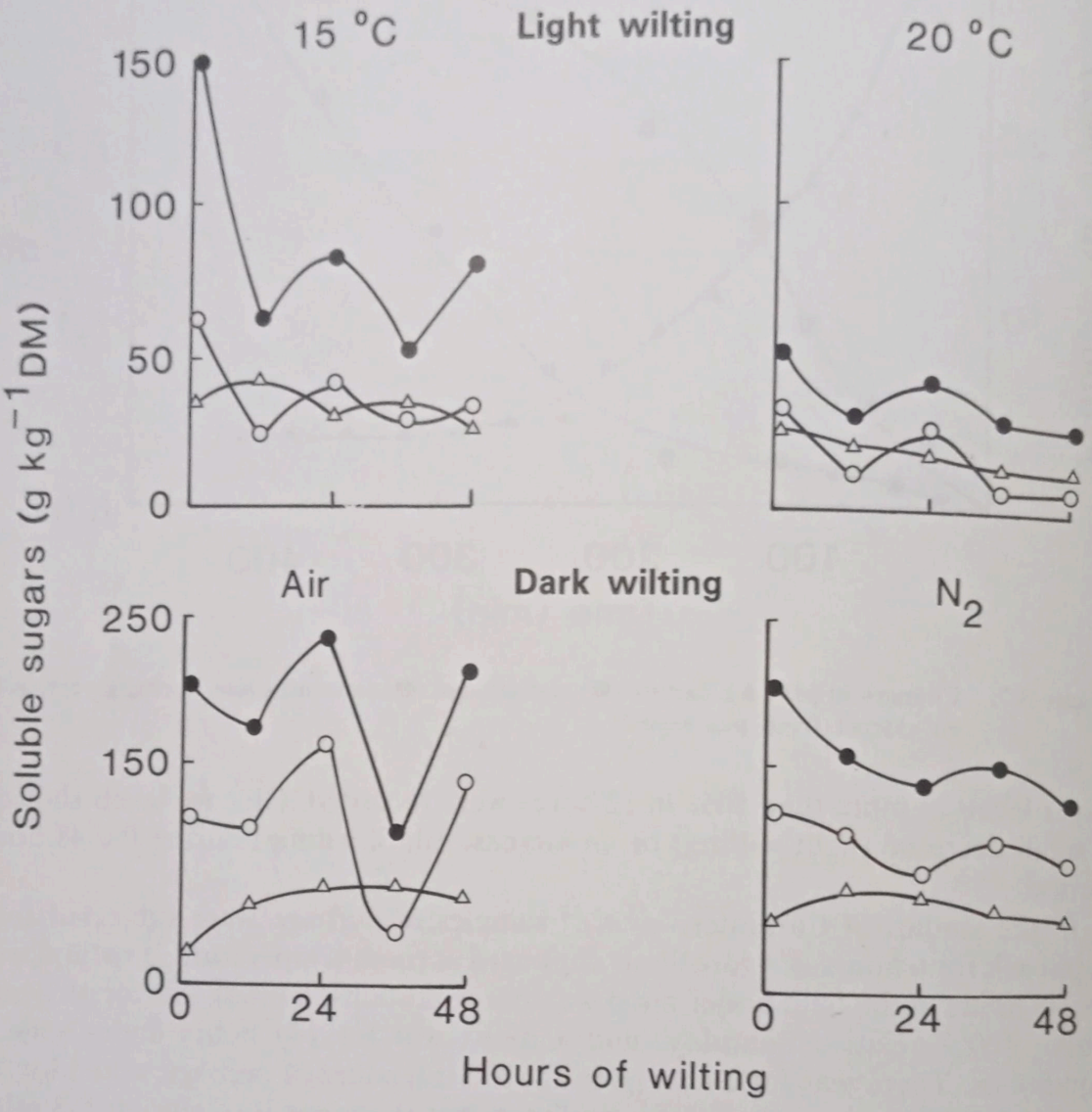


Figure 3.8. **Changes in soluble sugars during wilting under controlled conditions. ● = Total WSC; ○ = fructose; △ = glucose. (All determined after hydrolysis)[11]**

plant enzyme activities are mainly responsible for the production of this additional supply of sugars has come from studies where microbial fermentation has been restricted or prevented altogether. Using gamma-irradiated grass, Heron *et al.*[28] found that the WSC content of irradiated grass ensiled for 153 days was 65% higher than that of the original grass. Similar increases have been found by others using irradiated grass[11,18] or microbial inhibitors.[17,29-33]

Macpherson *et al.*[29] found that the increase in WSC observed during ensilage of grass which had been treated with sodium metabisulphite was mainly due to an increase in the glucose fraction. They suggested that the source of this had probably been cellulose since some hydrolysis of cellulose had occurred. The remainder was presumed to originate from the hydrolysis of hemicelluloses, a conclusion which was supported by the presence of pentoses in the silages. Although some breakdown of cellulose does occur during ensilage, it is usually considered to be small compared to that of the hemicelluloses.[22,34,35]

Table 3.1. Changes in the composition of ryegrass–clover during wilting[12]

	DM ($g\ kg^{-1}$)	pH	WSC ($g\ kg^{-1}$ DM)	WSC ($g\ kg^{-1}$ FW)*	Total-N ($g\ kg^{-1}$ DM)	Protein-N ($g\ kg^{-1}$ TN)	Ammonia-N ($g\ kg^{-1}$ TN)
Fresh	173	6.1	213	36.8	26.6	925	1.2
Wilted (6 hour)	349	6.0	215	75.0	28.3	878	1.1
Wilted (48 hour)	462	6.1	203	93.8	28.9	832	2.1
Moist wilted (48 hour)	199	5.9	211	42.0	29.9	752	2.6
Moist wilted (144 hour)	375	6.0	175	65.6	31.0	689	26.1

* FW Fresh weight

The hemicellulose content of grasses increases with maturity, varying from about 100 to 300 g kg^{-1} DM,[36-38] and significant hydrolysis has been found during ensilage.[22,30,34,35,39] McDonald *et al.*[34] found that as much as half the original hemicellulose content could be degraded.

There are three possible sources of hemicellulose breakdown:

1) hemicellulases present in the original herbage,
2) bacterial hemicellulases, and
3) hydrolysis by organic acids produced during fermentation.

Dewar *et al.*[20] studied numerous strains of lactic acid bacteria isolated from silages but could not detect any hemicellulase activity. This confirmed the findings of a previous investigation by Wylam (cited by McDonald *et al.*, 1960).[34] These findings are not conclusive, however, since lactic acid bacteria isolated from potato pulp silage have been found to have hemicellulase activity.[13]

In studies with hemicellulase enzymes isolated from perennial ryegrass, Italian ryegrass and cocksfoot, Dewar *et al.*[20] showed that up to 228 g sugars per kg substrate could be produced after a seven-day incubation period with hemicelluloses prepared from perennial ryegrass (Table 3.2). Analysis of the sugars produced indicated that they consisted mainly of xylose and arabinose. The action of plant hemicellulases is influenced by both temperature and pH and, in the above study, each of the enzymes showed an optimum pH of 6.0 but the temperature optima ranged from 30 to 43°C. The arabinose:xylose ratio in the original ryegrass hemicelluloses was approximately 1:4 whereas the ratio in the

Table 3.2. Enzymatic hydrolysis of grass hemicelluloses[20]

(a) Xylose (g kg^{-1} hemicellulose) produced after incubation of hemiocelluloses for seven days with grass enzymes at different temperatures

pH	Enzyme source								
	Perennial ryegrass 22°C			Italian ryegrass 30°C			Cocksfoot 37°C		
4.0	120	120	90	95	115	118	111	116	98
5.0	170	179	216	173	185	203	160	160	174
6.0	208	218	228	185	205	208	158	165	172

(b) Relative quantities of reducing sugars (g kg^{-1} total reducing sugars) resulting from the enzymolysis of hemicelluloses incubated for seven days at 37°C

Sugar	Enzyme source		
	Perennial ryegrass	Italian ryegrass	Cocksfoot
Xylose	426	473	601
Arabinose	440	401	269
Galactose	61	61	63
Glucose	72	65	67

products of enzymolysis was approximately 1:1 for the two ryegrass enzymes and about 1:2 for the enzyme prepared from cocksfoot. The change in this ratio indicated that arabinose had been split preferentially from the xylan chain by the enzymes.

Bousset *et al.*[18] failed to demonstrate hemicellulase activity *in vivo* but later concluded[40] that, although plant hemicellulases do exist, they are relatively inactive owing to compartmentalisation. They would be released only after plasmolysis, when their activity would be short-lived because of the rapid fall in pH during fermentation. Thus, under normal circumstances, hemicellulase activity would not be expected to contribute significantly to an increase in WSC. In those experiments where fermentation has been inhibited and the pH has remained high their activity would continue for much longer, leading to the large increases in WSC observed.

It has been suggested[20,22] that during the prolonged storage period, direct acid hydrolysis of cell wall polysaccharides may contribute to the increase in WSC. Dewar *et al.*[20] demonstrated considerable breakdown of hemicelluloses at pH 4 by incubating hemicellulose preparations at various pH levels (4 to 6) for 90 days (Table 3.3). Since they had already shown that short-term incubation at low pH reduced hemicellulose breakdown due to enzyme activity (Table 3.2), this long-term increase in xylose was assumed to be the result of acid hydrolysis. Morrison[22,23] noted increased hemicellulose breakdown when silages were made with certain acid additives, notably sulphuric and formic acids, but it is not known whether natural acid production in silages causes any hydrolysis. Morrison[23] also noted that cellulose was broken down to a greater extent in legumes than in grasses, and hemicelluloses to a lesser extent. This was attributed to differences in cell wall structure, particularly with respect to the content of arabinose. The arabinose residues are joined by furanosyl linkages, which are very acid labile, and grasses have a much higher content of these residues than do legumes.[41]

As discussed in Chapter 7, there is now considerable interest in the possibility of using polysaccharidases as silage additives in order to increase the WSC available.

It would appear from studies using sterile (gamma-irradiated) grass that in the anaerobic phase of ensiling only minor changes to the soluble carbohydrates occur as a result of plant enzyme activity,[11,18,42] such as hydrolysis of fructans, although the optimum pH for this activity is probably close to neutrality.[18]

Table 3.3. Effect of pH on the hydrolysis of ryegrass hemicellulose over a 90-day period[20]

pH	Temperature (°C)		
	22	30	37
4.0	71	76	84
5.0	46	48	48
6.0	38	44	44

Results expressed as xylose (g kg^{-1} hemicellulose).

Factors affecting the rate of respiration

Temperature

The rate of respiration is controlled mainly by temperature and thus the two are interdependent. The effect of temperature on the velocity of enzyme reactions has been well documented.[43] The typical progress curve of an enzyme reaction at various temperatures is shown in Figure 3.9.

Two different effects of temperature occur. Firstly, the initial velocity of the reaction increases and, secondly, the destruction of the enzyme, usually by denaturation at the higher temperatures, produces a continuous drop in active enzyme concentration. In nearly all cases enzyme inactivation becomes instantaneous at temperatures well below 100°C, and in the majority of cases below 70°C. There are some enzymes, however, such as those present in the thermophilic bacteria, which are heat stable. In plants the heat stability of enzymes is influenced mainly by the presence of accompanying proteins, the acidity at which they are held and the presence of substrate.[44] Within the range 0 to 30°C, the acceleration of respiration with temperature is roughly exponential.[44] Above 30°C the rate of increase per unit of temperature slowly decreases, but an increase in temperature still accelerates respiration until the enzymes are inactivated. Most temperate plants reach respiratory maxima near 35°C.[45] Holt *et al.*[46] found that the respiration rate of lucerne increased with temperature up to a maximum at 50°C.

In the early stages of ensilage, during the aerobic phase, temperature can have an important influence on the rate of respiration since much of the heat generated in the mass is retained, resulting in a progressive temperature increase with time. Temperatures of 40°C or more are not uncommon in farm silos.[47] The final

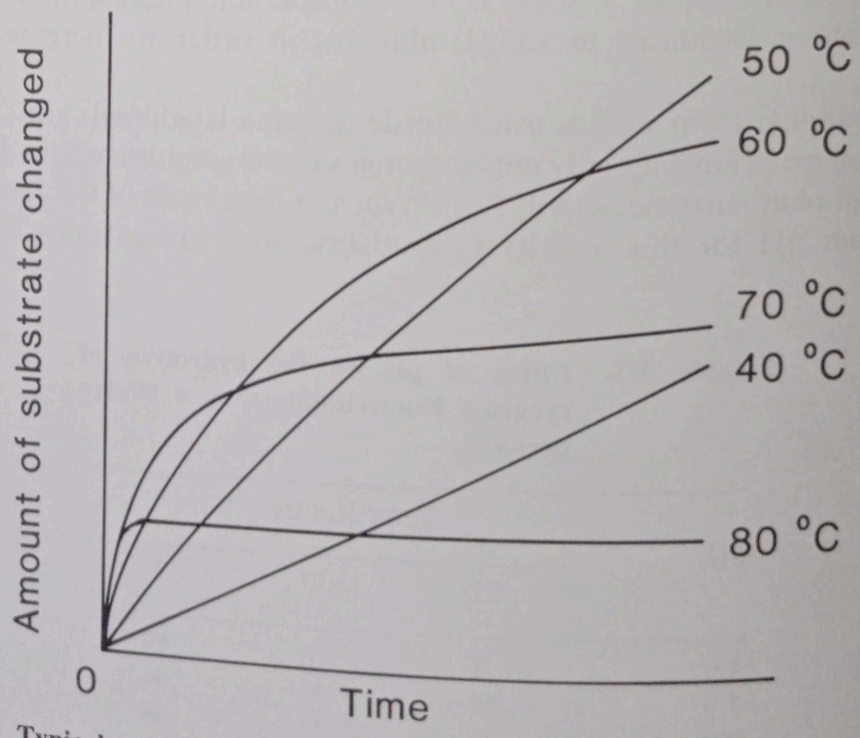


Figure 3.9. Typical progress curves of an enzyme reaction at various temperatures, showing dependence of apparent optimum temperature on time[43]

Table 3.4. Calculated specific heat capacities of ryegrass (kJ $kg^{-1}°C^{-1}$) at different dry matter levels*

DM ($g\ kg^{-1}$)	Specific heat capacity
150	3.85
200	3.73
250	3.62
300	3.50
350	3.39
400	3.27
450	3.16
500	3.04

* Assuming a specific heat capacity of 1.89 KJ kg^{-1} $°C^{-1}$ for ryegrass DM.

temperature is dependent on the quantity of air present, the insulating properties of the silo and the DM content of the crop, since the specific heat capacity of the plant material increases with increasing moisture content[48,49] (Table 3.4). The effect of ambient temperature is far less than that of plant respiration in controlling herbage temperature. A rise in temperature in the silo also accelerates the activities of other enzymes such as proteases, leading to further nutrient losses and a reduction in the digestibility of the silage[50] (see p. 156).

Oxygen and carbon dioxide concentrations

The effect of oxygen concentration on the intensity of respiration appears to vary in different tissues. In studies with wheat seedlings,[51] it was found that a decrease in oxygen concentration from that in normal air to 12.6 to 14.4% had no effect on carbon dioxide production, but caused a reduction of oxygen consumption by 5%. A further decrease in oxygen concentration to 9.6 to 10.4% resulted in carbon dioxide output being reduced by about 2.9%.

Increased concentration of carbon dioxide in the atmosphere brings about a very marked depression in respiration.[51] This effect is made use of in the gas storage of fruit and vegetables.

During the initial period of ensiling, oxygen is perhaps more important with regard to its effect on the extent, rather than the rate, of respiration. The former is controlled mainly by the sugar and oxygen supplies, but in practice sugars are usually abundant and the limiting factor is the oxygen supply. In a well-consolidated, sealed silo it is estimated that the trapped oxygen is of little significance and will lead to a temperature rise of only 3 or 4°C, with a loss of only 10 g kg^{-1} hexose.[46] Sprague[52] found that 90% of the oxygen initially present in large plastic silos (1 to 2 litre capacity) disappeared within 15 minutes and virtually none was left after 30 minutes. It was also noted that oxygen was more rapidly utilised in silos filled with direct-cut rather than pre-wilted herbage, which presumably reflected a reduction in the respiration rate of the wilted material owing to the increased DM content (see p. 61).

Hydrogen ion concentration

In general, enzymes are only active over a limited range of pH and, in most cases, for each enzyme there is a pH value at which the rate of enzymic activity is optimal. The effect of pH on enzymes, like all pH effects, is a result of changes in the state of ionisation of the components of the system as the pH alters. The free enzyme, the enzyme-substrate complex or the substrate may undergo such changes.[2] In silage studies, pH determinations are usually carried out on whole plant macerates, and with most fresh plant materials pH values usually lie within the range 5.5 to 6.5. Such determinations, however, ignore the pH variations which normally occur within the living plant. For example, vacuolar pH is usually between 5.0 and 5.5, while bulk cytoplasmic pH is usually about 7.0 to 7.5.[53] The pH of phloem is between 7.0 and 8.5, with values of about 8.0 occurring in most species.[54] Bowling[54] measured the profile of vacuolar pH across the intact sunflower root and found it rose from less than 6.0 near the epidermis to nearly 7.0 in the protoxylem. Studies using hornwort (*Phaeoceros* sp.) showed that light reduced the chloroplast pH from 6.2 to 4.6 but there were only transient changes in the cytoplasm, which had a steady value of pH 6.6 to 6.8.[54]

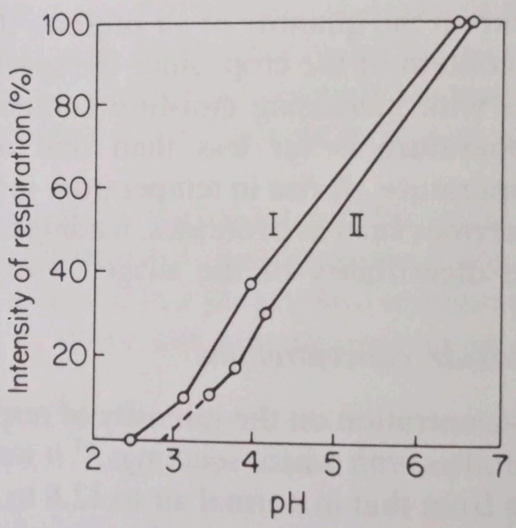


Figure 3.10. Results of experiments with green pea (curve I) and red clover (curve II) indicating how intensity of respiration depends on pH[55]

The aerobic phase of ensiling consists of the period in the field during wilting and the initial period in the silo, which may last only a few hours. During field wilting overall pH values of the crop do not appear to be affected to any extent, as the results of a wilting experiment shown in Table 3.1 indicate.[12] There were no significant changes in the pH values in any of the treatments. In the silo, lactic acid bacteria grow rapidly on the released plant juices and even in the aerobic phase produce lactic and acetic acids (see p. 99) which result in a rapid fall in pH.

Although no direct measurements of respiration rates in silos appear to have been made, there is evidence that the respiration rate declines as the pH falls. As early as 1933, in experiments with red clover and green peas (*Pisum sativum*), Virtanen[55] showed that at pH 3.5 respiration was only about 20% of the normal and it ceased completely at pH 3.0 (Figure 3.10). Indirect studies in which temperature measurements were made confirm this relationship. In an experiment with wilted perennial ryegrass (323 g DM kg^{-1}), formic acid was applied at the rate of 3.3 g kg^{-1} prior to ensiling, which lowered the initial pH from 6.2 to

4.9. Temperatures over the first eight days in the silo were consistently about 4°C lower in the acid-treated grass than those occurring in the untreated material.[56] More recent work at Edinburgh has demonstrated differences of up to 10°C (Figure 3.11). Hill[57] has reported that the application of acrylic acid reduced the respiration rate of sorghum and lucerne because it caused rupture of the cell membrane.

Dry matter content

The effects of water stress on respiration have been studied extensively but the results are conflicting. Different workers have found that water stress causes an increase, a decrease, or has no effect on the respiration rate of plants.[27,58,59] In single leaf studies with Italian ryegrass, Clark[11] showed that respiration rate increased dramatically as the DM content was increased during wilting from about 200 g kg^{-1} to 700 g kg^{-1}. In contrast, Greenhill[60] found a reduction in respiration rate in ryegrass leaves as DM content increased and he concluded that respiration ceased at DM levels of 600 to 700 g kg^{-1}. Holt *et al.*[46] found that respiration stopped in lucerne leaves when they reached a DM of 500 to 650 g kg^{-1} but some respiration still occurred in stems dried to 730 to 780 g kg^{-1}. Meidner[61] demonstrated a decrease in respiration rate with increasing water deficit in maize. Heath[62] found that respiration rate increased with reduced leaf moisture content,

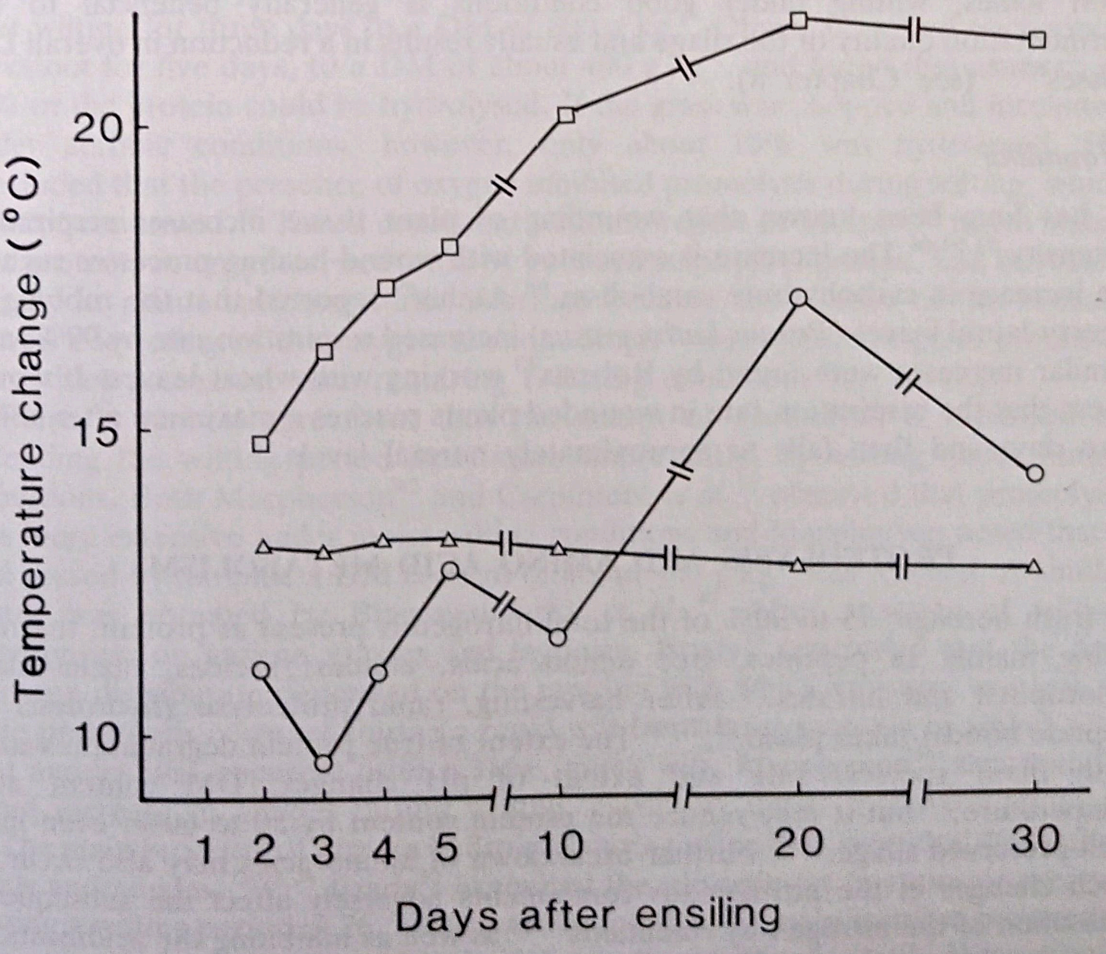


Figure 3.11. Effect of formic acid on temperature changes in 100 tonne capacity silos of Italian ryegrass (DM : 165 g kg^{-1}; WSC : 199 g kg^{-1} DM) Untreated (□), treated with 0.85% formic acid at 5 l t^{-1} (△), ambient temperature (○)

but eventually fell when the moisture content became sufficiently low. On the whole, the general opinion seems to be that respiration decreases with increasing DM.

Mitchell and Shepperson[63] considered that respiration losses were directly proportional to the initial moisture content and inversely proportional to the rate of drying. Thus, under favourable wilting conditions, when the DM increase is rapid, respiration losses would be expected to be low. Under moist wilting conditions, however, losses may be significant. This was confirmed by Honig,[64] who found that respiration intensity (respiration loss per hour) of wilting grass increased with decreasing DM content and increasing temperature according to quadratic functions. He observed that it was especially high at low DM contents directly after cutting, and can thus be decreased most effectively by speeding up the drying process during the early stages of wilting.

The temperature during wilting affects the losses incurred, as the respiration intensity of most plant materials increases rapidly with rising temperature, especially at low DM levels.[60,64] The stage of maturity of the harvested plant is also important as younger plants have a higher rate of respiration.[27,60] Since immature plants also have a lower content of WSC, the respiration losses are relatively more important. When ensiling wilted herbage, respiration losses can be reduced by chopping the material finely, as this allows it to compact more easily and hence reduces the amount of air trapped. Despite some unavoidable DM losses, wilting under good conditions is generally beneficial to the fermentation quality of the silage and usually results in a reduction in overall DM losses[65-67] (see Chapter 8).

Wounding

It has long been known that wounding of plant tissue increases respiration intensity.[51,59,68] The increase is associated with wound-healing processes such as an increase in carbohydrate catabolism.[68] Audus[69] reported that the rubbing of cherry-laurel leaves (*Prunus laurocerasus*) increased respiration rate by 99%, and similar increases were found by Roberts[70] working with wheat leaves. It would seem that the respiration rate in wounded plants reaches a maximum after about two days and then falls to approximately normal levels.[51]

PROTEOLYSIS AND AMINO ACID METABOLISM

In fresh herbage, 75 to 90% of the total nitrogen is present as protein, the rest being mainly in peptides, free amino acids, amides, ureides, nucleotides, chlorophyll and nitrates.[71] After harvesting, rapid proteolysis (hydrolysis of peptide bonds) takes place.[56,72-80] The extent of true protein degradation varies with plant species, rate and extent of pH changes, DM content and temperature,[81] but it may reduce the protein content by 50 to 60%, even in a well-preserved silage.[82,83] Further breakdown of amino acids may also occur.[71] Such changes in the nitrogenous components adversely affect the subsequent utilisation of the nitrogen by ruminants[84-87] as well as inhibiting the acidification process of fermentation.[88] Macpherson and Slater[75] considered that rapid, extensive proteolysis was only halted by attainment of a high DM content or a low pH, as the material was dried or ensiled respectively.

As early as 1917 Lamb,[89] working with maize treated with chloroform, toluene and cresol to inhibit bacterial growth, concluded that proteolysis and part of ammoniogenesis were caused by plant enzyme action. In 1921, Hunter[90] confirmed these results using identical methods. More recently, in experiments using aseptically grown grass,[82,91] microbial inhibitors[29,30,73,74] and gamma-irradiated grass,[11,18,28] a number of workers have also shown that proteolysis is predominantly the result of plant enzyme activity, but that further degradation of the amino acids is brought about mainly by microbial activity. Kemble[82] found that after 16 days' incubation the non-protein nitrogen content of aseptic grass had increased to 600 g kg^{-1} TN. Gouet *et al.*,[42] ensiling lucerne directly and after irradiation, found that protein degradation in both followed parallel courses and that activity in the irradiated material was only about 10% less than in the non-irradiated control. Heron *et al.*[28] obtained similar results with ensiled perennial ryegrass. In the non-irradiated material 66% of the original protein was broken down during ensiling, and in the irradiated material the value was 58%.

The wilting period

During wilting substantial proteolysis takes place.[12,56,73,92-94] Henderson *et al.*[56] found that during a 31 hour wilt to a DM content of 323 g kg^{-1}, 20% of the initial protein was hydrolysed. Kemble and Macpherson[94] also found a 20% decrease after wilting for three days to a DM of 600 g kg^{-1}. Ohyama[79] wilted unchopped cocksfoot for five days, to a DM of about 400 g kg^{-1}, and found that as much as 50% of the protein could be hydrolysed. If the grass was chopped and incubated under aerobic conditions, however, only about 10% was hydrolysed. He concluded that the presence of oxygen inhibited proteolysis during wilting, which he later confirmed.[95] This is consistent with the report of Mothes,[96] which stated that proteolytic enzymes, activated by reduced sulphydryl groups, had increased activity in plants suffering water shortage because the closure of the stomata caused a lowering of the oxygen tension. Singh[76] suggested that plant proteases were thiol proteases, thus requiring reducing conditions for activity.

There is general agreement that the extent of proteolysis is increased by extending the wilting period and, more importantly, by wilting under humid conditions. Both Macpherson[93] and Carpintero *et al.*[12] observed that proteolysis was more extensive under moist wilting conditions and Macpherson noted that it had ceased by the time a DM content of about 400 g kg^{-1} was reached. A similar result was obtained by Blagoveshensky *et al.*.[97] After a series of wilting experiments on various grasses and legumes, Brady[73] concluded that the final nitrogen distribution depended on the rapidity with which moisture was lost, as little proteolysis occurred during a rapid wilt but a large increase in amino acids and amides was apparent after a slow, moist wilt. Macpherson[93] also found a rapid increase in amides during wilting, especially under moist conditions.

The main products of protein hydrolysis during wilting are peptides, free amino acids and amides.[73,93,94] Brady[73] examined the nitrogenous fractions of ryegrass during a wilting period of 26.5 hours and found that the main increase occurred in the amino-N fraction, with only relatively small amounts of volatile-N (ammonia-N) and amide-N being formed (Table 3.5). Kemble and Macpherson[94] showed that all the amino acids they measured, other than proline, were found in amounts

Table 3.5. Nitrogenous changes in ryegrass during wilting[73]

Wilting time (hours)	DM ($g\ kg^{-1}$)	Total-N ($g\ kg^{-1}$ DM)	$g\ kg^{-1}$ total N				
			Total soluble-N	Soluble NPN	Amino-N	Ammonia-N	Amide-N
0	161	20.2	170	89	26	1	8
2.5	192	18.3	164	114	59	2	8
6.5	268	17.3	171	127	71	2	7
26.5	390	17.9	189	173	92	6	13

less than those calculated from the amount of protein degraded. The deficit was variable and presumably reflected different rates of subsequent amino acid breakdown, as it has been shown that protein breakdown is uniform.[98] After 8 days' wilting, free proline was 50% in excess of the total proline in the original herbage, indicating substantial proline synthesis.

In a second experiment Kemble and Macpherson[94] compared dry and moist wilting (Table 3.6). Similar results were obtained for dry wilting, but for moist wilting little change in DM was observed and no proline was synthesised. Instead there was an increase in the amide fraction. The accumulation of proline has also been noted in growing crops suffering water shortage.[59] The mechanism of such accumulation in leaves has been studied by Boggess and co-workers.[99-101] They concluded that proline plays a role in the detoxification of ammonia in leaves during periods of water shortage, a function which is performed by amide synthesis under normal conditions of growth or moist wilting.[94] Proline can be produced by reductive amination of α-oxoglutarate to form glutamate, which is then reduced to proline via γ-glutamate semialdehyde (Figure 3.12). Arginine is the main amino acid in herbage,[102,103] and proline can also be formed by the deamination of arginine via ornithine.[100] Ashbell *et al.*[104] found that during the wilting of wheat there was an increase in free amino acids and that, although the composition of amino acids remained similar to that in the fresh material, there were increases in asparagine, glutamine, proline and leucine and decreases in glutamate and arginine. Anderson[67] reported that arginine appeared to have been

Table 3.6. Proteolysis in grass (DM: 810 g kg^{-1}; TN: 20.7 g kg^{-1} DM) during wilting under different conditions[94]

	Dry wilting		Moist wilting	
Original fresh weight (g)	69.6		69.6	
Fresh weight after 3 days (g)	19		68.6	
	Amino acid nitrogen (g kg^{-1} TN)			
	Found	Calculated	Found	Calculated
Soluble N	306	—	386	—
Volatile base	5	—	15	—
Glutamine	26	—	36	—
Asparagine	48	—	104	—
Glycine	0.9	11.5	1.5	16.8
Serine	8.1	10.1	12.4	13.4
Threonine	4.8	7.4	6.1	10.6
Alanine	4.1	12.6	2.6	17.3
Proline	39.1	8.5	5.3	11.3
Tyrosine	0.8	5.0	1.2	6.8
Valine	5.8	8.5	7.6	12.2
Methionine	0.8	2.4	0.3	3.8
Phenylalanine	4.3	5.8	2.6	9.3
Leucine	2.0	8.4	2.0	13.2
Isoleucine	3.6	5.3	3.6	8.4
Peptide N	30	—	40	—

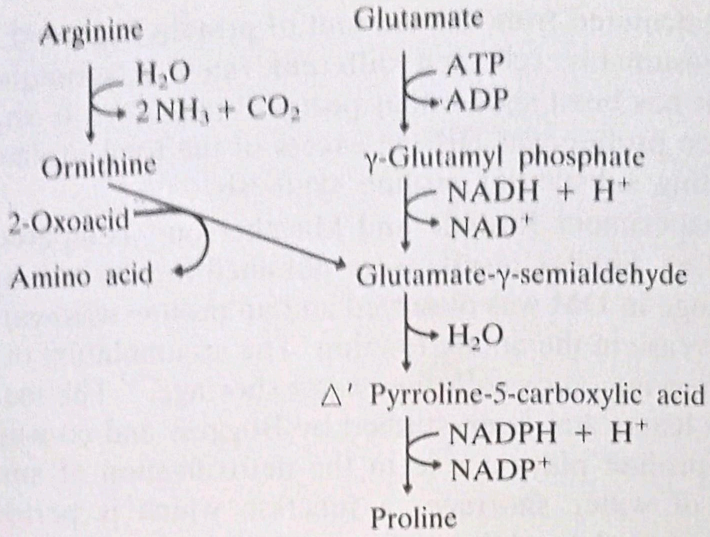


Figure 3.12. Formation of proline during wilting

converted to ornithine in both direct cut and wilted silages, especially in the latter.

Ammonia-N accounts for less than 1% of the TN in fresh herbage[71] and, although little increase is usually found during wilting, this does not preclude some ammoniogenesis taking place, since any formed would be rapidly absorbed by the system described above. If wilting is prolonged, substantial ammonia may be formed but this is most likely to be due to microbial activity.

Nitrate levels decrease during wilting[67] but it is thought unlikely that this is as a result of plant enzyme activity, since it is known that plant nitrate reductase is rapidly inactivated in excised leaves.[105]

The ensiling period

When herbage is ensiled, either directly or after wilting, proteolysis continues and within 12 to 24 hours the protein content may fall from about 800 g kg^{-1} TN to less than 600 g kg^{-1} TN.[10,73,80,82] By the end of ensilage this may have decreased to 300 g kg^{-1} TN or less.[78,83,106,107] Confirmation that this change is brought about by plant enzymes has been obtained by ensiling microbe-free herbage. Kemble[82] found that the protein content fell to 40 g kg^{-1} after only 16 days' ensilage of aseptically grown grass. Similar results have been obtained using γ-irradiated grass. Gouet *et al.*[42] ensiled sterile and non-sterile lucerne and after 46 days the protein decreased from 800 g kg^{-1} TN to only 280 and 220 g kg^{-1} TN respectively. Heron *et al.*[28] obtained equivalent values of 858, 359 and 293 g kg^{-1} TN for perennial ryegrass.

Wilting under good conditions does not appear to have much effect on the overall decrease in protein after ensilage[56,66,73,84] but it may reduce further amino acid metabolism, especially deamination.[84,108] Prolonged wilting in moist conditions, however, will increase the level of low molecular weight nitrogenous compounds in both the herbage and final silage. The net effect on silage composition is to increase the non-protein nitrogen (NPN) content, especially ammonia-N. Nevertheless some of the individual NPN components do decrease, such as nitrate-N, accumulated amide and some free amino acids, particularly arginine.[67] These changes, especially those resulting in ammonia, are not all brought about by plant enzymes.

Table 3.7. Total amino acid nitrogen (g kg^{-1} TN) in grass and silage[28]

	Grass	Silage*
Aspartic acid + asparagine	66.9	40.8 (61.1)
Threonine	30.5	19.5 (64.4)
Serine	34.2	20.8 (60.8)
Glutamic acid + glutamine	59.3	31.7 (53.4)
Proline	30.6	33.1 (108.4)
Glycine	65.6	66.9 (102.0)
Alanine	69.6	84.3 (121.3)
Valine	43.5	38.6 (88.8)
Isoleucine	22.8	23.2 (101.8)
Leucine	45.6	46.5 (102.1)
Tyrosine	14.5	10.3 (71.2)
Phenylalanine	23.4	22.6 (96.3)
Lysine	61.9	30.5 (49.2)
Arginine	88.7	23.5 (26.5)
Total	657.1	492.3

*The data in parentheses show the percentage recovery after ensilage.

The main products of protein breakdown during ensilage are amino acids and ammonia,[84] the proportion of each depending on the extent of further amino acid breakdown.[48,71] The total amino acid composition of herbage is consistent regardless of species[102,103] but, although proteolysis is uniform,[98] the further breakdown of amino acids is not. Arginine, which is the main amino acid in fresh herbage, is rapidly and almost completely broken down during wilting and ensiling.[109,110] Other amino acids are broken down to different extents and some are known to increase (Table 3.7). Many of these changes are brought about by microbial activity but plant enzymes are known to catabolise certain amino acids.[71] They are able to decarboxylate aspartic and glutamic acids to alanine and γ-aminobutyric acid (GABA) respectively (Figure 3.13),[71] both of which are found to increase during ensiling.[67,75,82,111] Macpherson and Slater[75] reported the appearance of considerable quantities of GABA in incubated microbe-free grass. In a study using ^{14}C-labelled amino acids, Ohshima[111] found that plant enzymes decarboxylated glutamate more readily than aspartate. He suggested that during ensilage plant enzymes would break down the glutamate, and microbial enzymes would break down the aspartate. Voss[88] reported that plant decarboxylases were active during the early stages of ensilage and bacterial decarboxylases during later ensilage.

Brady[73] and Gouet and Fatianoff[30] both reported some deamination of amino acids by plant enzymes, but Kemble[82] could find no evidence of this with ensiled microbe-free timothy and Gouet *et al.*[42] found no ammonia production in sterile ensiled lucerne. On the other hand, Mabbitt[91] also ensiled microbe-free timothy and, unlike Kemble, found substantial quantities of ammonia produced, as did Heron *et al.*[28] with irradiated ryegrass. Macpherson[74] also reported ammonia production if grass sap was incubated with toluene, and Ohshima[111] demonstrated deamination of histidine in red clover juice.

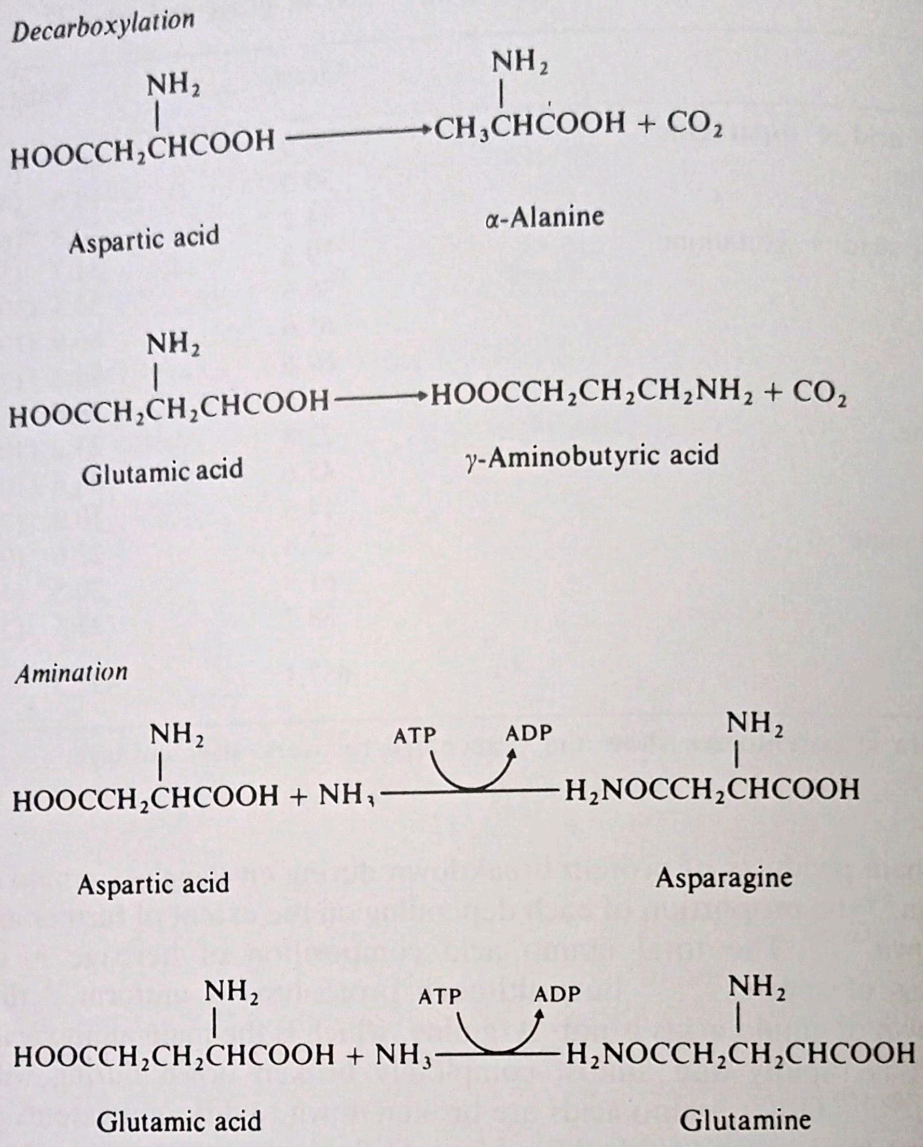


Figure 3.13. Decarboxylation and amination of glutamic and aspartic acids

In many studies the absence of ammonia has been taken to mean that deamination has not occurred. However, this may not be the case as any ammonia formed could combine with α-oxoglutarate to produce glutamate, or with glutamate or aspartate to form their respective amides, and it would thus not be detected as free ammonia. Increases in amide concentration may take place during wilting,[73,94] but during ensiling the amide concentration generally decreases[78,82,111] and it is difficult to detect if transient amide formation has occurred. It should be noted that many papers have been published where ammonia production has been referred to as a measure of proteolysis. This is an incorrect assumption as it is an indication of further amino acid breakdown only. Intensive proteolysis can occur without there being any significant increase in ammonia content.

Nitrate is rapidly reduced during ensilage.[112-114] Bousset-Fatianoff *et al.*[115] have used irradiated lucerne and ryegrass to show that this is not the result of plant enzyme activity.

Unlike the situation after wilting, where most of the nitrogen can be accounted for as peptide, amino acid, amide or ammonia, large quantities of the nitrogen in silage is not accounted for by these fractions.[73,82,116]

Factors affecting proteolysis

Dry matter

The extent of proteolysis during ensiling is influenced by several factors, including DM content, pH and temperature. During wilting, since there is little change in pH, any reduction of proteolysis depends on reaching a high enough DM. Macpherson[93] found that a DM of 400 to 450 g kg^{-1} was necessary but reports on the effects of DM on proteolysis during ensiling have been inconsistent.[73,92,117-119] McDonald[85] considered that, although wilting may lead to an inhibition of proteolysis during ensiling if a high DM is reached, it was unlikly to have much effect at the DM levels usually attained in the UK (250 to 300 g kg^{-1}). In fact, lightly wilted material may show increased levels of proteolysis due to the inhibition of acidification.[119]

Temperature

Since plant proteases have high temperature optima (p. 73), a rise in temperature in the silo will tend to increase their activity. The degree of heating is normally controlled by respiration, therefore it is important that the herbage should be well-compacted and the silo filled rapidly and well-sealed to prevent entry of air.

pH

It is well known that the rate of fall of pH is important in determining the extent of proteolysis. If it is slow to fall, then more protein will be broken down.[73] Macpherson[74] stated that once a pH of 4.3 was reached further proteolysis would be negligible, but subsequently several workers have shown that even direct acidification to a pH below 4 will not prevent proteolysis, although it will reduce it. Carpintero *et al.*[12] acidified a ryegrass-clover mixture with formic acid, achieving an initial pH of 3.5. The protein nitrogen in the untreated control fell from 819 to 265 g kg^{-1} TN after 50 days, whereas in the treated herbage it decreased to 462 g kg^{-1} TN (Table 3.8). Similar results were obtained with sulphuric acid.

Table 3.8. The effect of formic acid on protein-N and ammonia-N contents of ryegrass-clover ensiled for 50 days[12]

	pH		Total-N (g kg^{-1} DM)	Protein-N (g kg^{-1} TN)	Ammonia-N (g kg^{-1} TN)
	Initial	After 50 days			
Original grass	5.85	—	19.3	819	—
Silages					
Control	5.85	3.87	18.2	265	95
Formic acid (g kg^{-1})					
0.4	5.40	3.77	17.8	285	79
1.0	4.90	3.67	18.5	325	59
2.0	4.45	3.81	19.3	358	46
4.1	4.05	3.88	19.2	401	12
7.7	3.50	3.80	18.6	462	12

In an experiment using sterile buffered aqueous grass extract, Heron *et al.*[121] showed that ryegrass proteases were active at pH levels well below 4. The extract was buffered over a wide pH range, incubated at 20°C or 37°C for 120 days and the extent of proteolysis measured. The results were used to prepare a mathematical model with two components: 1) acid hydrolysis at low pH and 2) plant protease activity at higher pH. The estimated curves are shown in Figure 3.14. It was concluded that direct acid hydrolysis of plant proteins would be insignificant at a normal silage pH but the activity of the plant proteases would continue, although at a reduced rate. Despite Virtanen's claim[55] that plants do not contain proteases active below pH 4, it is now known that many plant proteases have pH optima below this (p. 72).

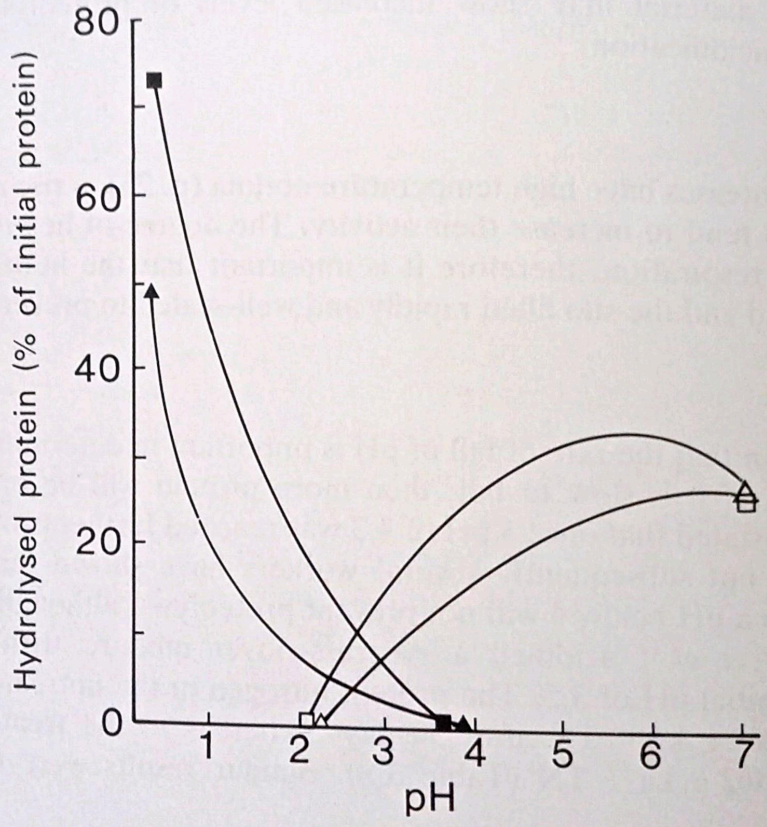


Figure 3.14. Effect of pH on proteolysis in irradiated grass extract incubated for 120 days. Plot of estimated acid hydrolysis (closed symbols) and plant protease activity (open symbols). Incubated at 37°C (■, □) or 20°C (▲, △)[121]

Species

The effect of species on the extent of proteolysis is not clear cut. Brady[73] and Ohyama[79] found that the pattern of proteolysis was similar regardless of species, but McKersie[122] found that species did have an effect. He suggested that the extent of proteolysis during ensiling depended mainly on the 'proteolytic potential' and the rate of fall of pH. The former is a measure of the total protease activity, the substrate availability and the substrate affinity. It varies between species and will probably also be affected by crop management and environmental conditions. Ryan[123] considered it not unexpected that the proteases from different plants would have evolved independently and therefore could exhibit different properties.

Proteolytic enzymes

Proteolytic enzymes are always present in leaves,[124] being active during germination, tissue differentiation and senescence as well as in the continuous turnover of cellular proteins.[123,125] Water deficiency induces premature senescence in many tissues,[59] and numerous studies of senescence in detached leaves are relevant to the situation within the swath during wilting.[11] Many of the initial changes during ensilage can also be explained in terms of senescence.

Senescence brings about a change in the balance of anabolic and catabolic processes such that the latter predominate.[126,127] These changes are reflected in the leaf ultrastructure, which shows a rapid degradation of the cell organelles.[128-130] Chlorophyll loss is one of the more visible signs of senescence but degradation of nucleic acid, protein, lipid and carbohydrate also occurs.[59,131,132] Proteolysis is very rapid,[133,134] especially in grasses and other monocotyledons.[11] It is not possible yet to say definitely whether the decrease in protein results from increased degradation, decreased synthesis, or a combination of both but evidence seems to indicate that increased degradation is mainly responsible.[59,125] This increase could be the result of increased synthesis of proteases, activation of existing proteases, removal of inhibitors or increased access to substrate. According to Brady,[59] the potential for protein breakdown in the plant is always high and it is the regulation of these existing proteases which is important in determining the extent of proteolysis. Nevertheless, some proteases would appear to be synthesised during senescence, especially in the initial stages.[133]

Ribulose bisphosphate carboxylase (RuBPCase) is the most abundant protein in green leaves, accounting for up to 40% of the total leaf protein, and is found only in the chloroplast stroma.[135] It is broken down rapidly in senescing leaves and may account for most of the initial protein loss.[133,136] Fairbairn *et al.*[137] found that when the pH remained high (>4.69) all of the RuBPCase present initially in lucerne was broken down within two days of ensiling. Choe and Thimann[136]

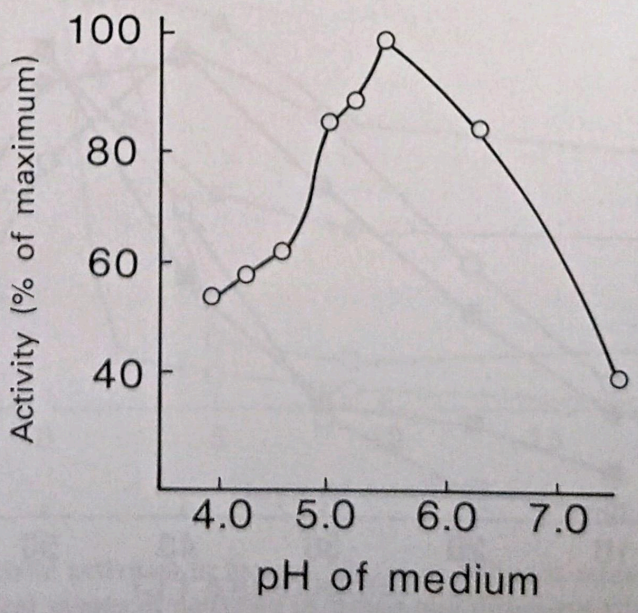


Figure 3.15. Protease activity at various pH values expressed as a percentage of the maximum activity found. (Portions of the same sample of partially purified tobacco sap were used for all determinations)[143]

found that isolated chloroplasts retained more of their protein than those in intact leaves, which suggests that the enzymes responsible for hydrolysis originate in the cytoplasm. This was substantiated by experiments which showed that senescence was inhibited by cycloheximide, an inhibitor of cytoplasmic protein synthesis.[133,138,139] These experiments also showed that some protein synthesis is necessary for senescence to occur.

In the later stages of senescence, when the chloroplast membranes have to a large extent been broken down and dispersed, the tonoplast (vacuolar membrane) is ruptured.[129] There is evidence that the cell vacuoles of some plants contain a variety of hydrolases, including proteases, which are then released.[140]

The earliest studies of the proteolytic enzymes of leaves were carried out at the beginning of the century[141,142] but, other than investigations by Tracey[143], Brady[144] and Singh[76] on tobacco (*Nicotania tabacum*), white clover and wheat leaves respectively, very little further interest was shown until fairly recently. The results of the early studies suggested that plant proteases had high temperature optima and were active over a wide pH range, with an optimum between 5 and 6 (Figure 3.15). Most recent studies on leaf proteases have been related to senescence, and the greatest interest has been shown in cereals, especially wheat and oats. Wheat, oat and maize leaves have been shown to contain proteases active in neutral and acidic conditions.[134,138,139,145-151] Proteases with pH optima ranging from 3.5 to about 7 have been found in maize.[134] It appears that enzymes active at acid pH predominate, and hence the widely held opinion that the achievement of pH 4.0 prevents further proteolysis during ensilage is unlikely to hold true, as the results of Carpintero *et al.*[12] have already shown (Table 3.8).

Since indications are that there are several different proteases in leaves, it is conceivable that each may have a specific and possibly interdependent role in both protein turnover and terminal degradation.[125] Drivdahl and Thimann[149] suggested that the presence of several proteases would be an advantage to the senescing plant, given the range and structural complexity of the proteins to be

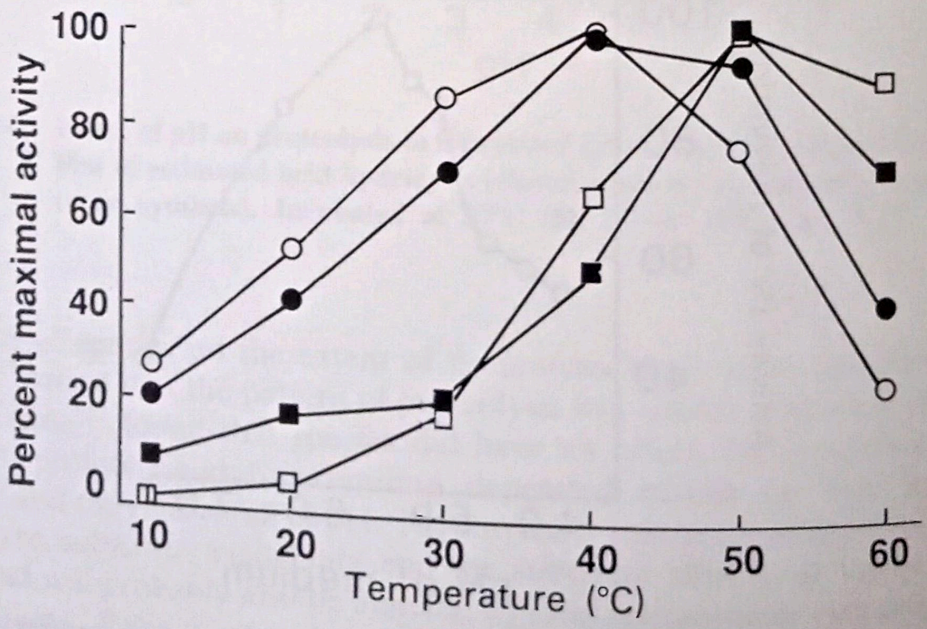


Figure 3.16. Effect of temperature on the activity of soluble acid proteinase (□), neutral proteinase (■), carboxypeptidase (●) and amino peptidase (○)[154]

degraded. Many proteases have specificities for particular peptide bonds and substrates.

Proteases can be classified into four main types based on their reaction mechanisms, ie carboxyl, thiol, serine and metal proteases.[152] In plant leaves most proteases have acid pH optima and belong to the thiol and carboxyl groups.[125,153] It is of interest to note that, in contrast to plants, animals and microorganisms utilise mainly serine proteases.[153]

In general, the proteases seem to have high temperature optima, in the region of 45 to 55°C (Figure 3.16).[134,144,146,147,149] Brady[144] concluded that inactivation at high temperature was unlikely to occur during ensilage. Macpherson[74] compared incubation of grass juice at 18°C and 30°C. At the higher temperature the pH dropped faster but the rate of proteolysis was increased, so that the final outcome after 14 days was very similar. Ohyama *et al.*[155] ensiled grass at 15 and 30°C and concluded that proteolysis was affected by temperature during the early stages of ensilage and by pH during the later stages.

Since appreciable protease activity is found even in young leaves,[145,146] there must be some mechanism by which the activity in non-senescing cells is controlled, such as by compartmentalisation or inhibition. Inhibitors against endogenous proteases have been found but in most cases they have been associated with the seeds, where they presumably play a role in protein mobilisation during germination.[156] Hydrolases, including proteases, are present in the vacuoles of many plants[123,146,157] but little is known about the mechanism of intracellular protein degradation, especially with regard to protein turnover. In most plants the vacuole pH is maintained at a lower level (5.0 to 5.5) than the

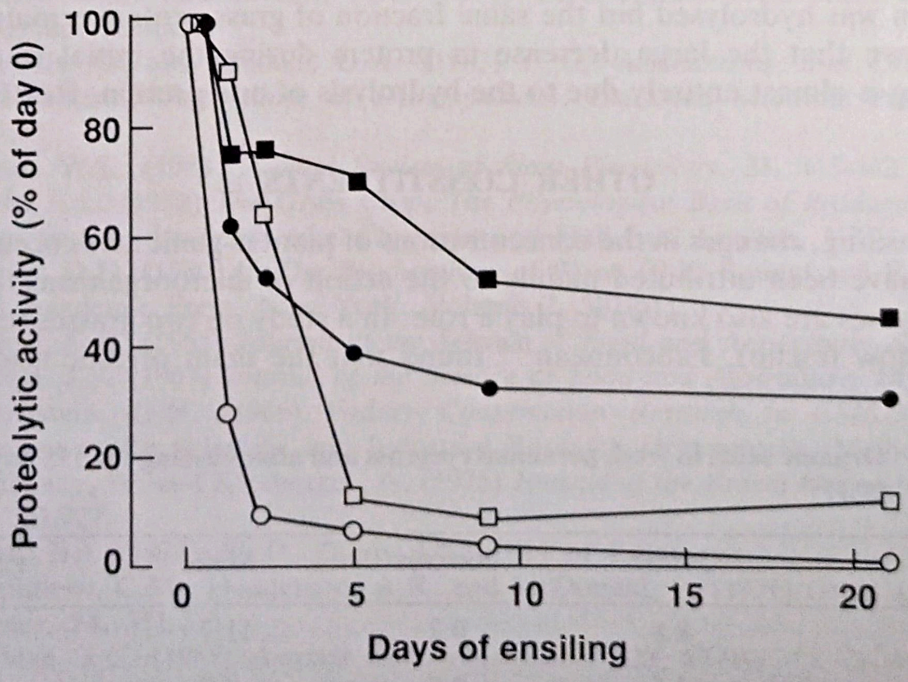


Figure 3.17. Proteolytic activities in lucerne silages at different stages of ensiling. Values represent means of activities in wilted and direct-cut silages: acid proteinase against haemoglobin, pH 4.5 (○); acid proteinase against azocasein, pH 5.5 (●); aminopeptidase against azocasein, pH 7.0 (□); carboxypeptidase against haemoglobin, pH 5.2 (■)[160]

cytoplasm (7.0 to 7.5) by active transport across the tonoplast,[53,158] which could explain why so many plant proteases have acidic pH optima. If compartmentalisation of proteases does occur, it is conceivable that they will eventually be released during ensilage, either as a result of mechanical disruption during harvesting or by cellular disintegration under anaerobic conditions. There is also evidence for the existence of zymogen-type pro-enzymes in plants,[156] which could account for the rapid increase in protease activity often observed.[124,125] McKersie[122] stated that the amount of protein hydrolysed during ensiling was dependent largely on two factors:

1) the rate of acidification, and
2) the "proteolytic potential", ie the total protease activity, and the substrate availability and susceptibility.

Proteolysis during ensilage does not proceed to completion, even when the pH is not inhibiting.[29,42] In fact, the protein content does not usually fall below 250 g kg^{-1} TN. Macpherson[74] stated that the age of a silage made little difference to the extent of proteolysis as it appeared to proceed to the same point, and even in an eight-year old silage 21% of the nitrogen remained as protein.[159] McKersie and Buchanan-Smith[160] have shown that, although the general level of activity declines as the period of ensiling progresses, each of the proteolytic enzymes declines independently (Figure 3.17). They also found that the cessation of proteolysis in ensiled lucerne after only a few days was not due to the loss of enzyme activity, and that the pH was not inhibiting. Therefore it would appear that not all the proteins are readily available; nor are all proteins degraded to the same extent. Proteases are known to hydrolyse different proteins at different rates.[139,143] Ohyama and Masaki[95] found that gluten was more susceptible to proteolysis than grass protein and, in some silages, proteolysis continued until all the gluten was hydrolysed but the same fraction of grass protein remained. It is now known that the large decrease in protein during the initial stages after harvesting is almost entirely due to the hydrolysis of one protein, RuBPCase.[137]

OTHER CONSTITUENTS

During ensiling, changes in the concentrations of plant organic acids occur. These changes have been attributed mainly to the action of microorganisms, although plant enzymes are also known to play a role. In a study on two grasses (cocksfoot and meadow fescue), Fauconneau[161] found that the main organic acids in the

Table 3.9. Organic acids in fresh perennial ryegrass and after ensiling for 175 days (g kg^{-1} DM)[29]

	Quinic	Succinic	Malic	Citric
Grass	8.8	0.2	11.7	7.3
Silage I*	12.5	0.6	0.7	0.8
Silage M	11.2	0	6.6	10.5
Silage IM	11.7	trace	1.7	10.8

*Inoculum of lactobacilli (I); 40g kg^{-1} sodium metabisulphite (M); inoculum + metabisulphite (IM).

grasses were malic, citric and quinic with, in addition, malonic acid in lucerne. Playne and McDonald[162] found the main acids in Italian ryegrass to be malic and citric, while in red clover they were malic and glyceric.

Macpherson *et al.*[29] ensiled perennial ryegrass with and without the addition of metabisulphite and/or an inoculum of lactic acid bacteria (Table 3.9). They found an increase in citric acid in the presence of the bacterial inhibitor, metabisulphite, and a considerable decrease in malic acid, particularly when lactobacilli were also present. Quinic acid increased in all treatments and there was a small amount of succinic acid produced in the presence of the inoculum alone.

In a study with timothy grass, grown and ensiled under sterile conditions, Playne *et al.*[163] showed that over a ten-day incubation period at 30°C the pH increased from 6.2 to 7.2; buffering capacity, acetic acid, and succinic acid also showed marked increases, while malic acid showed a decrease. Increases in succinic acid are known to occur during the normal ensilage process,[162,163] but only part of this increase can be attributed to plant enzyme action, as inoculation studies with *Lactobacillus plantarum* have shown that it also produces this acid.[163]

Some destruction of carotenes occurs during ensilage and part of this loss is thought to result from the action of plant enzymes. One such enzyme, lipoxygenase, oxidises in a highly specific way fatty acids and lipids containing the cis-cis-penta-1,4-diene unit. The hydroperoxide radicals formed oxidise the carotenoids and other oxy-labile compounds.[164]

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Chapter 4

Microorganisms

Silage is produced by the process of a natural lactic acid fermentation and therefore it follows that the role of microorganisms in this process is of paramount importance. The involvement of bacteria has been recognised since the early part of this century.[1,2] Although Lamb[2] considered the yeasts to be of even more significance, it is now known that they play only a minor part during the fermentation stage but are one of the causative agents of losses during aerobic deterioration, especially after opening of the silo. In 1918 studies on alfalfa silage[3] showed that the coliform bacteria had a role during fermentation, and in 1919 the butyric acid bacteria (clostridia) were also implicated.[4] In more recent years, other groups of bacteria (*Bacillus* spp., acetic acid bacteria, listeria, propionic acid bacteria) have been recognised to assume importance under certain conditions.

The presence of bacteria on the aerial parts of plants has long been recognised.[5,6] In an examination of the epiphytic microflora on sorghum, maize and lucerne, Miskovic and Rasovic[7] found that the total number of microorganisms was higher on the leaves than on the stems in all phases of development. The total number of bacteria on fresh grass has been shown to vary between 10^6 and 10^9 g^{-1} DM.[8] Similar numbers have been observed by Moon and Henk[9] using a scanning electron microscope, and they also noted that the bacteria were located primarily on the outer surfaces of the leaves rather than on the internal surfaces.

The great majority of these bacteria are strict aerobes which contribute little or nothing to silage preservation[10-15] and, since anaerobiosis is achieved rapidly after the silo is sealed, their growth is soon inhibited.[16] Of those organisms capable of anaerobic growth, the coliforms (members of the *Enterobacteriaceae* family) are the most abundant.[12,17,18] Species of *Bacillus* and *Clostridium* are also found in small numbers on fresh material.[12,16,19-21] The latter, being obligate anaerobes, occur only in endospore form and probably result from soil contamination and/or slurry application.[15,16,22] Lactic acid bacteria, which are the most important species during ensiling, are usually present on grass in numbers 1000 times lower than their main competitors, fungi and enterobacteria.[23]

After ensiling, the microorganisms capable of anaerobic growth (lactic acid bacteria, enterobacteria, clostridia, some *Bacillus* spp., yeasts) begin to grow and compete for available nutrients. The changes in the first few days are critical to the success or failure of the subsequent fermentation. If conditions are suitable, the lactic acid bacteria will quickly acidify the environment to such an extent that the competing organisms will not be able to survive and the end result will be a stable, low pH silage. If the pH is not lowered quickly enough the undesirable microorganisms (mainly enterobacteria, clostridia and yeasts) will be able to compete for nutrients and, in so doing, reduce the chances even further of obtaining a stable silage, since many of their products do not aid preservation. As will be seen later, they are also capable of producing end-products which may

reduce the nutritive value of the silage. Although of little importance during fermentation, the yeasts are the main causative agents of aerobic deterioration, along with *Bacillus* spp..

LACTIC ACID BACTERIA

This term describes a group of bacteria from several genera which are noted for their ability to produce lactic acid. The origins of the name are not known but the term "Milchsauerbacillus" was used by Hueppe in 1884[24] to describe some of the bacteria responsible for acidification in milk and other dairy products. Although lactic acid bacteria seem to coexist with plants, their role on the plant surface is still unknown. It has been suggested that they may protect plants from pathogenic microorganisms by producing antagonistic compounds such as acids, bacteriocins and anti-fungal agents.[25] This may well be true, as lactic acid bacteria are found in high numbers on damaged plant parts.[26]

The first attempt at classifying these bacteria was made by Orla-Jensen[27] who defined them as micro-aerophilic, Gram-positive, asporogenous, usually non-motile organisms which ferment sugars mainly to lactic acid. They were further divided on the basis of their fermentative metabolism (homofermentative or heterofermentative). Since then considerable attention has been devoted to the

Table 4.1. Some lactic acid bacteria of importance during ensiling

Genus	Glucose fermentation	Morphology	Lactate	Species
Lactobacillus	Homofermentative	Rod	DL	*L. acidophilus*
			L(+)	*L. casei*
			D(L)*	*L. coryniformis*
			DL	*L. curvatus*
			DL	*L. plantarum*
			L(+)	*L. salivarius*
	Heterofermentative	Rod	DL	*L. brevis*
			DL	*L. buchneri*
			DL	*L. fermentum*
			DL	*L. viridescens*
Pediococcus	Homofermentative	Coccus	DL	*P. acidilactici*
			DL	*P. damnosus (cerevisiae)*
			DL	*P. pentosaceus*
Enterococcus	Homofermentative	Coccus	L(+)	*E. faecalis*
			L(+)	*E. faecium*
Lactococcus	Homofermentative	Coccus	L(+)	*L. lactis*
Streptococcus	Homofermentative	Coccus	L(+)	*S. bovis*
Leuconostoc	Heterofermentative	Coccus	D(−)	*L. mesenteroides*

* About 15 to 20% of the total lactic acid is the L(+) isomer.

further classification of this group,[28-45] based on cell morphology (rods or cocci) and their metabolism. In addition, Dellaglio and Torriani[46] have used DNA hybridisation techniques to aid in the identification of isolates.

Although Orla-Jensen's original description of the group as a whole still holds true, there have been considerable changes to the genera originally assigned. Of the original six genera (*Betabacterium, Streptobacterium, Thermobacterium, Betacoccus, Streptococcus* and *Tetracoccus*) only one, *Streptococcus*, remains. Recently, evidence has accumulated to indicate that the streptococci can be divided into three genetically distinct groups:

1) Streptococci *sensu stricto*, which comprises the majority of streptococci,
2) Enterococci formed by the typical enterococci, e.g. *Streptococcus faecalis* and *Streptococcus faecium,* and
3) Lactococci, which encompasses all the lactic streptococci, e.g. *Streptococcus lactis*.[47]

These taxonomic changes have now been officially recognised and published in The Approved Lists of Bacterial Names,[48,49] therefore it is now technically incorrect to refer to *S.faecalis* etc.. Thus, in this chapter the genera *Streptococcus, Enterococcus* or *Lactococcus* will be used as appropriate when referring to a single species. Although technically incorrect, the term 'streptococci' will continue to be used when no further distinction has been made or when referring to members of all three of the above groups together. The other lactic acid bacteria are assigned to the genera *Leuconostoc, Pediococcus* and *Lactobacillus*.[50]

Lists of lactic acid bacteria commonly associated with silage have been published by several authors,[51-55] and Table 4.1 lists some of the species found. Nilsson and Nilsson[19] found that the predominant species during ensilage were streptococci and lactobacilli, with *Lactobacillus plantarum* being most frequently isolated. According to Langston and Bouma,[42] the dominant organisms in most silages are *L. plantarum, Lactobacillus brevis* and *Pediococcus* spp., whereas Gibson *et al.*[12] reported that *L. plantarum* and *Lactobacillus acidophilus* were dominant in a homofermentative fermentation. Moon *et al.*[56] examined wheat silage and found that *L. plantarum, Lactobacillus cellobiosus, Leuconostoc mesenteroides* and *Lactococcus lactis* dominated initially but after two to four days they were superseded by *Enterococcus faecium, Streptococcus bovis* and *L. plantarum*, and eventually *L. plantarum* dominated completely. Dellaglio *et al.*[45] considered that *L. plantarum, Lactobacillus buchneri, Pediococcus acidilactici* and *Pediococcus pentosaceus* played an important role in the preservation of grass and lucerne silages.

Changes have also been noted in the ratio of homofermentative to heterofermentative strains. Beck[57] found that, in well-preserved silages, acidification was initiated by homofermentative strains, particularly *L. plantarum* and *Lactobacillus curvatus*, but after only four days 85% of the strains were heterofermentative, e.g. *L. buchneri* and *L. brevis,* and these remained dominant until the end. Dellaglio and Torriani[46] also reported that fermentation was initiated by homofermentative lactic acid bacteria which dominated until day 60, after which there was a gradual decline with a concomitant increase in heterofermentative species. Beck[58] considered that the eventual dominance of heterofermentative strains was due to their greater tolerance to acetic acid. Rauramaa *et al.*[59] also found that in most silages heterofermentative lactic acid bacteria dominated at the

end of the preservation period. Seale *et al.*[60] found that there was a progression from leuconostocs to pediococci and eventually to lactobacilli in ensiled lucerne. In the untreated control only homofermentative lactobacilli were detected, but in the silages to which glucose or fructose had been added heterofermentative strains were also found. Recently, Ruser[61] published some of the results from a survey of grass and maize crops in Germany (Table 4.2), which indicated that on forage-harvested grass the homofermentative species dominated, and of the heterofermentative species, leuconostocs were isolated most frequently.

Clearly there is considerable variation in the actual species dominating in particular silages and both numbers and species present are bound to be affected by the many variables associated with ensiling. There is evidence to show that members of most of the genera of lactic acid bacteria are represented at some stage during ensiling, with *L. plantarum* being the most frequently found species.[20,37,44,45,56,62-67] Of all the lactic acid bacteria, *L. plantarum* can most easily and successfully colonise a freshly ensiled forage as this species can ferment a wide variety of substrates, is highly competitive and produces large amounts of acid quickly.[23] It should be noted that the species designation *L. plantarum* encompasses strains with a wide physiological variation, such that it has been proposed that some of them should be named as new species.[23] Recently, a new species of facultatively heterofermentative lactobacillus, *Lactobacillus graminis*, has been found to dominate in some grass silages.[68,69]

A distinctive feature of the lactic acid bacteria is their high acid tolerance. The pH range for growth is about 4.0 to 6.8, although some species, such as *Pediococcus damnosus (cerevisiae)*, will grow at pH 3.5.[50] Streptococci generally acidify the medium to approximately pH 4.5 to 5.0, although the minimum pH for growth and fermentation differs among strains, probably because of a slightly different complement of enzymes and transport carriers.[70] The enterococci associated with silage (*Enterococcus faecalis* and *E. faecium*) are able to initiate growth at pH 9.6 and may reduce the pH to as low as 4.0.[50] Lactobacilli grow best

Table 4.2. Numbers and relative frequency of lactic acid bacteria isolated from forage harvested perennial ryegrass and maize crops in 1986[61]

	Grass	Maize
Total number of isolates	991	370
% of total		
Unidentified	23	22
homofermentative	21	20
heterofermentative	2	2
Leuconostocs	32	46
Lactobacilli	24	31
obligate heterofermentative	7	6.5
facultative heterofermentative	15	24
obligate homofermentative	2	0.5
Streptococci	4	1
Pediococci	17	0
Total homofermentatives (%)	59	46

in slightly acidic media with an initial pH of 6.4 to 4.5, and generally decrease the pH to lower values than streptococci, reaching as low as pH 3.5 for some species. *P. acidilactici* and *P. pentosaceus* have similar pH profiles, with a pH optimum of 6.0 to 6.5, and they usually reduce the pH to below 4.0, whereas the pH optimum of *P. damnosus* is slightly lower at pH 5.5.[50]

Most of the lactic acid bacteria normally maintain a pH differential (higher inside) across the cytoplasmic membrane, although the internal pH decreases along with the external pH. The pH gradient is probably maintained using an H^+ATPase but eventually the accumulation of acidic end-products of fermentation lowers the internal pH below the threshold level, resulting in cessation of growth and metabolism. The level of this threshold is characteristic for each organism.

The temperature range is very variable, growth occurring within the range 5 to 50°C, but the optimum for most strains is about 30°C.[50] Most species of lactobacilli grow at 15°C but not at 45°C. *Lactobacillus fermentum* is an exception as it grows at 45°C but not at 15°C. The two species of enterococci commonly found in silage (*E. faecalis* and *E. faecium*) both grow within the temperature range 10 to 45°C. *P. damnosus, P. pentosaceus* and *P. acidilactici* have temperature optima of 25, 30 and 40°C respectively. Stirling[8] found that maximum numbers of lactic acid bacteria were obtained earlier in silages incubated at 30°C than at 22°C but it was demonstrated that they retained viability for much longer at the lower temperature.[13] The same conclusions were reached by Ohyama *et al.*[71] when comparing temperatures of 30 and 15°C.

The lactic acid bacteria have very limited synthetic abilities and therefore have complex nutrient requirements, especially with respect to amino acids and vitamins.[50,72] They have been described as facultative anaerobes,[73,74] micro-aerophilic[72,75] and aerotolerant.[76] However, none of these labels is completely satisfactory and they should be used with caution since a specific strain can behave as a strict anaerobe, a facultative anaerobe, a micro-aerophile or an aerotolerant anaerobe depending on the growth substrate and/or other environmental conditions.[77] They are most often described as facultative anaerobes, as in the current edition of Bergey's Manual of Systematic Bacteriology,[50] in that they can grow under aerobic or anaerobic conditions but, unlike other such organisms which change from fermentative to respiratory pathways in the presence of oxygen, their metabolism remains fermentative.[74,76] They do not usually possess cytochromes etc. and thus must obtain their energy from substrate level phosphorylation rather than oxidative phosphorylation. A few species, for example *E. faecalis* and *L. lactis*, can synthesise cytochromes if cells are grown aerobically in the presence of hemin.[30] As well as utilising carbohydrates, some lactic acid bacteria, for example most heterofermentative lactobacilli, can obtain energy from the conversion of carbamyl phosphate to carbon dioxide and ammonia, the first step in arginine fermentation.

Under anaerobic conditions the lactic acid bacteria can ferment a wide range of substrates (Table 4.3) using a variety of pathways. The pathway followed for fermentation of hexose sugars is used as a basis for their identification. In 1957, using enzymatic studies, Buyze *et al.*[78] divided the lactic acid bacteria into three physiologically distinct groups:

1) **Obligate homofermenters** ferment hexoses almost exclusively to lactic acid but pentoses and gluconate are not fermented. They contain fructose bisphosphate aldolase but not phosphoketolase.

Table 4.3. Some carbohydrate reactions of typical silage lactic acid bacteria[50]

	L. acidophilus	*L. brevis*	*L. buchneri*	*L. casei*	*L. coryniformis*	*L. curvatus*	*L. fermentum*	*L. plantarum*	*L. salivarius*	*L. viridescens*	*E. faecalis*	*E. faecium*	*L. lactis*	*S. bovis*	*P. acidilactici*	*P. damnosus*	*P. pentosaceus*	*L. mesenteroides*
Arabinose	−	+	+	−	−	−	d	d	−	−	−	d	d	d	d	−	+	+
Cellobiose	+	−	−	+	−	+	d	+	−	−	+	+	ND	ND	a	a	a	d
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	a	a	a	+
Galactose	+	d	d	+	+	+	+	+	+	−	+	+	+	+	a	a	a	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	d	d	d	d	d	+	+	+	−	+	+	+	+	d	−	d	(d)
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	d	+	+
Mannitol	−	−	−	+	+	−	−	+	+	−	+	+	d	d	−	−	−	d
Mannose	+	−	−	+	+	+	$+_w$	+	−	+	+	+	ND	+	a	a	a	+
Melibiose	d	+	+	−	d	−	+	+	+	−	−	+	ND	ND	a	a	a	d
Raffinose	d	d	d	−	d	−	+	+	+	−	−	ND	−	ND	a	ND	a	d
Sucrose	+	d	d	+	+	−	+	+	+	d	+	d	d	+	−	d	−	+
Xylose	−	d	d	−	−	−	d	d	−	−	ND	ND	d	d	+	−	d	d

\+ Positive reaction by 90% or more strains.
d Positive reaction by 11-89% of strains.
− Negative reaction by 10% or less strains.
(d) Delayed reaction.
$+_w$ Positive to weak reaction.
ND No data available.
a Most strains ferment cellobiose, fructose, galactose and mannitol but not melibiose. Raffinose may be fermented by *P. acidilactici* and *P. pentosaceus*.

2) **Faculative heterofermenters** possess FBP aldolase and also ferment hexoses almost exclusively to lactic acid, but they are in addition able to ferment pentoses to lactic and acetic acids using an inducible phosphoketolase.
3) **Obligate heterofermenters** ferment hexoses to lactic acid, acetic acid/ethanol and carbon dioxide, and possess phosphoketolase but not FBP aldolase.

Thus the presence of aldolase helps to differentiate the bacteria, as does the stereoisomer of lactic acid produced during fermentation (Table 4.1). Unlike higher animals and plants which produce exclusively the L(+) isomer, species of lactic acid bacteria produce either D(−), L(+) or a mixture of both (DL). Normally each isomer is made by a stereospecific lactate dehydrogenase and mixtures arise when both enzyme systems exist, although their respective activities may not be equal and are affected by environmental conditions such as pH.[79] In some species, such as *Lactobacillus casei*, only L(+) is formed initially but the activity of lactate racemase converts half of this to the D(−) form.[80] Lactate racemase is induced by the presence of L(+) lactate and has also been found in pediococci.[81] Leuconostocs produce only the D(−) isomer and this can help in differentiation from strains of heterofermentative lactobacilli which may exhibit a coccobacilli cell morphology, since most lactobacilli form racemic mixtures of the two isomers.[44] Most species produce at least some of each isomer even if the majority is of one type; for example *L. casei* produces about 5% of the D(−) form.[80]

Schaadt and Johnson[82] stated that, in general, most of the lactic acid produced during ensilage is in the D(−) form, and there is concern that this isomer is nutritionally inferior to the L(+) form because it is metabolised more slowly by the host animal for gluconeogenesis.[81,83-85] Unlike the L-isomer which is oxidised in the cytosol, the D-isomer must first cross the mitochondrical membrane to be oxidised within the mitochondrion. However, even on silage diets the lactate concentration in the rumen is low since it is rapidly metabolised by the rumen microorganisms.[86] Gill *et al.*[86] found that only 10% of the rumen lactic acid is absorbed from the rumen, the rest being metabolised mainly to acetate as well as propionate and butyrate. These volatile fatty acids are than absorbed from the rumen and used for production. They found that both isomers were metabolised at equal rates in the rumen, as did Chamberlain *et al.*.[87]

Until recently it was thought that rumen bacteria were responsible for the metabolism of lactate but Newbold *et al.*[88,89] demonstrated that protozoa can account for the disappearance of a large amount of the lactate, removing L-lactate at a faster rate than D-lactate.

Rauramaa *et al.*[59] reported that in untreated and cellulase-treated grass silages the ratios of L(+) to D(−) lactic acid were 1.23 and 1.14 respectively compared to 1.04 and 0.98 for inoculated and formic acid-treated silages, indicating that species such as *E. faecalis, E. faecium* and *L. casei* had probably been more active in the former silages. Vanbelle *et al.*[55] suggested that it would be beneficial if lactic acid bacteria used for inoculants produced increased amounts of the L(+) isomer. Ataku *et al.*[90] inoculated ensiled herbages with *L. casei* and succeeded in increasing the proportion of L(+) lactic acid in the silage. *L. plantarum*, which is the most commonly isolated species in silage, is reported to produce both isomers, with a slight excess of the D(−) isomer.[79]

Sources and development of lactic acid bacteria

The numbers of lactic acid bacteria present on some growing crops have been reported to be extremely small.[11,19,26,39,60,91-99] Stirling and Whittenbury[26] carried out an extensive survey on the numbers of lactobacilli, leuconostocs and pediococci occurring on growing plants. They were all scarce (usually less than 10^2 g^{-1}) and, when present, were mostly found on the sheath material at the base of grasses or on partially decayed material. Of 400 colonies isolated, 80% were found to be leuconostocs, the remainder being lactobacilli and pediococci. Leuconostocs were also the most widely distributed. The lactobacilli were least common and were found only on grasses. Langston *et al.*[20] found that the dominant lactic acid bacteria on cocksfoot were streptococci and Mundt[100] found that streptococci occurred mainly on the flowering parts of plants. Stirling and Whittenbury[26] also found that on nine farms examined only five were shown to have lactic acid bacteria on the growing crops, and on two of these numbers were very low (Table 4.4). More recently, Speckman *et al.*[96] surveyed the numbers of lactobacilli on maize crops in the USA and showed that 42, 63, 69 and 77% of samples had counts below 100, 500, 1000 and 2000 g^{-1} respectively.

Stirling[28] reported that numbers of lactic acid bacteria were not affected by season but, in contrast, Lindgren *et al.*[97] found that the numbers varied throughout the season, being lowest at the beginning and the end. Holden[101] also noted an increase in counts between the first and second cuts, and found that in the latter case there were much larger numbers of bacteria contained within the structure of the plant (endophytes).

In view of the above finding, it is perhaps surprising that in most silages lactic acid bacteria eventually dominate the fermentation, reaching maximum numbers (about 10^9) within two to four days, followed by a slow, steady decline. In earlier work it appeared that there was a very large increase in numbers of lactic acid bacteria between harvesting and actual ensiling of the crop.[26,91,102,103] This was attributed to inoculation from the farm equipment,[13,26,103,104] along with

Table 4.4. The incidence of lactic acid bacteria on silage crops on various farms during harvesting[26]

Farm	Incidence of lactic acid bacteria*	
	On growing crop	At silage pit
A	0	10
B	0	670
C	50	860
D	0	280†
E	0	20,000†
F	4	170†
G	240	900,000†
H	126	2,600†
I	5	5,200‡

* As colony forming units ml^{-1} of macerated plant material on acetate agar.
† Molasses added.
‡ Chopped fodder beet added.

multiplication in the sap liberated during harvest. Fenton[67] examined the numbers of lactic acid bacteria in grass from the standing crop through to the second day after ensiling. She found that numbers were very low on the standing crop (10 to 250 g^{-1}) but they increased 10-fold immediately after mowing. The numbers then remained fairly steady until after ensiling, when rapid multiplication began. An examination of various pieces of harvesting machinery showed that the majority of lactic acid bacteria present before harvest were streptococci (80 to 100% of the total). After harvest the total numbers had increased from 10^6 to 10^{10} metre^{-2} but the proportion of streptococci had decreased to less than 50% and other lactic acid bacteria, especially lactobacilli, had become dominant. Once again it was concluded that machinery would inoculate the herbage.

However, recent work by Pahlow and Ruser[105] has demonstrated that it is not possible to achieve some of the large increases in numbers reported from this source, nor are they the result of bacterial multiplication as the time span involved is too short. It has been suggested that in the past the counts reported for the standing crop have been underestimates of the true count, due to the bacteria losing viability before samples of herbage reached a laboratory for analysis. It was noted that the highest counts were obtained for shorter chop material and that these apparent increases after harvesting were only found with lactic acid bacteria, not with other types of microorganisms. Pahlow and Ruser[105] have proposed that the explanation for such observations lies with the method used by some lactic acid bacteria to protect themselves against the deleterious effects of oxygen. Instead of superoxide dismutase, they utilise the manganese ion (Mn^{2+}) as an oxygen scavenger. This is a unique feature of lactic acid bacteria associated with plants,[106] and is reflected in the high manganese content and requirement of such bacteria.[107,108]

In addition, lactic acid bacteria are unusual among bacteria in that several of their enzyme systems, such as DNA-dependent RNA polymerase, require manganese for activity rather than magnesium.[109,110] Since plants have a high content of manganese it is assumed that they act as reservoirs for the accumulation of manganese by epiphytic lactic acid bacteria. *L. plantarum* requires a high Mn^{2+} content for growth and can accumulate intracellular concentrations as high as 30 mM.[108] Thus manganese is only available in plentiful supply if the plant juice is released. Pahlow and Ruser[105] suggested that only in such circumstances can lactic acid bacteria survive the stressful conditions of harvest. As standing crop samples are obtained by manual harvesting methods, with the minimum release of nutrients, the lactic acid bacteria may lose their viability.

It is interesting to note that the counts for epiphytic lactic acid bacteria quoted in more recent years are often considerably higher than those reported previously. Recently, Pahlow and colleagues[61,111,112] carried out a survey of the epiphytic lactic acid bacteria on forage harvested grass and maize crops over a two-year period and found that numbers for most grasses were in the range 10^4 to 10^5 g^{-1} (Figure 4.1.) with somewhat higher counts being observed in maize. These counts were markedly higher than previously commonly accepted, and more in accord with those reported at the EUROBAC conference in 1986.[113] It may be that increasing use of inoculant additives in the field and improvements in harvesting machinery, which have led to shorter chop length etc., have encouraged higher numbers in recent years.

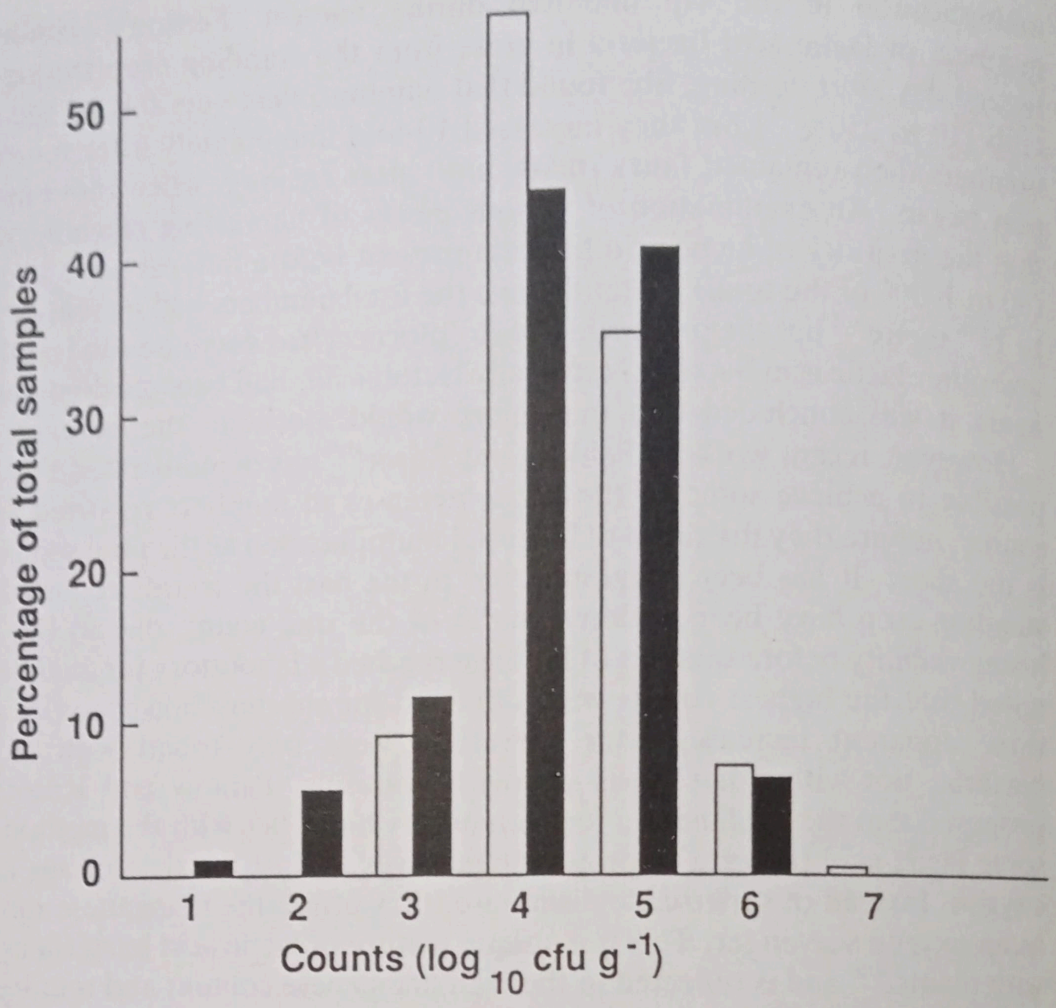
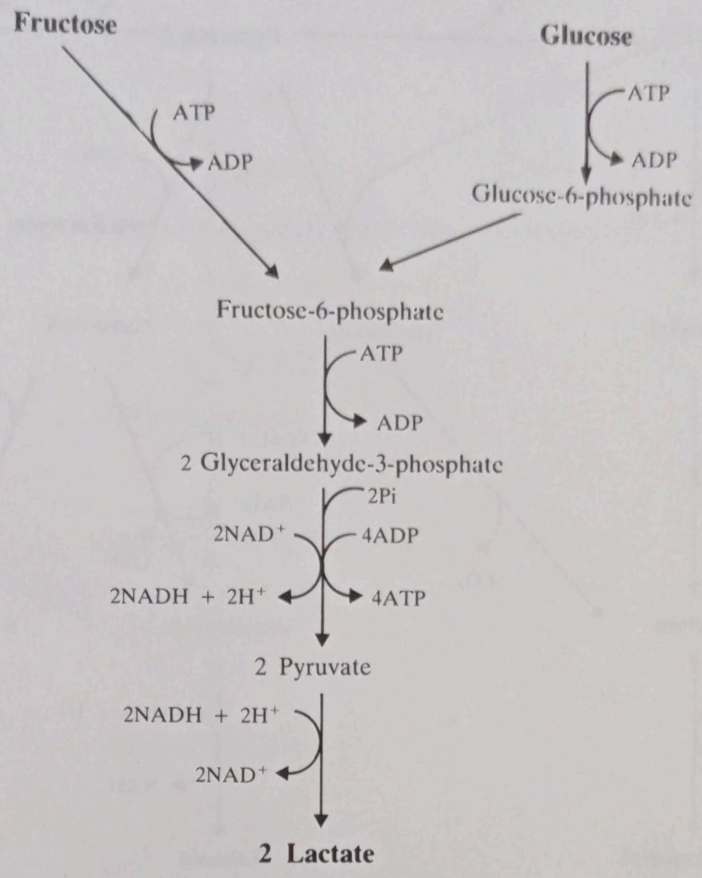


Figure 4.1 Numbers of epiphytic lactic acid bacteria on perennial ryegrass □ 1986, n=105; ■ 1987, n=104[61]

Another possible explanation for the difference was proposed by Rooke,[114] who suggested that it may be due to the use of different incubation media. Earlier experiments tended to use low pH agars such as Rogosa[115] or Tween acetate agar,[116] which do not include streptococci in the count. Now the tendency is to use either one higher pH medium such as MRS agar[117] or several different specific media, and thus all types of lactic acid bacteria are counted. In view of the fact that streptococci and leuconostocs tend to predominate on fresh crops, this may well contribute to the explanation. Rooke[114] found significantly lower numbers using Rogosa agar compared to MRS agar. Pitt and Leibensperger[118] summarised the literature with respect to epiphytic lactic acid bacteria on chopped grass, lucerne and maize crops. They found a mean of 5×10^3 g^{-1} with a range of <10 to 10^7 g^{-1}, but low pH media had been used in most of these studies.

Homolactic fermentation of hexoses

Gibbs *et al.*[119] used ^{14}C-glucose to show that the Embden-Meyerhof-Parnas (glycolytic) pathway (see Figure 3.1) was the mechanism involved in the initial stage of glucose fermentation by homolactic organisms. The two pyruvate molecules formed from either glucose or fructose are subsequently reduced to two molecules of lactic acid by the pathway shown in Figure 4.2.[119-121] This pathway



Sum: Glucose (or fructose) + 2ADP + 2Pi → 2 Lactate + 2ATP + $2H_2O$

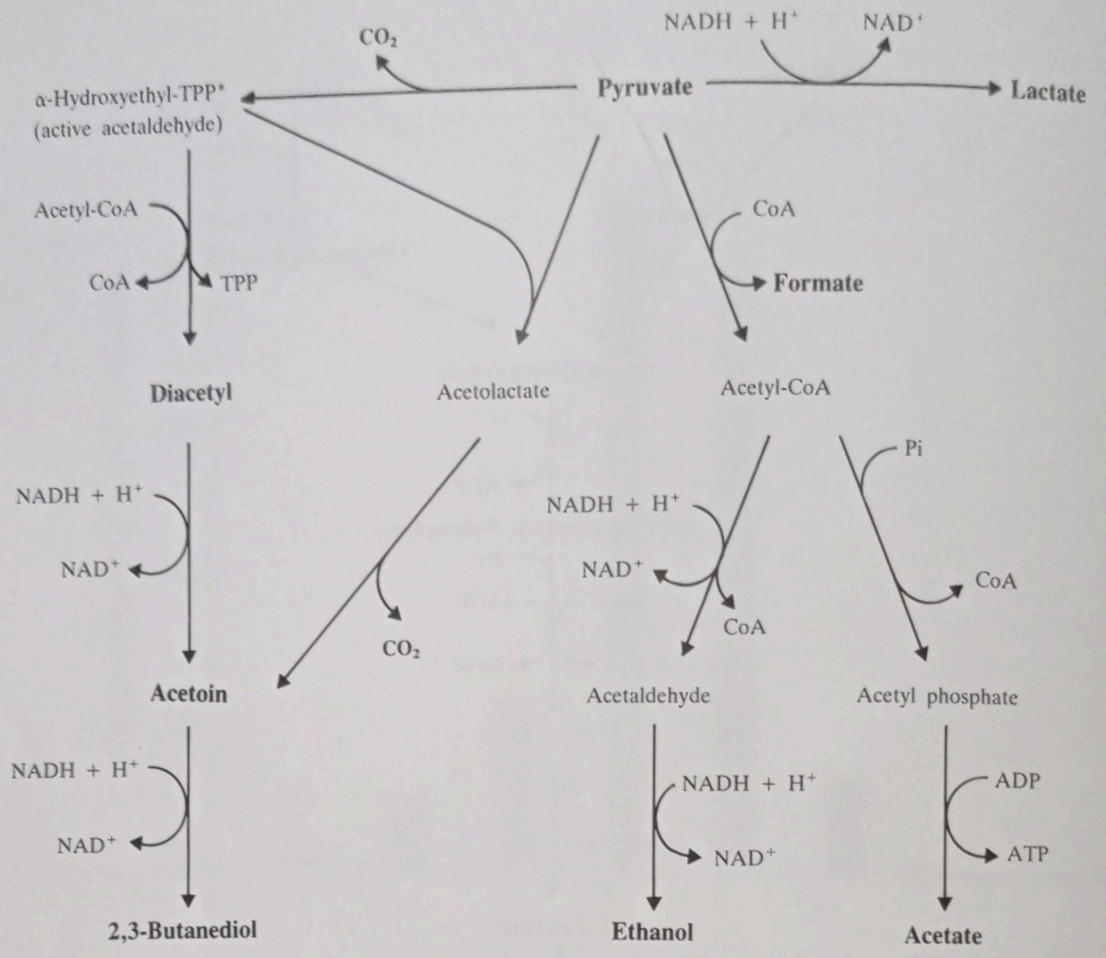
Figure 4.2. Fermentation of glucose and fructose by homofermentative lactic acid bacteria

results in no DM, and negligible energy losses.[122] Although the parameters controlling homofermentation are not completely understood, some homolactic species have been reported to convert to a mixed fermentation when the pH is altered[123] or when substrate is limiting.[124]

Streptococci, pediococci and some lactobacilli utilise the glycolytic pathway for sugar metabolism. In conditions of low oxygen tension the main product is lactic acid, allowing the reoxidation of NADH. However, many of these bacteria possess the genetic capacity to make other products from pyruvate, such as acetate, carbon dioxide, formate, ethanol, acetoin, diacetyl and 2,3-butanediol (Figure 4.3). Their formation can be demonstrated under conditions of low glucose availability and is attributed to low intracellular concentrations of fructose-1,6-bisphosphate, an essential activator of lactate dehydrogenase.[125] Under such conditions homofermentation effectively becomes heterofermentation, with ethanol, acetate and formate as the main products.[124,125]

Heterolactic fermentation of hexoses

Leuconostocs and some lactobacilli carry out a heterolactic fermentation, the products of which depend on the hexose substrate utilised (Figure 4.4).[85,121,126-129] Thus the relative proportions of these sugars in a crop will affect the final outcome of fermentation.



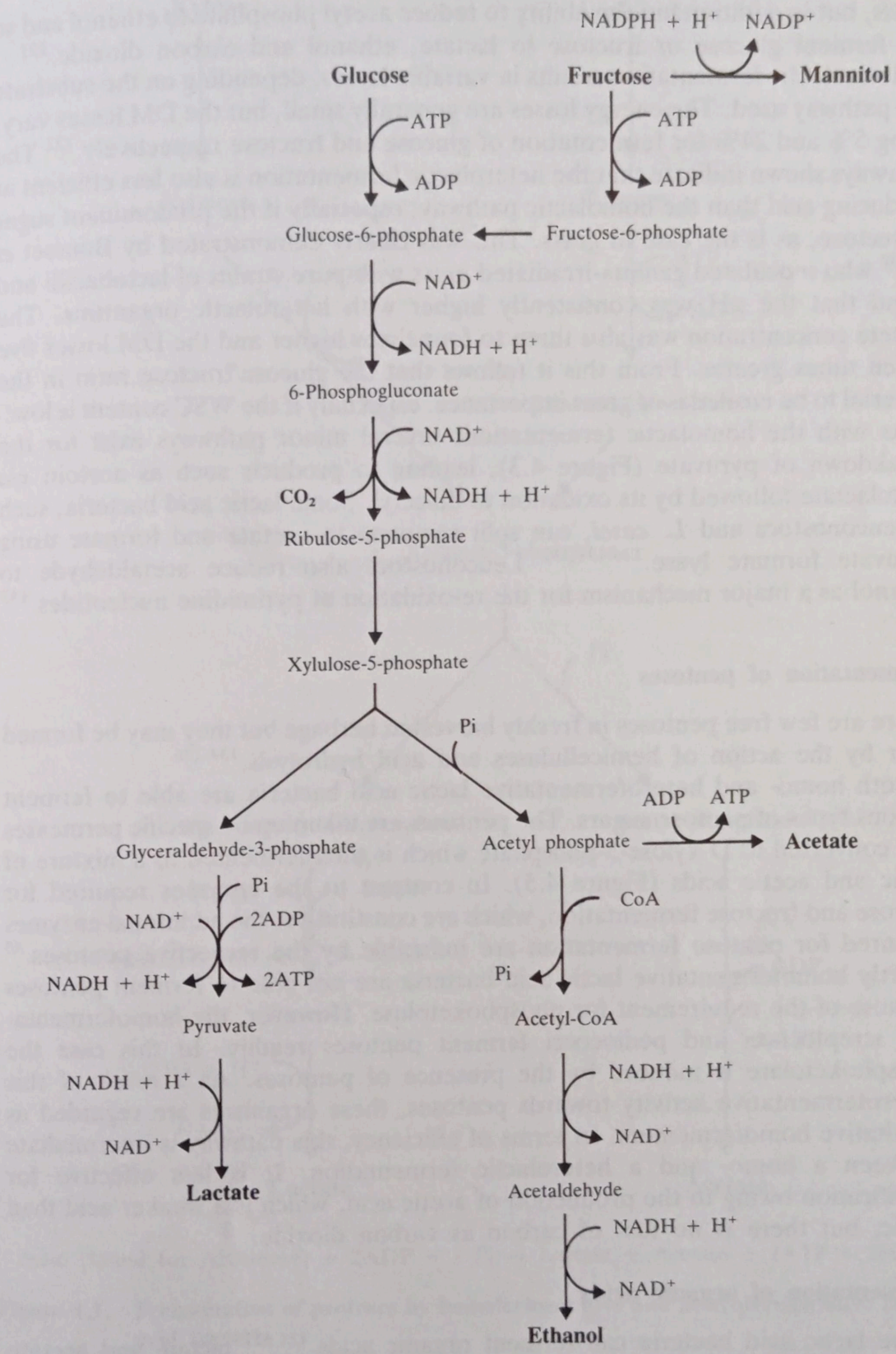
*TPP Thiamine pyrophosphate

Figure 4.3. Pathways of pyruvate fermentation by lactic acid bacteria

Both glucose and fructose are degraded by the 6-phosphogluconate pathway to glyceraldehyde-3-phosphate and acetyl phosphate. The glyceraldehyde is oxidised to pyruvate, which is then reduced to lactate as in the homolactic pathway. The acetyl phosphate acts as a hydrogen acceptor and is reduced to ethanol or acetate. The products of this fermention are therefore one mole each of lactate, ethanol/acetate and carbon dioxide (Reaction 1 in Figure 4.4). The ratio of acetate to ethanol depends on the oxidation-reduction potential of the system.

If an additional hydrogen acceptor such as oxygen or fructose is available no ethanol is formed, but oxygen is reduced to hydrogen peroxide and fructose is reduced to mannitol. The reduction of two moles of fructose to two moles of mannitol is coupled to the oxidation of one mole of fructose to produce one mole each of lactate, acetate and carbon dioxide (Reaction 2 in Figure 4.4).

Members of the *Lactobacillus brevis-buchneri* complex do not ferment glucose anaerobically to ethanol as they lack the necessary enzyme, acetaldehyde dehydrogenase.[121] Glucose will be fermented to acetate, however, provided that a suitable hydrogen acceptor is present. Fructose can function as a hydrogen acceptor and is reduced to mannitol.[16,121] In this pathway the reduction of two moles of fructose to two moles of mannitol is coupled with the oxidation of one mole of glucose to one mole each of lactate, acetate and carbon dioxide (Reaction 3 in Figure 4.4). *L. mesenteroides* carries out a similar fermentation to that of *L.*



Sum: (1) Glucose + ADP + Pi → Lactate + Ethanol + CO_2 + 2ATP + H_2O
(2) 3 Fructose + 2ADP + 2Pi → Lactate + Acetate + 2 Mannitol + CO_2 + 2ATP + H_2O
(3) Glucose + 2 Fructose + 2ADP + 2Pi → Lactate + Acetate + 2 Mannitol + CO_2 + 2ATP + H_2O

Figure 4.4. Fermentation of glucose and fructose by heterofermentative lactic acid bacteria

brevis, but in addition has the ability to reduce acetyl phosphate to ethanol and so can ferment glucose or fructose to lactate, ethanol and carbon dioxide.[121]

Heterolactic fermentation results in variable losses, depending on the substrate and pathway used. The energy losses are generally small, but the DM losses vary, being 5% and 24% for fermentation of glucose and fructose respectively.[122] The pathways shown indicate that the heterolactic fermentation is also less efficient at producing acid than the homolactic pathway, especially if the predominant sugar is fructose, as is the case in grass. This was clearly demonstrated by Bousset *et al.*[130] who inoculated gamma-irradiated grass with pure strains of lactobacilli and found that the pH was consistently higher with heterolactic organisms. The acetate concentration was also three to four times higher and the DM losses five to ten times greater. From this it follows that the glucose/fructose ratio in the material to be ensiled is of great importance, especially if the WSC content is low.

As with the homolactic fermentation, several minor pathways exist for the breakdown of pyruvate (Figure 4.3), leading to products such as acetoin *via* acetolactate followed by its oxidation to diacetyl. Some lactic acid bacteria, such as leuconostocs and *L. casei*, can split pyruvate to acetate and formate using pyruvate formate lyase.[124,131,132] Leuconostocs also reduce acetaldehyde to ethanol as a major mechanism for the re-oxidation of pyrimidine nucleotides.[133]

Fermentation of pentoses

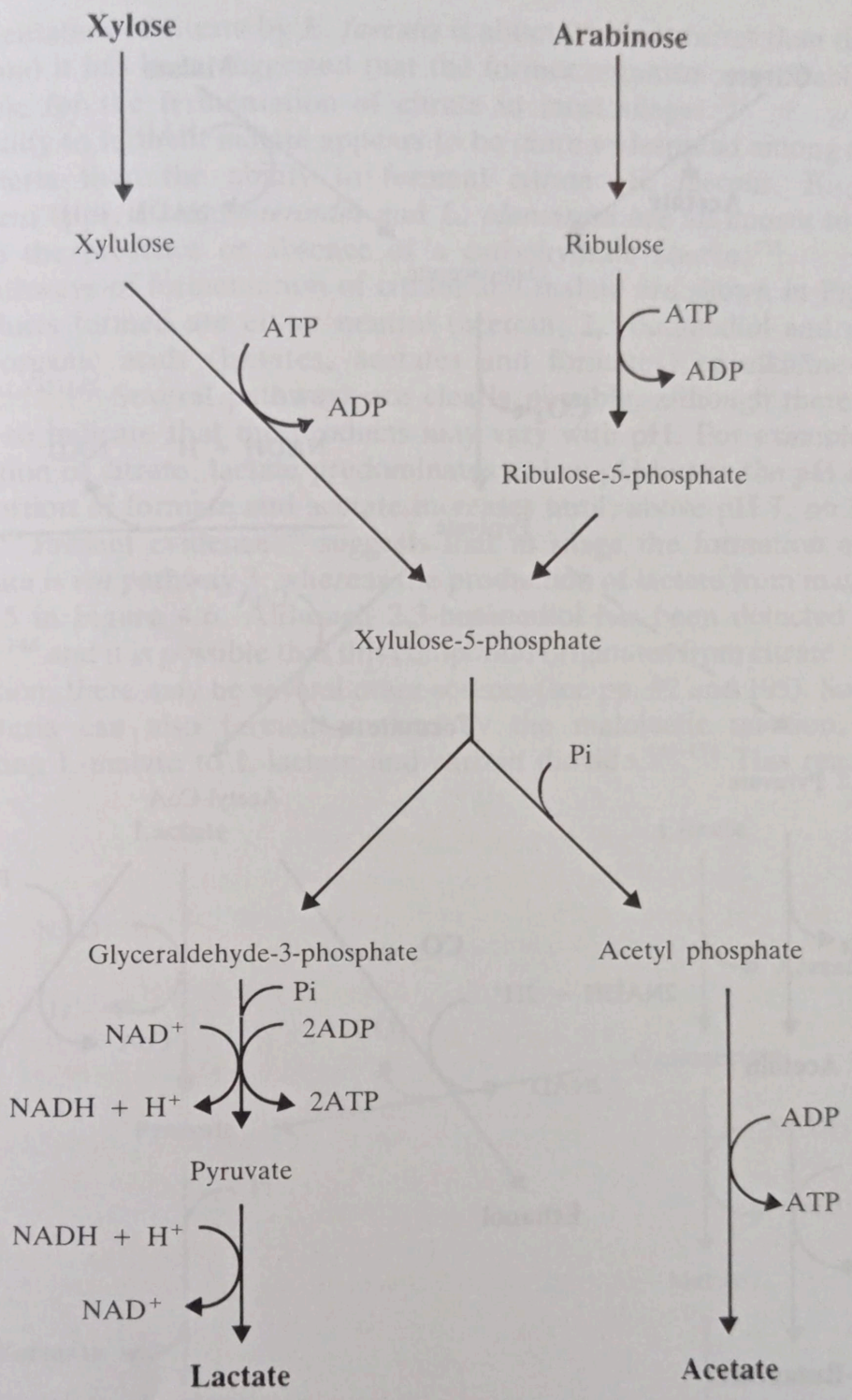
There are few free pentoses in freshly harvested herbage but they may be formed later by the action of hemicellulases and acid hydrolysis.[134-136]

Both homo- and heterofermentative lactic acid bacteria are able to ferment various types of pentose sugars. The pentoses are taken up by specific permeases and converted to D-xylose-5-phosphate which is then fermented to a mixture of lactic and acetic acids (Figure 4.5). In contrast to the enzymes required for glucose and fructose fermentation, which are constitutive, the additional enzymes required for pentose fermentation are inducible by the respective pentoses.[85] Strictly homofermentative lactic acid bacteria are not able to ferment pentoses because of the requirement for phosphoketolase. However, the homofermentative streptococci and pediococci ferment pentoses readily. In this case the phosphoketolase is induced by the presence of pentoses. As a result of this heterofermentative activity towards pentoses, these organisms are regarded as facultative homofermenters. In terms of efficiency, this pathway is intermediate between a homo- and a heterolactic fermentation. It is less effective for acidification owing to the production of acetic acid, which is a weaker acid than lactic, but there is no loss of carbon as carbon dioxide.

Fermentation of organic acids

Some lactic acid bacteria can ferment organic acids,[121,137] lactate and acetate being the main products.[135] Their breakdown during ensilage leads to a large increase in buffering capacity since most acids exist as salts, and thus their degradation results in the liberation of an excess of cations which must be neutralised by fermentation acids, such as lactic and acetic.[138]

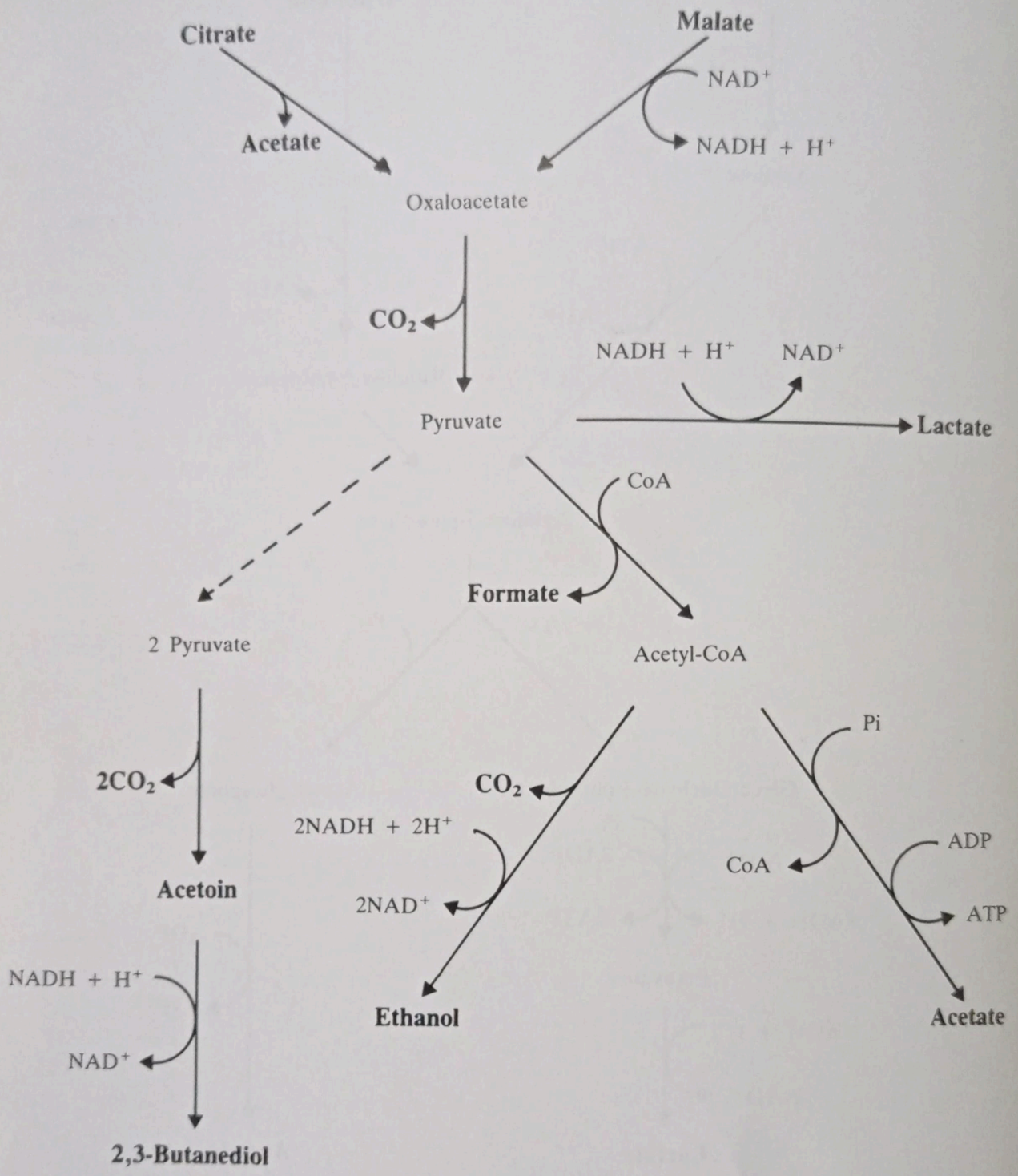
Of the acids generally present in herbage, citric and malic acids are quantitatively the most important and these are known to be rapidly metabolised



Sum: Xylose (or Arabinose) + 2ADP + 2 Pi → Lactate + Acetate + 2ATP + $2H_2O$

Figure 4.5. Fermentation of pentoses by homofermentative and heterofermentative lactic acid bacteria

in ensiled herbage.[139-141] Some lactic acid bacteria, for example *E. faecalis* and *E. faecium*, are able to ferment citrate on its own but others such as *L. brevis* can only use it in the presence of an energy source such as a fermentable carbohydrate.[137] *L. mesenteroides, Pediococcus* spp. and *L. plantarum* do not appear to ferment citrate in the presence or absence of a soluble carbohydrate.[121]



Sum: (1) Citrate + ADP + Pi → 2 Acetate + Formate + CO_2 + ATP
(2) 2 Citrate + 2H → 2 Acetate + 2,3-Butanediol + $4CO_2$
(3) 2 Citrate + ADP + Pi → 3 Acetate + Lactate + $3CO_2$ + ATP
(4) Citrate + 4H → Acetate + Ethanol + Formate + CO_2
(5) Malate → Lactate + CO_2
(6) 2 Malate → 2,3-Butanediol + $4CO_2$ + 2H
(7) Malate + ADP + Pi → Acetate + Formate + CO_2 + 2H + ATP
(8) Malate + 2H → Ethanol + Formate + CO_2

Figure 4.6. Fermentation of citrate and malate by homofermentative and heterofermentative lactic acid bacteria

The fermentation of citrate by *E. faecalis* is about 25 times faster than that by *E. faecium* and it has been suggested that the former organism is probably mainly responsible for the fermentation of citrate in most silages.[121]

The ability to ferment malate appears to be more widespread among the lactic acid bacteria than the ability to ferment citrate. *E. faecalis*, *E. faecium*, *Pediococcus* spp., *L. mesenteroides* and *L. plantarum* are all known to ferment malate in the presence or absence of a carbohydrate source.[121]

The pathways of fermentation of citrate and malate are shown in Figure 4.6. The products formed are either neutral (acetoin, 2,3-butanediol and ethanol), salts of organic acids (lactates, acetates and formates) or alkaline-released cations.[121,142-145] Several pathways are clearly possible, although there is some evidence to indicate that the products may vary with pH. For example, in the fermentation of citrate, lactate predominates at low pH but as the pH increases the proportion of formate and acetate increases until, above pH 7, no lactate is formed.[145] Present evidence[146] suggests that in silage the formation of lactate from citrate is *via* pathway 3, whereas the production of lactate from malate is *via* pathway 5 in Figure 4.6. Although 2,3-butanediol has been detected in some silages[147,148] and it is possible that this compound originates from citrate or malate fermentation, there may be several other sources (see pp. 92 and 105). Some lactic acid bacteria can also ferment malate by the malolactic reaction, thereby dissimilating L-malate to L-lactate and carbon dioxide.[149-151] This reaction has

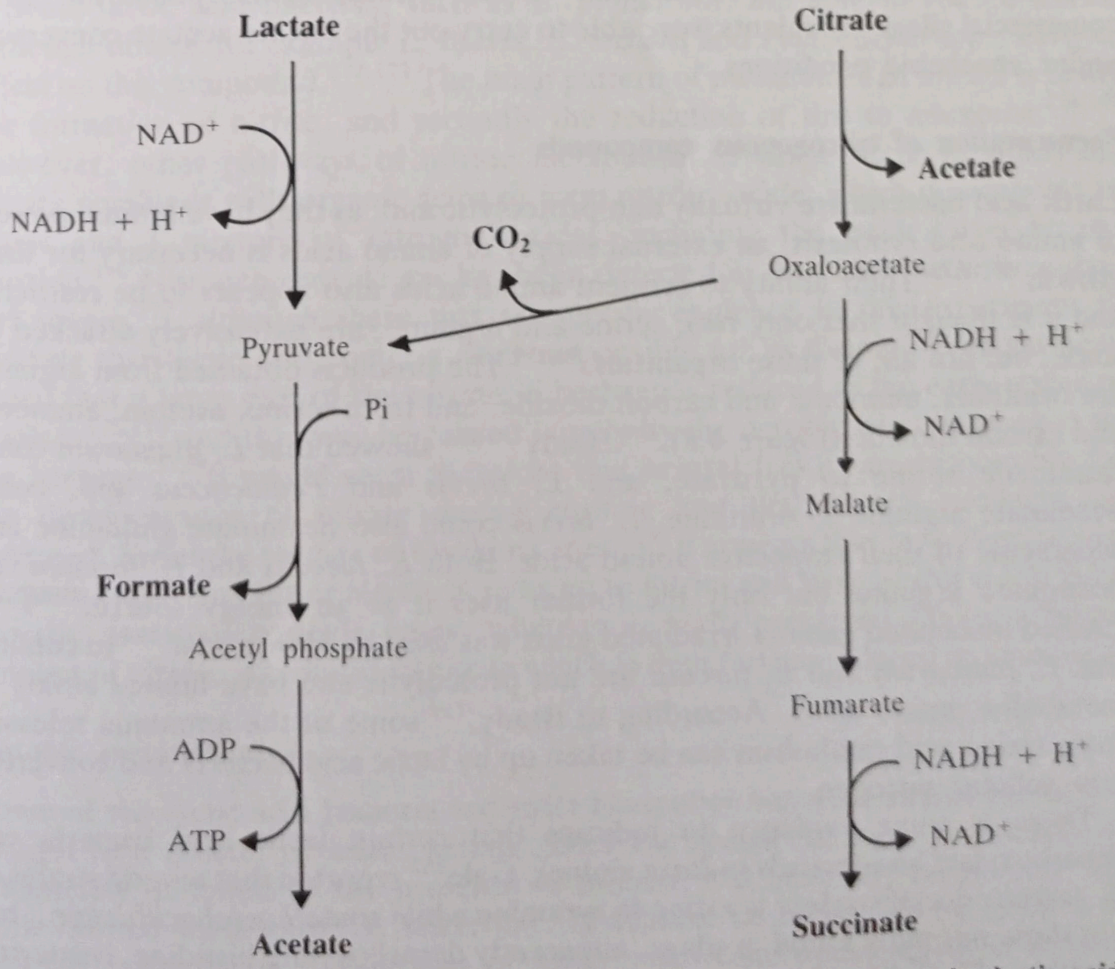


Figure 4.7. Proposed pathway for the fermentation of lactate to acetate by lactic acid bacteria[158]

been used recently by the wine industry to make wine less acidic,[152] but is obviously not desirable in silage.

There is now evidence that under conditions of hexose limitation certain lactic acid bacteria can utilise lactic acid as an energy source under anaerobic conditions.[153] This possibility was first put forward by Peterson and Fred[154] in 1920 and reiterated in 1943 by Stone and his colleagues.[155] In 1954 Wurtz[156] suggested that certain mutant strains of *L. plantarum* could convert lactate to acetate, but later Beck[58,157] suggested that only heterofermentative species such as *L. brevis* and *L. buchneri*, together with some *Pediococcus* spp. were able to carry out this reaction.

Until recently nothing was known of the possible mechanism behind the reaction but Lindgren and his co-workers have suggested a pathway involving citrate acting as electron acceptor.[153] They found that, in the absence of fermentable substrate, strains of *L. plantarum* are able to ferment lactate with the production of formate and acetate. This activity only occurs in the presence of citrate and results in the formation of succinate, indicating that oxaloacetate formed from citrate is acting as electron acceptor. The pathway proposed by Lindgren[158] for lactate-citrate metabolism is shown in Figure 4.7. In this process lactate is replaced by acetate, a weaker acid. This brings about an increase in the pH and has the potential to turn a stable, low pH silage into a poor quality, high pH silage and allow undesirable microorganisms to become active again (see p. 109). Lindgren *et al.*[153] found that several strains of *L. plantarum* from commercial silage inoculants were able to carry out the lactate/acetate conversion under anaerobic conditions.

Fermentation of nitrogenous compounds

Lactic acid bacteria are virtually non-proteolytic and, as they have limited powers of amino acid synthesis, an external supply of amino acids is necessary for their growth.[50,58,72] Their ability to ferment amino acids also appears to be restricted and it is thought that only two, serine and arginine, are extensively attacked by some, but not all, of these organisms.[50,159] The products obtained from arginine are ornithine, ammonia and carbon dioxide, and from serine, acetoin, ammonia and carbon dioxide (Figure 4.8).[160] Brady[159,161] showed that *L. plantarum* could deaminate serine to pyruvate, and *L. brevis* and *Pediococcus* spp. could deaminate arginine to ornithine. *L. brevis* could also deaminate glutamine and asparagine to their respective amino acids. Both *E. faecalis* and *E. faecium* can deaminate arginine but only the former uses it as an energy source.[50,162,163] Ensiled inoculated gamma-irradiated grass was used by Heron *et al.*[164] to confirm that *L. plantarum* and *E. faecalis* are not proteolytic and have limited ability to metabolise amino acids. According to Brady,[159] some of the ammonia released from amino acid catabolism can be taken up by lactic acid bacteria and converted into cellular nitrogen.

There is some evidence to indicate that certain lactic acid bacteria can decarboxylate amino acids to form amines. Gale[165] reported that several strains of *E. faecalis* decarboxylate tyrosine to tyramine while some *Lactobacillus* spp., but not those normally found in silage, apparently decarboxylate histidine, lysine and ornithine.[166,167] The decarboxylation of histidine to histamine by pediococci has been observed by Mayer *et al.*[168] in sauerkraut. Amines have been found in low

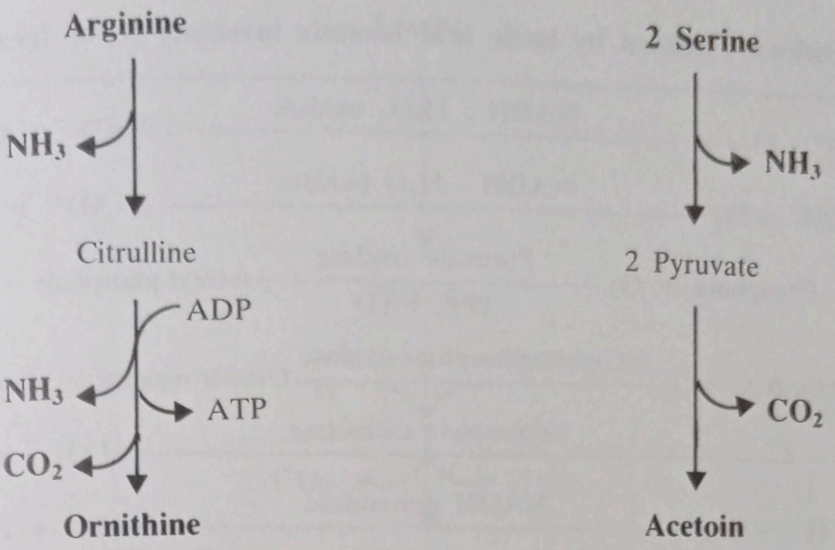


Figure 4.8 Fermentation of arginine and serine by lactic acid bacteria

pH silages by several workers. For example, Macpherson and Violante[169,170] reported significant amounts of cadaverine and putrescine in silages whose pH had fallen rapidly to 3.9 within 24 hours of ensiling. Hughes[171] examined low pH silages after six months and found that non-volatile amines accounted for 9.3% of the total nitrogen, cadaverine and putrescine being dominant.

Some lactic acid bacteria, such as *L. plantarum*, are able to reduce nitrate although others, for example *L. brevis, E. faecalis* and *Pediococcus* spp., have no effect on this compound.[172,173] The main pattern of metabolism of nitrate is firstly the formation of nitrite, and secondly the reduction of this to ammonia.[174,175] However, other pathways of nitrate metabolism in silage occur in which the nitrate combines with organic acids to form nitrous oxide, which decomposes to water and a mixture of nitrogen oxides, including the oxide, dioxide and trioxide.[176] Nitrogen dioxide gas has been detected in one study with three-day-old silages,[177] although there was no specific evidence in this experiment to indicate that lactic acid bacteria were responsible for its formation. It has been noted that a large part of the nitrate in herbage is reduced in the early stages of ensilage[178,179] and this may be caused largely by the aerobic bacteria present on the herbage.[178] It would seem therefore that several factors are responsible for the disappearance of nitrate during ensiling and that several products are obtained. From the studies of Ohshima *et al.*,[180] it appears that in well-preserved silages a small amount of nitrate is reduced to nitrite and most of this is lost from the silo as nitrogen oxide gases, whereas in badly-preserved silages a larger amount of nitrate is reduced to nitrite which is then further reduced to ammonia.

Aerobic metabolism

Some of the lactic acid bacteria are strict anaerobes but most strains can utilise oxygen to a greater or lesser extent. Since the growth of some is partially or completely inhibited by the presence of oxygen, it is generally considered that their normal metabolism is anaerobic. Oxidation of substrates by lactic acid bacteria leads to the production of some superoxide anion which is rapidly dismuted, either spontaneously or through superoxide dismutase or high intracellular Mn^{2+}, forming the more stable hydrogen peroxide. The enzymes so

Table 4.5. Pathways utilised by lactic acid bacteria involving O_2 or its metabolites

1) $NADH + H^+ + O_2$	$\xrightarrow{NADH : H_2O_2 \text{ oxidase}}$ $NAD^+ + H_2O_2$
2) $2NADH + 2H^+ + O_2$	$\xrightarrow{NADH : H_2O \text{ oxidase}}$ $2NAD^+ + 2H_2O$
3) Pyruvate + Phosphate + O_2	$\xrightarrow[TPP,\ FAD]{\text{Pyruvate oxidase}}$ Acetyl phosphate + CO_2 + H_2O_2
4) α-Glycerophosphate + O_2	$\xrightarrow{\text{α-Glycerophosphate oxidase}}$ Dihydroxyacetone phosphate + H_2O_2
5) $2O_2^- + 2H^+$	$\xrightarrow{\text{Superoxide dismutase}}$ $H_2O_2 + O_2$
6) $NADH + H^+ + H_2O_2$	$\xrightarrow{\text{NADH peroxidase}}$ $NAD^+ + 2H_2O$

TPP Thiamine pyrophosphate.
FAD Flavin adenine dinucleotide.

far identified which carry out the reactions with oxygen all produce water or hydrogen peroxide and are mainly pyridine nucleotide oxidases as well as pyruvate oxidase and α-glycerophosphate oxidase (Table 4.5).

In the absence of oxygen, lactic acid bacteria use lactate, acetaldehyde and alcohol dehydrogenases to regenerate NAD^+ for the dehydrogenation reactions during glycolysis. In the presence of oxygen, however, NADH oxidases and NADH peroxidases can be used to regenerate NAD^+. These mechanisms have a sparing effect on pyruvate, acetyl CoA and acetaldehyde and can alter the profile of end-products. For instance, leuconostocs produce mainly lactate, ethanol and carbon dioxide in the absence of oxygen, whereas in its presence acetate rather than ethanol is produced. This substitution doubles the amount of ATP which can be produced from glucose metabolism and results in a much faster growth rate.[181] Under conditions of glucose limitation and aeration, *L. lactis* produces acetate and acetoin in addition to lactate, whereas in anaerobic conditions lactate, acetate, ethanol and formate result.[182] Aerobic growth also allows lactic acid bacteria to metabolise substrates such as glycerol, mannitol, sorbitol and lactate which they do not utilise in the absence of oxygen.[77] Thus, although oxygen is toxic for some strains, it is an important electron donor for many others, allowing re-oxidation of larger amounts of reduced pyrimidine nucleotides than in the absence of air, and consequently allowing a wider range of substrates to be used. In addition, it allows utilisation of pathways which are not able to be used under anaerobic conditions, but which permit additional ATP to be generated, thus improving both yield and growth rate.

It is known that lactic acid bacteria can oxidise lactate to acetate and carbon dioxide under aerobic conditions using stereospecific, NAD-independent, flavin-containing lactate dehydrogenases or lactate oxidases. The probable pathway (Figure 4.9) is thought to be *via* dehydrogenation of lactate to pyruvate, followed by oxidation to acetyl phosphate with pyruvate oxidase, and finally conversion of acetyl phosphate to acetate with synthesis of ATP.[77] When *L. plantarum* is growing aerobically with adequate levels of glucose, lactic acid is the only major product during active growth, i.e. when the cells have low levels of lactate-oxidising enzymes. When the glucose level becomes limiting, the lactate oxidation

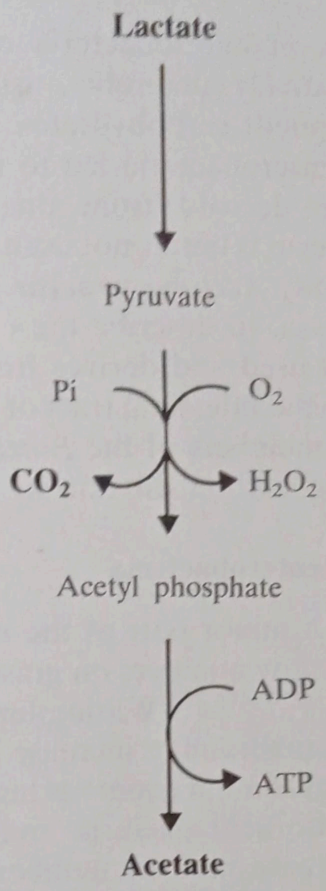


Figure 4.9 Probable pathway of oxygen-dependent lactate utilisation in *Lactobacillus plantarum*[77]

pathway is derepressed and acetate rather than lactate accumulates. Thus, when all the glucose has been used, the bacteria convert the lactate already formed into acetate.[183]

Production of antimicrobial compounds

It is well known that lactic acid bacteria are able to produce a variety of antimicrobial substances, and there have been numerous reports of bacteriocin production.[50,184-193] Such strains may be included in silage inoculants in the future, as they will be more competitive. There is also a possibility that the improved animal performance seen with some inoculants where there has been no significant effect on the fermentation characteristics of the silage[194-196] is the result of a probiotic-like effect. This could be directly due to the inoculant, which may produce substances during ensiling which exert an effect on the rumen population, or it could be an indirect effect due to the inoculant bringing about changes in the microbial population of the silage; for example, inoculated silages often have high yeast counts.[96]

ENTEROBACTERIA

The enterobacteria have long been of general interest because they include a number of important pathogens which attack man, animals and plants. Until recently they were not thought to be of any importance during the silage fermentation and only a few, generally non-pathogenic, species were thought to be present.

Beck[58] defined the species of enterobacteria occurring in silage as Gram-negative, non-sporing, facultatively anaerobic, usually motile, non-pathogenic, rod-shaped bacteria which ferment carbohydrates. Early studies by Ruschmann and Harder[197] on the silage enterobacteria led to their being called 'acetic acid bacteria', a descriptive title derived from one of the main products of fermentation. However, this term is better not used since it is more correctly used to describe bacteria of the family *Acetobacteraceae*. Later, Allen and Harrison[198] used the term 'coliform bacteria' to describe the enterobacteria associated with silage. This term is often still used and derives from the fact that many of this group are also associated with the intestinal tract of man and other animals. They are differentiated from other members of the *Enterobacteriaceae* by their ability to ferment lactose, producing carbon dioxide in the process.

Sources and development of enterobacteria

The enterobacteria represent a minor part of the microflora of grass. Pedersen and Guttormsen[199] found very low numbers on grass, whereas others have found 10^4 g^{-1},[11] or more than 10^5 g^{-1}.[97,114,200] Wilting tends to decrease the counts of enterobacteria.[200,201] Their numbers may increase substantially during the first few days of ensiling, reaching maximum counts as high as 10^8 to 10^{10} g^{-1}.[12,13,97,202] The development of the lactic acid bacteria and the subsequent fall in pH normally lead to a rapid decrease in their number.[22,57,97,202-204] Under certain circumstances, however, they may persist, for example when fermentation is delayed or when formic acid is applied,[65,97,202,205] and they may also increase during aerobic deterioration.[206] Their presence is undesirable as they compete with the lactic acid bacteria for nutrients. They also produce endotoxins[202,207] and may be responsible for much of the ammonia produced during ensiling.[60,208,209]

Little is known about the types of enterobacteria present on grass and silages.[92,99,198,210,211] Heron *et al.*[212] identified enterobacteria isolated from grass and silages ensiled with a range of different treatments. They found that the types of enterobacteria appeared to follow a pattern of succession. *Erwinia herbicola* and *Rahnella aquitilis* dominated the fresh grass but after ensiling these were rapidly superseded by *Hafnia alvei* and, finally, by *Escherichia coli*. *Serratia fonticola* appeared to occur early in fermentation if the pH fell very rapidly. The same progression of *E. herbicola, H. alvei* and finally *E. coli* was observed in a similar study by Spoelstra.[211] The finding that *E. herbicola* is the predominant species of enterobacteria on grass but not in silage is in agreement with other reports.[92,211,213] This suggests that the total number of enterobacteria present initially on grass will have no bearing on events after ensiling. Indeed, Spoelstra[211] found that inoculation with *E. herbicola* had no effect on nitrate degradation nor on any other parameter observed. In their study, Kazubiak and Muszyńska[99] did not detect any *Erwinia* spp. on fresh grass. Instead they showed that *E. coli*, *Enterobacter cloacae* and *Citrobacter* spp. were dominant, with several other species represented. They found that application of pig slurry did not change the types of species which dominated but did lead to a wider range of other species being present. During the period between application and mowing, *E. coli* and *E. cloacae* remained dominant on the untreated grass but they were joined by *H. alvei* while *Citrobacter* decreased. On the slurry-treated grass *E. coli* and *H. alvei* were dominant.

Table 4.6. Counts (log_{10}cfu g^{-1})* of microorganisms in two samples of slurry[199]

	Early May	Late June
Total viable	7.98	7.86
Total coliforms	4.52	5.54
Faecal coliforms	4.15	4.85
Proteolytic anaerobes	<4.30	<3.30
Saccharolytic anaerobes	4.89	4.51
Faecal streptococci	n.d.	6.38

* cfu Colony forming units.
n.d. Not determined.

The increased use of liquid manure as a fertiliser on grassland intended for silage has led to some concern about its possible adverse effects on fermentation. Unfortunately, there appears to be little published information available on this subject. In an examination of the microbial flora of two samples of slurry (Table 4.6), Pedersen and Guttormsen[199] found that the coliform bacteria were less than 1% of the total viable count, the most important organisms present being faecal streptococci. When herbage was harvested from slurry-treated plots several weeks after application, counts of coliforms were below maximum numbers occasionally found in fresh material and it was concluded from the study that this group of bacteria was unlikely to influence to any great extent the fermentation of slurry-treated herbage. Kazubiak and Muszyńska[99] examined pig slurry over a period of three years and found that coliforms and clostridia ranged from 6.9 to 7.9 and 4.7 to 8.0 log_{10}cfu g^{-1} respectively. Rooke[114] examined slurry-treated grass over two seasons and found that slurry application in the winter and spring did not necessarily lead to an increase in the enterobacteria count when the grass was harvested for silage. However, slurry can contain high counts of spore-forming anaerobes and the possibility of these organisms dominating the fermentation in treated material cannot be ruled out.[214]

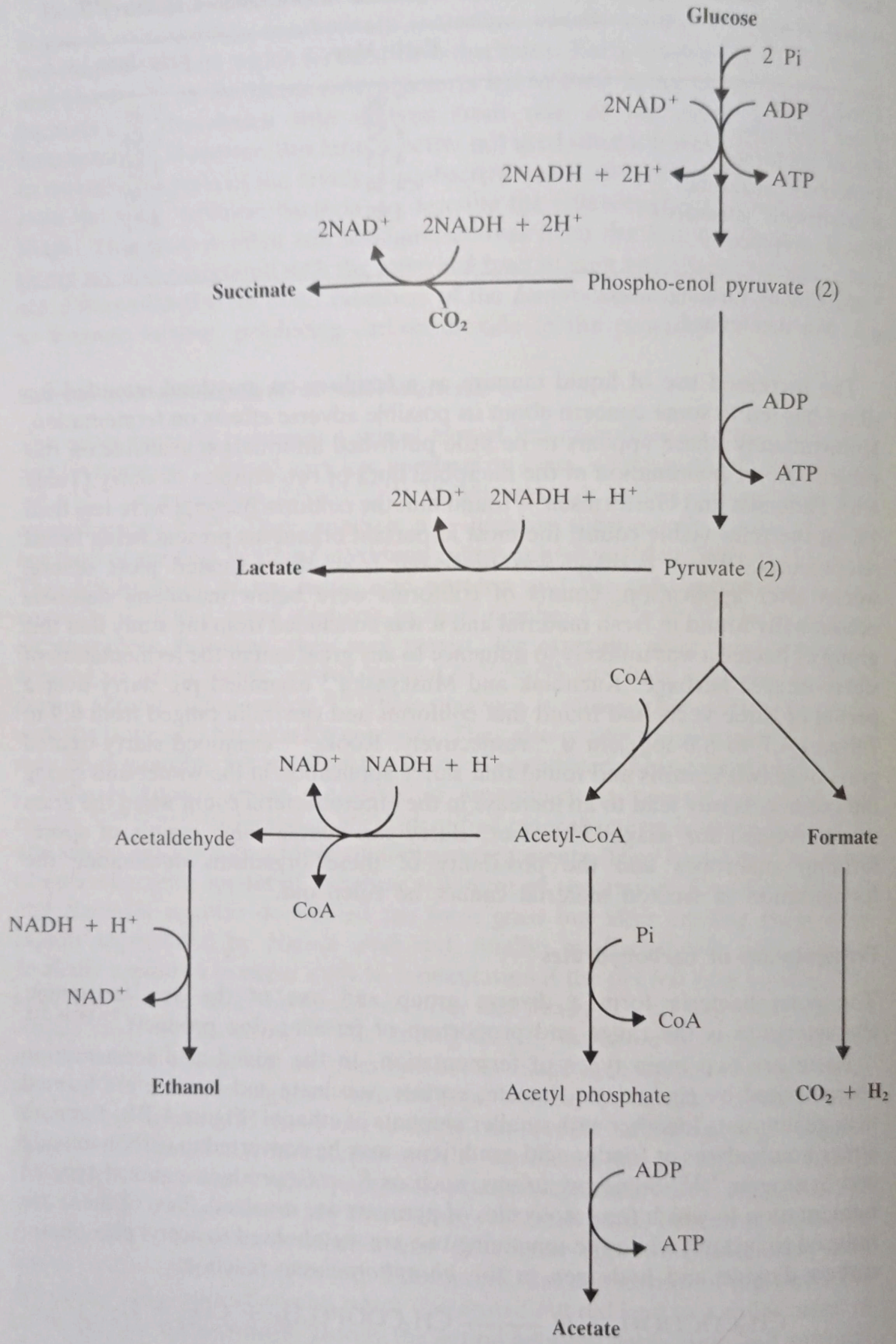
Fermentation of carbohydrates

The enterobacteria form a diverse group and one of the key taxonomic characteristics is the range and proportion of fermentation products.[73,74,76,215]

There are two main types of fermentation. In the mixed acid fermentation characterised by *Escherichia*, lactate, acetate, succinate and formate are formed in large amounts together with smaller amounts of ethanol (Figure 4.10). Formate either accumulates or, under acid conditions, may be converted to carbon dioxide and hydrogen.[76,120] Some organisms, such as *E. coli*, produce a mixed type of fermentation in which four molecules of pyruvate are involved. Two of these are reduced to lactate, while the remaining two are metabolised to acetyl phosphate, carbon dioxide and hydrogen in the *phosphoroclastic* reaction:

$$\underset{\text{Pyruvic acid}}{CH_3COCOOH} + Pi \longrightarrow \underset{\text{Acetyl phosphate}}{CH_3COOPO_3H_2} + CO_2 + H_2$$

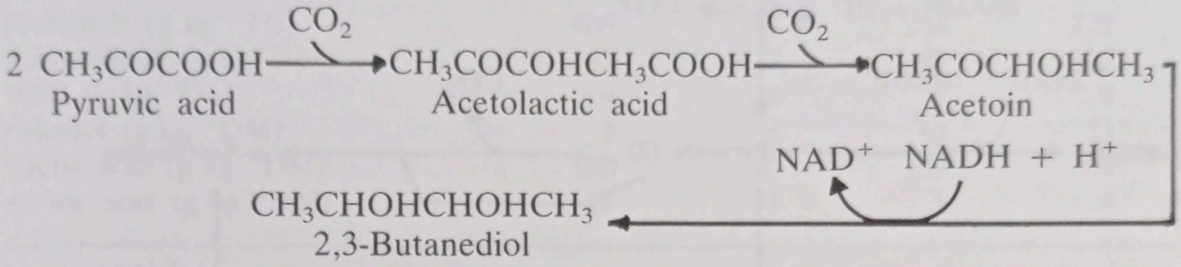
One molecule of the resulting acetyl phosphate is reduced to ethanol while the other is converted to acetate.



Typical product ratios: 4 acid : 1 neutral
1 CO_2 : 1 H_2

Figure 4.10. Mixed acid fermentation by enterobacteria

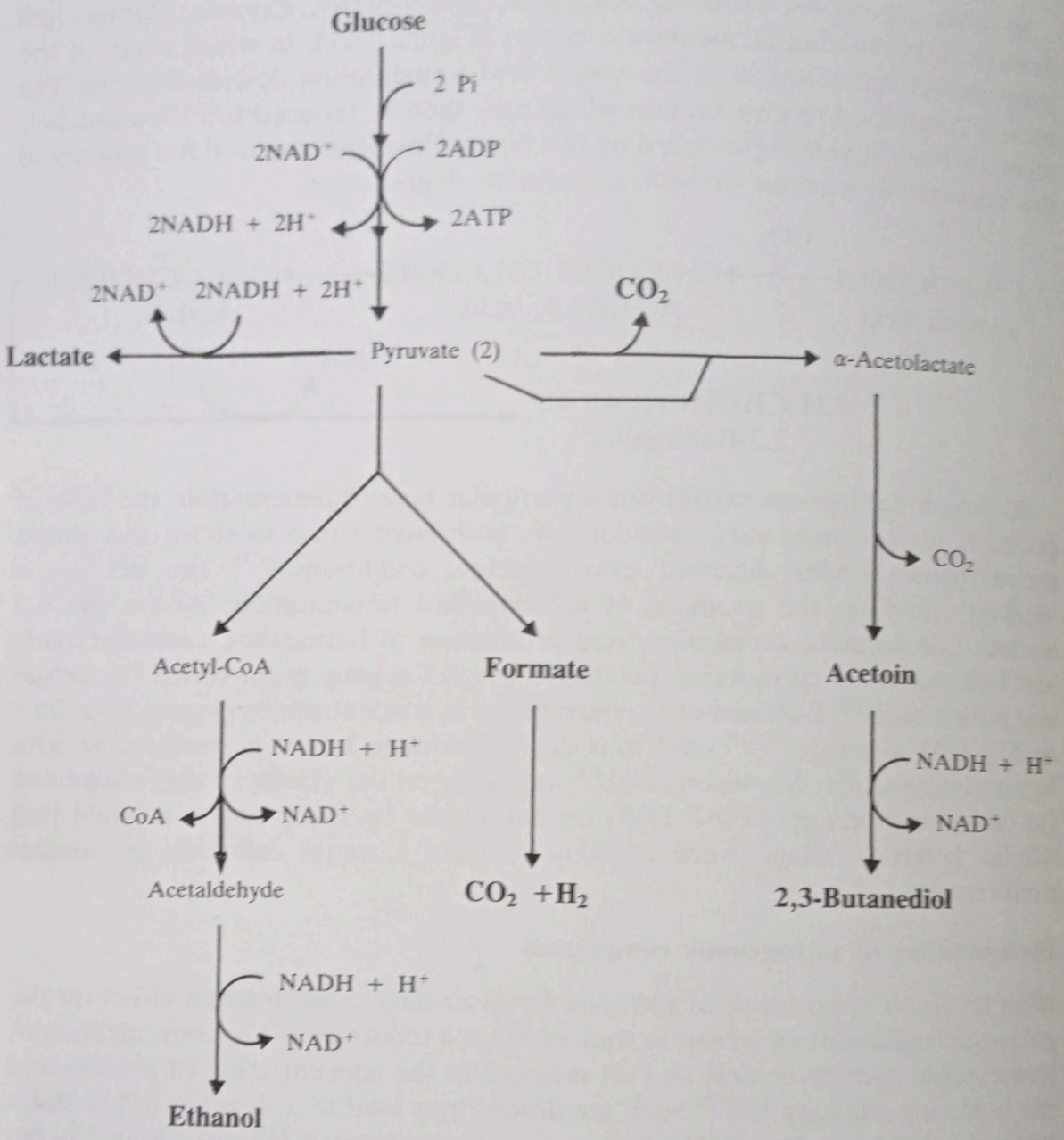
In other organisms, including *Klebsiella, Enterobacter, Erwinia, Hafnia* and *Serratia*, a butanediol fermentation occurs (Figure 4.11), in which some of the pyruvate is catabolised as in the mixed acid fermentation described above, but most is condensed to give acetoin which may then be reduced to 2,3-butanediol. More carbon dioxide is produced by this type of fermentation as it is a product of the butanediol reaction as well as formate degradation.



Although each genus carries out a particular type of fementation, the ratio of products formed may vary considerably, both from strain to strain and within strains grown under different environmental conditions.[58,73] The pH has a marked effect on the products of a butanediol fermentation. Above pH 6.3 acetate and formate accumulate and production of butanediol, carbon dioxide and hydrogen does not occur, but below pH 6.3 acetate is converted to acetoin and butanediol.[120] Butanediol has been found in concentrations ranging from 0.87 to 5% DM in silage[147,216] and thus can accumulate to levels comparable with fermentation acids. Mathison *et al.*[217] investigated the effects of this compound (at concentrations up to 5% DM) on hay intake by sheep and concluded that similar levels in silage were unlikely to have a major influence on animal performance.

Fermentation of nitrogenous compounds

High levels of application of nitrogen fertiliser have a detrimental effect on the potential ensilability of a crop as they result in a reduction in the concentration of fermentable carbohydrates, and an increase in the concentration of protein and the buffering capacity.[218-220] Such conditions may lead to a slow fall in pH and a higher final pH, conditions which may allow enterobacteria to persist longer in the silage. Enterobacteria have weak proteolytic activity but can deaminate and decarboxylate some amino acids,[58] and most species can reduce nitrate.[172,221] Although it is generally assumed that proteolytic clostridia are responsible for the formation of ammonia during ensilage, in many of these high ammonia silages clostridia are not detected.[60,222] Recently, work at Edinburgh[208] has shown that enterobacteria are capable of producing large quantities of ammonia during ensiling. Two separate experiments were carried out, one with sterile lucerne and another with non-sterile ryegrass, in which herbage was inoculated with a single strain of *Hafnia alvei* isolated originally from silage and identified using the API 20E system (API-bioMérieux (UK) Ltd, Basingstoke). In both cases there was a very large increase in ammonia production during the early stages of ensiling when the enterobacteria were added (Table 4.7). A similar experiment was carried out by Heron *et al.*[223] using several different species of enterobacteria, including *H. alvei,* isolated from silage but, although increases in ammonia were observed, they were considerably lower than those found previously at Edinburgh.[208]



Typical product ratios: 1 acidic : 6 neutral
5 CO_2 : 1 H_2

Figure 4.11. Butanediol fermentation by enterobacteria

Fertilisation also causes an increase in the nitrate content, which is broken down to a variable extent during the subsequent fermentation. It is preferable that high levels of nitrate should not exist after fermentation is complete, as reduction to nitrite in the rumen may lead to poisoning of the animal.[224] Although nitrate content does decrease during ensiling, the reduction is least in good quality silages and any measures taken to improve ensiling tend to lead to higher levels of residual nitrate.[201,225,226]

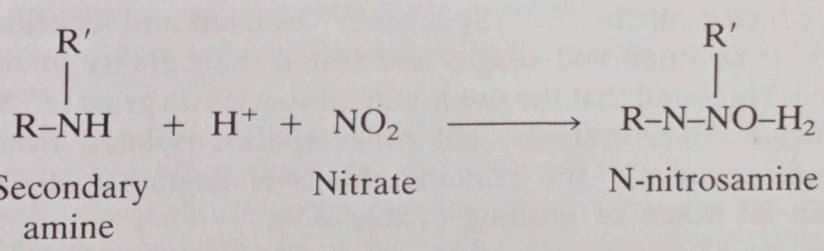
Nitrate reduction usually begins within a few hours of ensiling[227] and the final reduction products are ammonia or the gases nitric oxide (NO) and nitrous oxide (N_2O). The oxide gases are potentially hazardous to workers during silo filling and emptying operations as they may cause 'silo-filler's disease', which can result

Table 4.7. Ammonia production during ensilage of sterile lucerne (gamma-irradiated) and non-sterile ryegrass inoculated with *Hafnia alvei* at a rate of 10^6 g^{-1} [208]

	Lucerne		Ryegrass	
	U	E	U	E
pH	4.23	5.11	3.97	4.77
Protein-N (g kg^{-1}TN)	400	347	274	272
Ammonia-N (g kg^{-1}TN)	47	217	89	207
WSC (g kg^{-1}DM)	4	0	75	0
Ethanol (g kg^{-1}DM)	3	6	4	13
Lactic acid (g kg^{-1}DM)	100	40	139	69
Acetic acid (g kg^{-1}DM)	16	31	6	8

U Untreated.
E Treated with enterobacteria.

in serious respiratory problems or even death.[228] Normally these gases account for less than 1% of the total nitrogen in silage as their formation is inhibited if rapid acidification occurs.[228] There is some potential risk of the formation of volatile N-nitrosamines (carcinogens) during ensilage of high nitrate herbages, due to reactions between secondary amines and nitrite in low pH conditions.



Studies by Van Broekhoven and colleagues[229-231] have shown that, although conditions during ensiling seem favourable for the formation of such compounds, high concentrations are unlikely and the amounts transferred to milk would be insignificant.[232] This confirms the earlier findings of Terplan *et al.*[233] who found small amounts of nitrosamine in silages made from sugar beet leaves, grass and maize but could not detect any transference to milk. Tate and Alexander[234] were unable to detect nitrosamines in maize silage with or without added nitrite (250 ppm). During the reduction of nitrate, carbohydrates are used as the electron donor and the most important reactions are shown in Table 4.8. The extent to which the nitrate is reduced depends on the rate of acidification of the silage and thus reduction proceeds to a greater extent in poor quality silages.[235]

Bacteria have been shown to play a major role in nitrate reduction during ensiling[172,236] and strains of enterobacteria, clostridia and lactobacilli are able to reduce nitrate. Spoelstra[201] considered it likely that enterobacteria were principally responsible for nitrate reduction in silage for several reasons: they are active in the initial stages of ensiling, decline when the acidity increases and tend to persist in poor quality silages; the reduction of nitrate could make them more competitive by yielding additional energy; and the reduction of nitrite to nitrous oxide and ammonia would prolong their activity by increasing the buffering capacity. Most enterobacteria can reduce nitrate to ammonia *via* nitrite and some can also produce nitrous oxide.[201]

Table 4.8. Main conversions of nitrate-N occurring during ensiling[201]

Reaction	pH range	Method
1) $NO_3^- + AH_2 + H^+ \longrightarrow NO_2^- + A + H_2O$	>(4.5-5.0)	Enterobacteria
	>5.5	Plant enzymes
	>4.0	Lactobacilli
2) $2NO_2^- + 2AH_2 + 2H^+ \longrightarrow N_2O + 2A + 3H_2O$	>(4.5-5.0)	Enterobacteria
3) $NO_2^- + 3AH_2 + 2H^+ \longrightarrow NH_4^+ + 3A + 2H_2O$	>(4.5-5.0)	Enterobacteria
4) $3NO_2^- + 2H^+ \longrightarrow 2NO + NO_3^- + H_2O$	<6	Chemical

AH_2 Electron donor; e.g. carbohydrate, NAD

The conversion of nitrate to nitrite involves a membrane-bound nitrate reductase and is coupled to electron transport phosphorylation. Carbohydrates are used as the electron donor in this process which is generally referred to as nitrate respiration.[201] The nitrite then undergoes a dissimilatory reduction to ammonia. This reaction is not linked to electron transport phosphorylation but there is some evidence to suggest that it is coupled to ATP synthesis using a chemiosmotic system. The reduction of nitrite results in regeneration of oxidised coenzymes and thus the nitrate acts as an electron sink for the enterobacteria.[237,238] Enterobacteria have also been shown to produce nitrous oxide when reducing nitrite.[239-242] Spoelstra[211] isolated and identified species of enterobacteria from grass and silages and tested their ability to reduce nitrate during ensiling. He found that the predominant species on grass (*E. herbicola*) did not affect nitrate concentrations, but other species isolated from silages did increase nitrate reduction; for example, *H. alvei* degraded all of the nitrate present within 20 hours of ensiling (Table 4.9).

Table 4.9. Percentage of original nitrate degraded after 20 hours in grass ensiled after inoculation with enterobacteria[211]

	Number of strains	% degradation
Untreated	—	10-25
Citrobacter freundii	2	85-90
Erwinia herbicola	7	10-30
Escherichia coli	7	85-95
Hafnia alvei	2	100
Proteus morganii	1	93

CLOSTRIDIA

Clostridia are Gram-positive, sporulating, usually motile, rod-shaped bacteria, which grow under strict anaerobic conditions and ferment sugars, organic acids or proteins.[50] It was originally thought that clostridia were unable to grow except when oxygen was rigidly excluded but, although free oxygen inhibits their growth and may kill some strains in the non-sporing state, it is known that many of these bacteria can grow in the presence of air provided a sufficiently low oxidation-reduction potential is established. They can be divided into two major

physiological groups based on their substrates.[58] Saccharolytic clostridia, for example *Clostridium butyricum*, ferment mainly sugars and organic acids and possess little activity against proteins or amino acids. Proteolytic clostridia, such as *Clostridium sporogenes*, ferment mainly amino acids. Some clostridia, for example *Clostridium perfringens (welchii)*, have both types of activity.

Gibson[21] has identified those organisms which are likely to be of importance in silage and, although the genus includes over 60 species,[50] only about seven of these are considered to play a major role in silage fermentation. *Clostridium tyrobutyricum* is the species most frequently isolated in silage. With reference to silage, Gibson[21] preferred to designate the clostridia as lactate or amino acid fermenters (Table 4.10).

Sources and development of clostridia

Apart from their presence in silage, clostridia also occur in faeces and in soil. It was originally thought that the alimentary tract was the main habitat of these organisms and that their presence in soil and other materials was due to faecal contamination. It is now considered more likely that the primary habitat is the soil and that their presence in the digestive tract is through ingestion of soil-contaminated vegetable foods.[72] The presence of clostridia in silage is also probably the result of soil contamination, since clostridial numbers on green plant material are generally quite low.[12,19,21,60,197,243-246] An alternative source of clostridial contamination is from slurry since such material may contain significant levels of spores.[99,199,214,245]

After the filling of a silo, certain clostridia have been shown to multiply almost as early as do other silage bacteria and they participate in the exponential bacterial growth which occurs in the first few days.[12] The cell sap of plants is an ideal medium for their growth and, if the silage is adequately consolidated and the silo effectively sealed, anaerobic conditions are rapidly produced. Despite this, extensive clostridial growth usually only occurs in the later stages of ensiling and their metabolic products tend to appear only in more mature silages.[54] Most methods for their enumeration utilise pasteurised samples and thus only detect spores. Unfortunately, there is poor correlation between spore numbers and the products of clostridial fermentation, such as butyric acid and ammonia.[247] This is not altogether surprising, however, since such compounds are produced by the vegetative cells, not the spores. Clostridial activity is, therefore, best detected by biochemical means.

Clostridial growth is stimulated by high storage temperature, low DM content, low WSC content and high buffering capacity of the crop, and by delayed sealing of the silo. If a stable, low pH silage is not achieved, clostridial activity will be encouraged and a secondary fermentation will occur. As already mentioned in

Table 4.10. Clostridia of importance during ensiling[21]

Lactate fermenters	Amino acid fermenters	Others
C. butyricum	*C. bifermentans*	*C. perfringens*
C. paraputrificum	*C. sporogenes*	*C. sphenoides*
C. tyrobutyricum		

Chapter 1, the growth of clostridia is undesirable since they act against preservation by destroying lactic acid, leading to a pH rise, and they reduce the nutritional value of the product by catabolism of amino acids. As well as causing fermentation losses, their spores may contaminate milk and cause 'late blow' in hard cheeses like Emmental, Gouda and Edam.[247-251] *C. tyrobutyricum* is considered to be the species mainly responsible.[252,253]

Clostridia may also present a health hazard to animals fed on silage. Recently, Ricketts *et al.*[254] highlighted thirteen instances of botulism in horses fed big bale silage, and botulism resulting from the feeding of grass silage to cattle has also been reported.[255,256] *Clostridium botulinum* is very widely distributed in nature and at present there is no way of destroying it in the soil, therefore it is inevitable that it will occur in harvested plants. However, it is relatively uncommon in soil in the UK compared to that of the Baltic regions and parts of the USA. There are seven immunologically distinct strains (A to G) which cause botulism, but only strains C and D are associated with the symptoms in animals.[257] Both proteolytic and non-proteolytic strains are inhibited at a pH of about 4.6, but the latter can produce toxin at temperatures below 5°C whereas the former are inhibited below 10°C.[257] Botulism is not normally a health hazard to man as the silage would have to be ingested or the bacteria gain entry *via* a deep cut.

Two main factors operate in suppressing the growth of clostridia during ensiling. Firstly, the optimum pH for their growth is 7.0 to 7.4 and they cannot tolerate acid conditions.[258] If a sufficient amount of lactic acid is produced to lower the pH to a critical level their growth is inhibited. A pH of 4.2 is usually considered sufficiently low. They are inhibited at a higher pH with organic acids than with inorganic acids (Table 4.11).[259] In studies *in vitro*, Jonsson and Lindgren[259] found large differences in acid tolerance between different species; for example *C. tyrobutyricum* tolerated concentrations of acetic and lactic acid two times higher than *C. butyricum*. Such differences have also been noted

Table 4.11. pH for inhibition of clostridia in artificial media* using different acids[259]

	pH range†		
Acid	*C. tyrobutyricum*	*C. butyricum*	*C. sporogenes*
Hydrochloric	4.4-4.6	4.5-4.8	5.0-5.1
Formic			
55mM	5.0-5.4	5.4	6.2
110mM	5.4-5.6	5.6	6.4
Acetic			
80mM	4.8-4.9	5.0-5.3	5.3-5.5
250mM	4.9-5.3	5.3-5.8	5.6-5.8
Lactic			
55mM	4.7-4.9	5.2-5.3	5.4-5.6
220mM	5.2-5.3	>5.5	>5.8

* PYG medium.

† Number of replicates: *C. tyrobutyricum* (n=5); *C. butyricum* (n=4); *C. sporogenes* (n=3).

elsewhere.[21,260-262] Jonsson[263] has suggested that clostridia are probably inhibited *in vivo* at pH levels above 4.2 in most circumstances and that the limiting pH is dependent on the actual concentration of acids, particularly the undissociated acids, in the silage and is affected by the DM content, as suggested by Honig.[264] The exception is when silage is acidified directly with mineral acids, which do not have intrinsic antimicrobial properties.

Jonsson and Lindgren[259] demonstrated *in vitro* that the growth of the most acid-tolerant clostridia was inhibited by a pH just below 5.0 by 55mM lactic acid in the liquid phase, a level which corresponds to a lactic acid concentration of 15 g kg^{-1} DM in silage of DM 250 g kg^{-1}. However, in another experiment *in vivo*,[265] Jonsson found that *C. tyrobutyricum* appeared to be able to grow at a pH of 4.4 to 4.9, corresponding to a lactic acid concentration of more than 20 g kg^{-1} (equivalent to a concentration of 25 g kg^{-1} in the liquid phase if the DM is 200 g kg^{-1}). It was concluded that the growth had occurred in pockets of higher pH silage.

Secondly, clostridia like wet conditions for active growth and if crops are wilted, preferably to DM levels above 300 g kg^{-1}, their activity is usually restricted. Jonsson *et al.*[246] found that although a DM of 350 g kg^{-1} suppressed clostridial activity in big bale silage, a DM of above 400 was required for complete inhibition.

Clostridial growth is inhibited at a water activity (a_w) below 0.94[50,266] and they are more sensitive than lactic acid bacteria to low a_w. The critical pH level below which clostridia will not grow varies with the a_w of the plant material.[267] This was well illustrated by Wieringa,[268] who showed in studies with *C. tyrobutyricum* that this organism could grow in a broth adjusted with hydrochloric acid to pH 4.1. Tests with varying concentrations of sodium chloride (NaCl) in the broth showed that as the concentration was increased so also was the limiting pH value. With a concentration in the broth of 35 g NaCl kg^{-1}, pH 5.4 was the lower limit for growth. When the a_w of silage was decreased either by adding sodium chloride or by pre-wilting the herbage similar results were obtained.

Greenhill[269] has shown that the a_w of plant material increases with moisture content and has proposed the following relationship:

$$a_w = 1 - \frac{c}{m}$$

where a_w is the water activity of the plant juice measured in terms of relative vapour pressure, m is the moisture content expressed as g of water per g DM, and c is a constant, determined by the weight of molecules and ions in the plant juice. Greenhill[269] has tested a wide range of pasture species for a_w and the value of c changes according to a number of factors, for example becoming lower as the plant ages. However, for materials normally made into silage, the values of c fall within the following ranges:

Ryegrass	0.04 to 0.02
Clover and lucerne	0.05 to 0.03

A further factor of importance in the inhibition of clostridia is the buffering capacity of the crop, as the more highly buffered a crop is the more lactic acid must be produced to achieve the critical pH level for clostridial inhibition. Legumes have a two-fold disadvantage in being both highly buffered and having a

Table 4.12. Influence of temperature on the outcome of silage fermentation[275]

	Grass	Silage	
		42°C	20°C
DM (g kg^{-1})	152	162	169
pH	5.90	4.57	3.72
Total-N (g kg^{-1}DM)	20.4	21.2	20.9
Protein-N (g kg^{-1}TN)	824	448	421
Ammonia-N (g kg^{-1}TN)	—	128	67
WSC (g kg^{-1}DM)	83	7.5	11.7
Lactic acid (g kg^{-1}DM)	—	4.5	79
Acetic acid (g kg^{-1}DM)	—	45	27
Propionic acid (g kg^{-1}DM)	—	3.0	0.9
n-Butyric acid (g kg^{-1}DM)	—	5.1	0
i-Butyric acid (g kg^{-1}DM)	—	0.7	0

low WSC content, and as a consequence clostridia tend to dominate the fermentation of these crops unless they are wilted or ensiled with a suitable additive.[135,270,271]

The influence of temperature on clostridial growth is complex since, in practice, high temperatures in the silo are largely a result of excessive oxidation of sugars and they consequently lead to a reduction in fermentable substrate for the growth of lactic acid bacteria. High temperatures *per se*, however, do seem to encourage the growth of clostridia.[272,273] The optimum temperature for the growth of most of these organisms is about 37°C although some thermophilic species such as *Clostridium sphenoides* have an optimum temperature of 45°C and can grow at up to 50°C.

In general high temperatures in the silo favour clostridial growth.[71,274] In a study using 1 tonne capacity silos McDonald *et al.*[275] demonstrated that with ensiled grass of low DM content (152 g kg^{-1}), increasing the temperature to 42°C resulted in a clostridial fermentation, whereas in similar material kept at 20°C a lactic fermentation occurred (Table 4.12). Similar effects of temperature have been obtained by other workers.[71,272,273,276-278] In addition, oxidation reactions produce water which increases the a_w, making conditions in a high DM silage more suitable for clostridial growth.[245]

It is generally assumed that, since clostridia are strict anaerobes, they will not play any part during aerobic deterioration. However, recently Jonsson[265] has shown that ingress of air can indirectly bring about further growth of *C. tyrobutyricum*. Good quality silage in laboratory silos was exposed to air and high spore counts and butyric acid levels were found in the aerated, deteriorating silage on the surface, as well as in the low pH silage in the deeper, less-exposed areas. It appeared that *C. tyrobutyricum* was able to grow at a pH of 4.4 to 4.9 and a lactic acid concentration of more than 20 g kg^{-1}, which corresponds to a concentration of 25 g kg^{-1} in the liquid phase when the DM is 200 g kg^{-1}. This is a higher acid level than can be withstood in laboratory media (20 g kg^{-1} at pH 5.0).[259] It was surmised that the clostridia may be developing in micro-niches created by aerobic microorganisms like yeasts and *Bacillus* spp. which utilise lactic acid and amino acids. By so doing they reduce the oxygen concentration and increase the pH, making conditions ideal for clostridial development.

Table 4.13. Some carbohydrate reactions of typical silage clostridia[50]

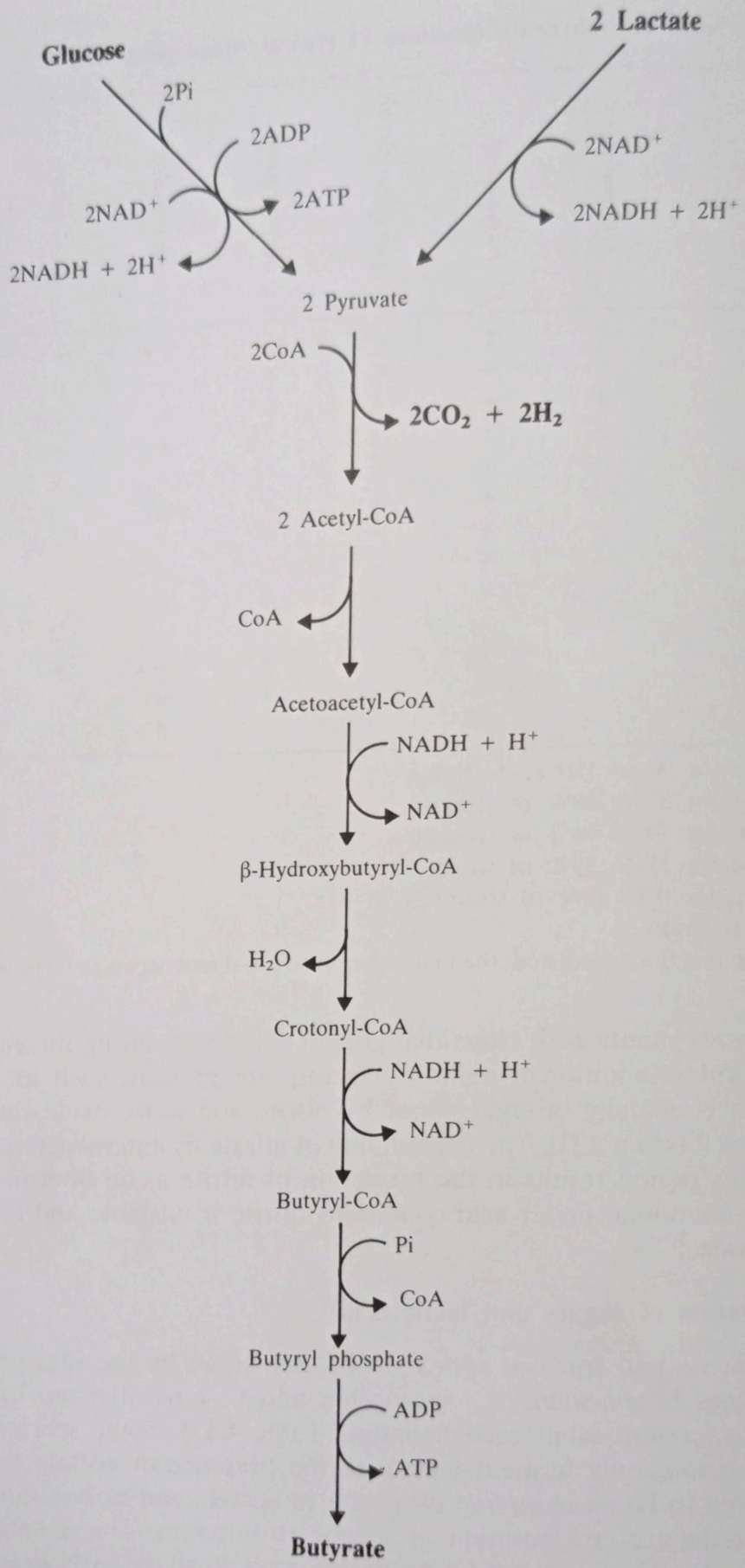
	C. bifermentans	*C. butyricum*	*C. paraputrificum*	*C. perfringens*	*C. sphenoides*	*C. sporogenes*	*C. tyrobutyricum*
Arabinose	−	±	−	−	−w	−	−
Cellobiose	−	+	+	∓	+	−	−
Fructose	d	+	+	+	+	−w	+
Galactose	−	+	+	+w	w+	−	−
Glucose	+	+	+	+	+	+	+
Lactose	−	+	+	+	w+	−	−
Maltose	w−	+	+	+	+	−w	−
Mannitol	−	∓	−	−	w+	−	−w
Mannose	−w	+	+	+	w+	−	+w
Melibiose	−	+	−	−w	d	−	−
Raffinose	−	+	−	d	+w	−	−
Sucrose	−	+	+	+	w−	−	−
Xylose	−	+	−	−	d	−	∓

\+ Positive for 90 to 100% of strains.
± Positive for 61 to 89% of strains.
d Positive for 40 to 60% of strains.
∓ Positive for 11 to 39% of strains.
− Positive for 0 to 10% of strains.
w Weak reaction.
Where two reactions are listed, the first is the more usual and occurs in 60 to 90% of strains.

As already mentioned, clostridial growth will occur during the early stages of ensiling unless additional inhibitory factors are present, such as nitrate. The inhibition is actually brought about by nitrite and nitric oxide rather than by nitrate itself (see p. 121). The degradation of nitrate by enterobacteria (see p. 106) during this period results in the formation of nitrite as an intermediary on the route to ammonia; under acid conditions nitrite is unstable and is degraded to nitric oxide.[279]

Fermentation of sugars and lactic acid

Both glucose and fructose appear to be fermented by the silage clostridia but *Clostridium bifermentans, C. sporogenes* and *C. tyrobutyricum* have a limited ability to ferment other carbohydrates (Table 4.13). Some species, such as *C. tyrobutyricum*, only ferment lactate in the presence of acetate.[21,58,280] This is considered to be an oxidation of lactate to acetate and carbon dioxide coupled with a reductive condensation of acetate to butyrate. The quantity of acetate produced from lactate is not sufficient to react with all the hydrogen derived from lactate, thus extra acetate is required if the process is to continue. Sugars and lactic acid are utilised by clostridia as an energy source *via* a similar pathway, with pyruvate as an intermediate, producing butyrate, carbon dioxide and hydrogen (Figure 4.12). It should be noted that not all saccharolytic clostridia produce



Sum: (1) Glucose + 3ADP + 3Pi → Butyrate + $2CO_2$ + $2H_2$ + 3ATP + $3H_2O$
(2) 2 Lactate + ADP + Pi → Butyrate + $2CO_2$ + $2H_2$ + ATP + H_2O

Figure 4.12 Fermentation of glucose and lactate by clostridia

butyrate. For example, *C. sphenoides* ferments glucose to ethanol, acetate, carbon dioxide, hydrogen and small amounts of lactate.

There are minor pathways resulting in the formation of formate, acetate, propionate, ethanol and butanol, but these are usually only produced in the later stages of fermentation after the pH has decreased.[73,74,120] As butyric acid is a much weaker acid than lactic acid and as only one mole of butyrate is produced from two moles of lactate, it follows that the pH will increase. This makes

Table 4.14. Catabolism of amino acids and amides by proteolytic clostridia[283]

1. **Deamination**
   - Arginine → citrulline + NH_3
     - ↳ ornithine + NH_3 + CO_2
   - Aspartic acid → fumaric acid + NH_3
     - ↳ acetic acid + pyruvic acid
   - Glutamic acid → mesacronic acid + NH_3
     - ↳ acetic acid + pyruvic acid
   - Histidine → urocanic acid + NH_3
     - ⟶ formiminoglutamic acid → formamide + glutamic acid
   - Lysine → acetic acid + butyric acid + $2NH_3$
   - Methionine → α-ketobutyric acid + methylmercaptan + NH_3
   - Phenylalanine → phenylpropionic acid + NH_3
   - Serine → pyruvic acid + NH_3
   - Threonine → α-ketobutyric acid + NH_3
   - Tryptophan → indolepropionic acid + NH_3
   - Tyrosine → *p*-hydroxyphenyl propionic acid + NH_3
   - Asparagine → aspartic acid + NH_3
   - Glutamine → glutamic acid + NH_3

2. **Decarboxylation**
   - Arginine → ornithine → putrescine + CO_2
   - Aspartic acid → alanine + CO_2
   - Glutamic acid → γ-aminobutyric acid + CO_2
   - Histidine → histamine + CO_2
   - Lysine → cadaverine + CO_2
   - Phenylalanine → β-phenylethylamine + CO_2
   - Serine → ethanolamine + CO_2
   - Troptophan → tryptamine + CO_2
   - Tyrosine → tyramine + CO_2

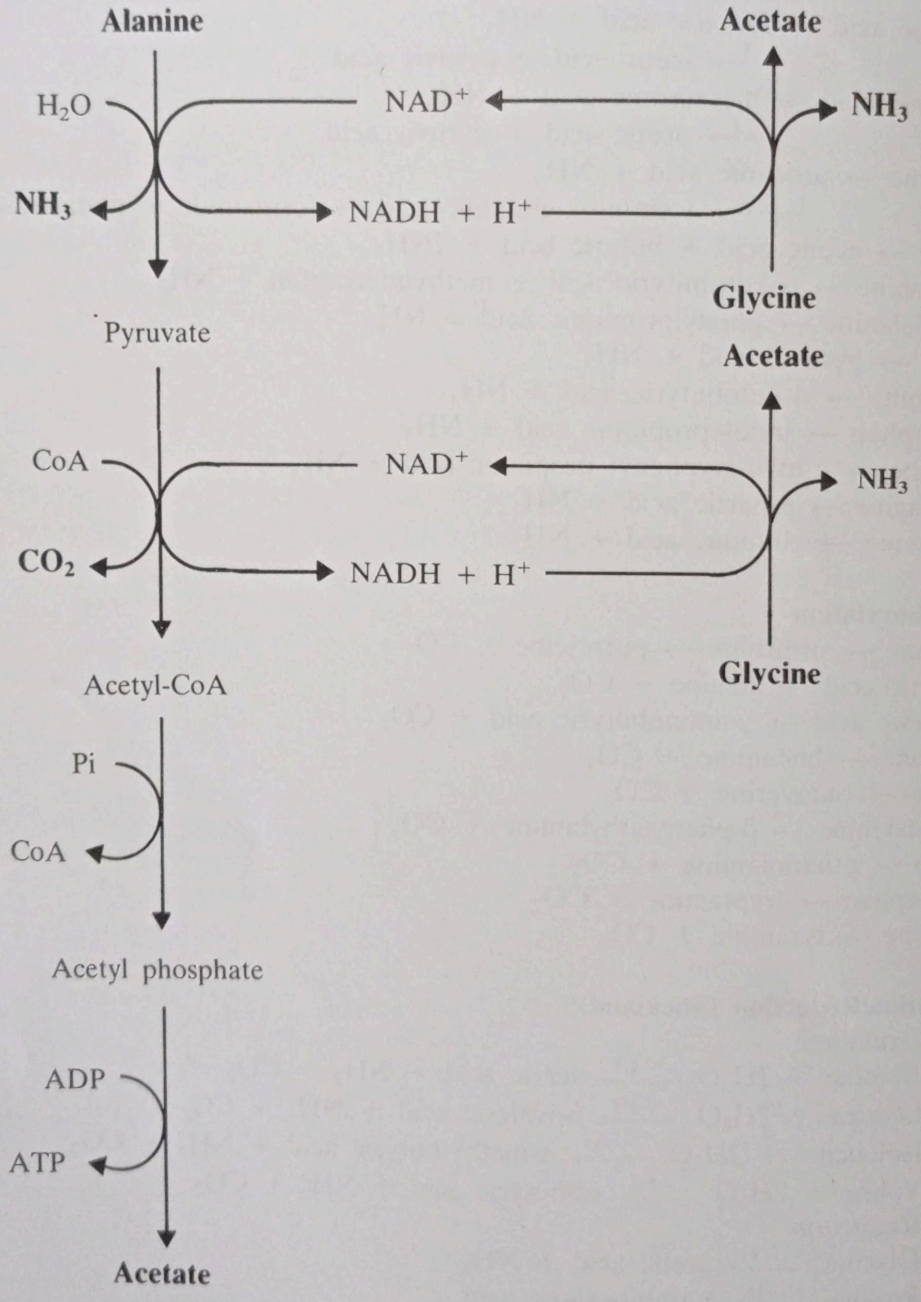
3. **Oxidation/Reduction (Stickland)***
   - (a) Oxidation
     - Alanine + $2H_2O$ $\xrightarrow{-4H}$ acetic acid + NH_3 + CO_2
     - Leucine + $2H_2O$ $\xrightarrow{-4H}$ isovaleric acid + NH_3 + CO_2
     - Isoleucine + $2H_2O$ $\xrightarrow{-4H}$ α-methylbutyric acid + NH_3 + CO_2
     - Valine + $2H_2O$ $\xrightarrow{-4H}$ isobutyric acid + NH_3 + CO_2
   - (b) Reduction
     - Glycine $\xrightarrow{+2H}$ acetic acid + NH_3
     - Proline $\xrightarrow{+2H}$ δ-aminovaleric acid
     - Ornithine $\xrightarrow{+2H}$ δ-aminovaleric acid + NH_3

* Some examples.

conditions more suitable for the growth of proteolytic clostridia, which are inhibited at a higher pH than saccharolytic clostridia.[16,281] Clostridia are usually considered to be solely responsible for the production of butyric acid but many other organisms, such as yeasts and *Bacillus* spp., produce small amounts.[54,282]

Fermentation of nitrogenous compounds

The proteolytic clostridia can selectively degrade many amino acids.[282] Three types of reaction occur, examples of which are given in Table 4.14.[283] The most widespread mechanism of amino acid catabolism is the fermentation of pairs of amino acids (Stickland reaction), one of which acts as electron donor and the other as electron acceptor.[73,74,173,284] Most amino acids act as either donor or acceptor; only tyrosine plays both roles.[74] The oxidised amino acids are converted



Sum: Alanine + 2 Glycine + ADP + Pi → 3 Acetate + $3NH_3$ + CO_2 + ATP

Figure 4.13. Example of a coupled oxidation-reduction (Stickland) reaction by clostridia

into fatty acids with one less carbon atom than in the original compound, while the reduced amino acid forms a fatty acid with the same number of carbon atoms.[173] In both parts of the reaction, except with proline, ammonia is liberated.[173,284] Typical of the Stickland type of reaction is the fermentation of alanine and glycine to yield acetic acid, ammonia and carbon dioxide (Figure 4.13).

Traditionally, the amount of ammonia present in silage has been taken as an indicator of the extent of proteolytic clostridial activity, as this compound was not thought to be produced in significant quantities by any other silage microorganisms or by plant enzyme activity. However, recently it has been found that enterobacteria are also capable of producing large amounts of ammonia during ensilage (see p. 105). Nevertheless, extensive ammonia production by clostridia was demonstrated by Kemble,[285] who examined the changes in nitrogen distribution occurring in ryegrass silages inoculated with clostridia and with lactobacilli. These changes are summarised in Table 4.15.

In the clostridial silages, the pH was reduced from 6.1 in the original herbage to 5.3 by day 3, but this then increased to 6.7 by day 21. The pH continued to increase thereafter until it reached pH 7.3 on day 147. These results show a clear relationship between pH and ammonia-N level, a relationship which has been confirmed by other workers.[170,270] By day 57, about 560 g kg^{-1} of the total nitrogen was in the form of ammonia, indicating extensive degradation of amino acids released through proteolysis. By comparison, the silage produced from similar ryegrass inoculated with lactobacilli had an ammonia-N content of only 30 g kg^{-1} TN. Hughes[286] also concluded from his studies that the main end-product of nitrogen metabolism in high pH clostridial silages was ammonia.

Not all amino acids are decomposed by clostridia and increases in alanine, histidine, isoleucine, lysine, tyrosine and valine have been obtained with specific organisms in pure culture studies (Table 4.16).[282] In a comparison of the nitrogenous changes in ensiled Italian ryegrass and lucerne, Ohshima *et al.*[287] obtained increases in alanine content, and decreases in histidine, lysine and tyrosine, while isoleucine, leucine and valine concentrations remained relatively stable in both silages despite the fact that the lucerne silage was badly preserved. Similar results have been obtained by other workers.[171,288,289] In the study by Ohshima *et al.*,[287] isobutyric acid (a derivative of valine), isovaleric acid (a metabolite of leucine), and an unidentified acid thought to be α-methylbutyric acid (a metabolite of isoleucine) were found in the badly preserved lucerne silages. Since valine, leucine and isoleucine concentrations had not decreased, these results suggest that the branched chain amino acids were synthesised during ensilage as well as being degraded by the Stickland reactions of clostridial enzymes. Similar trends were shown in the studies of De Vuyst *et al.*.[288]

The chemical changes occurring in the production of the badly-preserved lucerne silage in the study by Ohshima *et al.*[287] are shown in Table 4.17. The amines, α-aminobutyric acid, γ-aminobutyric acid, δ-aminovaleric acid, cadaverine, glucosamine, histamine, putrescine and tyramine, were present in the lucerne silage in measurable amounts. These amines, and also β-alanine, ethanolamine and tyramine, have been detected in silages by other workers.[169,171,288-294]

There is considerable interest in the presence of these amines in silage because of their potential toxicity to the animal. Histamine in particular has been singled

Table 4.15. Changes in pH and nitrogen distribution (g kg^{-1} TN) of ensiled ryegrass inoculated with (a) clostridia and (b) lactobacilli[285]

Days	pH	Soluble-N	Ammonia-N	Asparagine-N	Glutamine-N	α-amino acid-N (less 50% of amide-N)	Alcohol insoluble peptide-N
0 (a)	6.1	182	5	20	30	50	17
(b)	6.1	182	5	20	30	50	17
1 (a)	6.2	423	31	24	12	115	23
(b)	4.2	376	17	25	8	100	12
3 (a)	5.3	632	52	23	27	193	15
(b)	3.9	469	22	35	5	118	11
7 (a)	5.6	638	73	16	16	218	10
(b)	3.9	540	23	38	9	152	16
21 (a)	6.7	700	170	14	8	206	9
(b)	3.9	548	24	45	5	168	15
57 (a)	7.2	836	559	0	0	122	5
(b)	3.8	577	26	48	6	184	18
147 (a)	7.3	753	558	0	0	66	1
(b)	3.9	592	30	21	1	206	19

Table 4.16. Percentage loss (−) or gain (+) of amino acids by *Clostridium* spp[282]

Amino acids in medium				
Name	mM litre^{-1}	*C. bifermentans*	*C. sporogenes*	*C. tetanomorphum*
Alanine	5.7	+68	−35	−4
Arginine	2.2	−100	−100	−23
Aspartic acid	11.2	−81	−21	−72
Glutamic acid	20.9	−3	−7	−100
Glycine	4.4	−14	−43	−7
Histidine	1.5	−7	+20	−100
Isoleucine	2.3	0	−17	+74
Leucine	7.4	−54	−37	−35
Lysine	6.6	+32	+17	0
Methionine	2.4	−96	−83	−58
Phenylalanine	2.6	−27	−100	−8
Proline	12.2	−100	−100	−7
Serine	7.5	−100	−93	−95
Threonine	4.0	−100	−48	−75
Tryptophan	2.0	−100	−75	−100
Tyrosine	2.6	+12	−100	−100
Valine	3.8	+63	+34	+55

out for study, and experiments with sheep showed that intravenous injections of this amine completely stopped rumen motility and the ability of the animal to eructate.[295] In high concentrations, histamine can be lethal.[295] There is evidence that when the amine is given orally it is metabolised in the rumen, and levels as high as 1 to 3 g of histamine as the dihydrochloride have been added to the diets of sheep without apparent physiological effect.[296,297] However, it has been shown that under certain conditions, such as in the presence of formic or acetic acids, histamine can be absorbed, resulting in a depression in food intake.[291,298] Also, in trials with sheep it has been shown that animals generally select forages low in amines.[299] Thus amines may play a role in taste and smell responses. Histamine is usually present in smaller amounts than cadaverine, putrescine and tyramine.[171,286,294] This may be because it is produced solely as a result of microbial decarboxylase activity whereas the other amines are also produced by plant enzymes.[300] According to Voss,[301] the latter are active early in ensiling before the pH decreases and becomes inhibitory.

Hughes[286] has suggested that amines may be unstable in high pH silages so that the concentrations found may not, in fact, be any higher than those occurring in well-preserved, low pH silages where clostridial activity has been limited. There does not, however, appear to be any information in the literature defining the conditions or mechanisms under which amines are destroyed in silages.

Macpherson and Violante[170] examined the putrescine and cadaverine contents of a number of silages during the course of fermentation. In a ryegrass silage inoculated with *C. butyricum* and *C. sporogenes*, the concentrations of these two amines rose steadily to maximum values of 86 mg kg^{-1} and 180 mg kg^{-1} fresh weight respectively in the final sample (after 21 days; pH 5.25) without evidence of breakdown. These amounts contrasted sharply with those obtained in a silage inoculated with *L. plantarum*, in which putrescine and cadaverine contents were

Table 4.17. Chemical changes to lucerne after a 90-day ensiling period[287]

(a) Composition	Herbage	Silage
DM (g kg^{-1})	140	131
pH	6.3	7.0
Buffering capacity (mE kg^{-1} DM)	570	2530
WSC (g kg^{-1} DM)	54	0
Lactic acid (g kg^{-1} DM)	—	13
Acetic acid (g kg^{-1} DM)	—	114
n-Butyric acid (g kg^{-1} DM)	—	8
Isobutyric acid (g kg^{-1} DM)	—	0.2
n-Valeric acid (g kg^{-1} DM)	—	0.2
Isovaleric acid (g kg^{-1} DM)	—	0.2
Protein-N (g kg^{-1} DM)	731	260
Amino-N (g kg^{-1} TN)	28	278
Amide-N (g kg^{-1} TN)	99	0.3
Ammonia-N (g kg^{-1} TN)	1.8	292
Ornithine (g $16g^{-1}$ N)	0	trace
α-Aminobutyric acid (g $16g^{-1}$ N)	0	1.00
γ-Aminobutyric acid (g $16g^{-1}$ N)	0.71	6.48
δ-Aminovaleric acid (g $16g^{-1}$ N)	0	2.85
Cadaverine (g $16g^{-1}$ N)	0	2.33
Ethanolamine (g $16g^{-1}$ N)	0.10	trace
Glucosamine (g $16g^{-1}$ N)	0.04	0.25
Histamine (g $16g^{-1}$ N)	0	1.03
Putrescine (g $16g^{-1}$ N)	0	2.65
Tyramine (g $16g^{-1}$ N)	0	1.74

(b) Recoveries of amino acids	Herbage (g $16g^{-1}$ N)	Silage (% recovery)
Alanine	5.35	121
Arginine	4.86	19
Aspartic acid	17.3	16
Cysteine	1.16	31
Glutamic acid	9.35	28
Glycine	4.32	59
Histidine	2.21	29
Hydroxyproline	0.76	145
Isoleucine	3.90	89
Leucine	7.59	86
Lysine	6.20	26
Methionine	1.52	56
Phenylalanine	4.61	76
Proline	3.86	47
Serine	4.13	29
Threonine	3.74	32
Tryptophan	1.80	80
Tyrosine	3.36	27
Valine	5.36	97

49 mg kg^{-1} and 27 mg kg^{-1} respectively. Macpherson and Violante[170] concluded that the rate of fall of pH in the early stages of ensiling was the most important factor influencing the amine content of silages. Thus, it is not surprising that higher amine contents are found in silages made from crops with higher buffering capacities.[294]

Both α- and γ-aminobutyric acids are frequently found in clostridial silages.[292,302,303] In the study of Ohshima *et al.*[287] (Table 4.17), the γ-isomer was the amine present in highest concentration. It is generally accepted that γ-aminobutyric acid is formed by the decarboxylation of glutamic acid (Table 4.14) while α-aminobutyric acid is most likely to be derived from threonine.[302,303]

Some of the clostridia found in silages are able to reduce nitrate to ammonia, for example *C. tyrobutyricum*.[50,172] Here, nitrate is used as a 'hydrogen sink' and results in the regeneration of oxidised coenzymes for use in other fermentation reactions. In most cases nitrate reduction is not associated with ATP formation by a chemiosmotic mechanism and the process may be referred to as fermentative nitrate reduction. Thus, when clostridia use nitrate as an electron acceptor less butyrate and more acetate are produced.[201] In circumstances where nitrate is abundant but energy is limiting, nitrite may accumulate.[304]

Clostridial activity normally begins shortly after ensiling, but in crops with high nitrate contents clostridial activity in the early stages of ensiling is inhibited.[305,306] Spoelstra[306] found that, as long as nitrate remained in the silage, clostridial activity was prevented. Wieringa[219] has demonstrated that the nitrate content of herbage can have a marked influence on the development of clostridia in silage. In ensiled grasses with nitrate contents of 6 to 10 g kg^{-1} DM clostridia were inhibited, but in herbages with nitrate contents below or above these levels inhibition did not occur. The inhibition is due to the presence of nitrite and nitric oxide, reduction products of nitrate, which accumulate during the early stages of fermentation, and it is therefore only temporary.[218] Once the nitrite is removed, clostridia will multiply unless in the interim the lactic acid bacteria have produced sufficient lactic acid to reduce the pH of the silage below the critical level for clostridial growth. In an experiment by Bousset-Fatianoff *et al.*,[172] where γ-irradiated grass was inoculated with *C. tyrobutyricum*, almost all the nitrate was degraded, suggesting that self-inhibition by nitrite formation does not occur. Nevertheless, clostridial inhibition is generally considered to be due to the presence of these compounds and nitrite is used to prevent clostridial spoilage of meat products.

Table 4.18. Degradation of purines and pyrimidines by clostrida[173,309]

Purines	
Adenine	→ uric acid
	↓ ↑ → glycine + formic acid + NH_3 + CO_2
Guanine	→ xanthine
Pyrimidines*	
Cytosine	→ uracil → dihydrouracil → β-alanine + NH_3 + CO_2
Thymine	→ dihydrothymine → β-aminoisobutyric acid + NH_3 + CO_2

* With some clostridia, e.g. *C. sporogenes*, the pyrimidines are reduced to the dihydro-derivatives, but no evidence is available for further degradation of the latter.

It has been shown that the inhibitory effect of nitrite is enhanced by decreasing pH and a_w.[307] Since under acid conditions nitrite is converted to nitric oxide, Woods *et al.*[308] have suggested that the latter inhibits the reactions occurring during the phosphoroclastic split of pyruvate, an important ATP-yielding reaction in clostridia.

As nitrate and WSC levels in crops are negatively correlated,[219] it follows that the more nitrate present the lower will be the amount of fermentable carbohydrate and the greater will be the chance of eventually obtaining a dominant clostridial fermentation, especially since the decomposition of nitrate to ammonia leads to a rise in the buffering capacity.[219,235] In extreme cases the accumulated nitrite is not sufficient to prevent clostridial growth.[306]

Some species of clostridia are known to degrade purines and pyrimidines by pathways shown in Table 4.18.[173,309] The products β-alanine and β-aminoisobutyric acid arising from this degradation have been identified in clostridial silages.[310,311]

Reference was made in Chapter 3 to an increase in the buffering capacity of silages during the fermentation of lactic acid. Similarly, in a silage in which clostridia have been dominant, the buffering capacity within the pH range 4 to 6 increases quite dramatically. This is well illustrated in the results presented in Table 4.17.

FUNGI

Fungi are eukaryotic, heterotrophic microorganisms which grow either as single cells, the yeasts, or as multicellular filamentous colonies, the moulds. They are abundant in soil, on vegetation and in water, where they live largely on decaying plant material. They obtain nutrients for their growth by secreting extracellular enzymes (proteases, lipases, amylases, cellulases) which break down complex organic molecules to simple monomers which can then be absorbed through their cell membranes. The walls of fungi contain a mixture of fibrillar and amorphous components. The fibrillar ones include chitin and cellulose, which are both β (1→4)-linked polymers and confer rigidity on the wall. The amorphous components include proteins, mannans and β (1→3)-, β (1→6)- and α (1→3)-linked glucans.[312]

The majority of fungi are strict aerobes in that they require oxygen in order to grow although some, including a number of yeasts and a few filamentous fungi, can grow under anaerobic conditions, obtaining sufficient energy for their growth by fermentation.[312]

Yeasts

Ruschman and Graf[313] first identified the presence of yeasts in silage in 1932, but their importance was largely ignored until 1964, when Beck and Gross[314] demonstrated that they played a major role in the deterioration of silage on exposure to air. The majority of the yeasts found on fresh crops are non-fermentative species of the genera *Cryptococcus, Rhodotorula, Sporobolomyces* and *Torulopsis*, ranging in numbers from 10^1 to 10^7 g^{-1}.[206,315-318] Many of these yeasts are pigmented, the carotenoid pigments giving them protection from ultraviolet radiation.[319,320] Yeast numbers often increase during wilting, partly due to the favourable growth conditions but also as a result of soil contamination

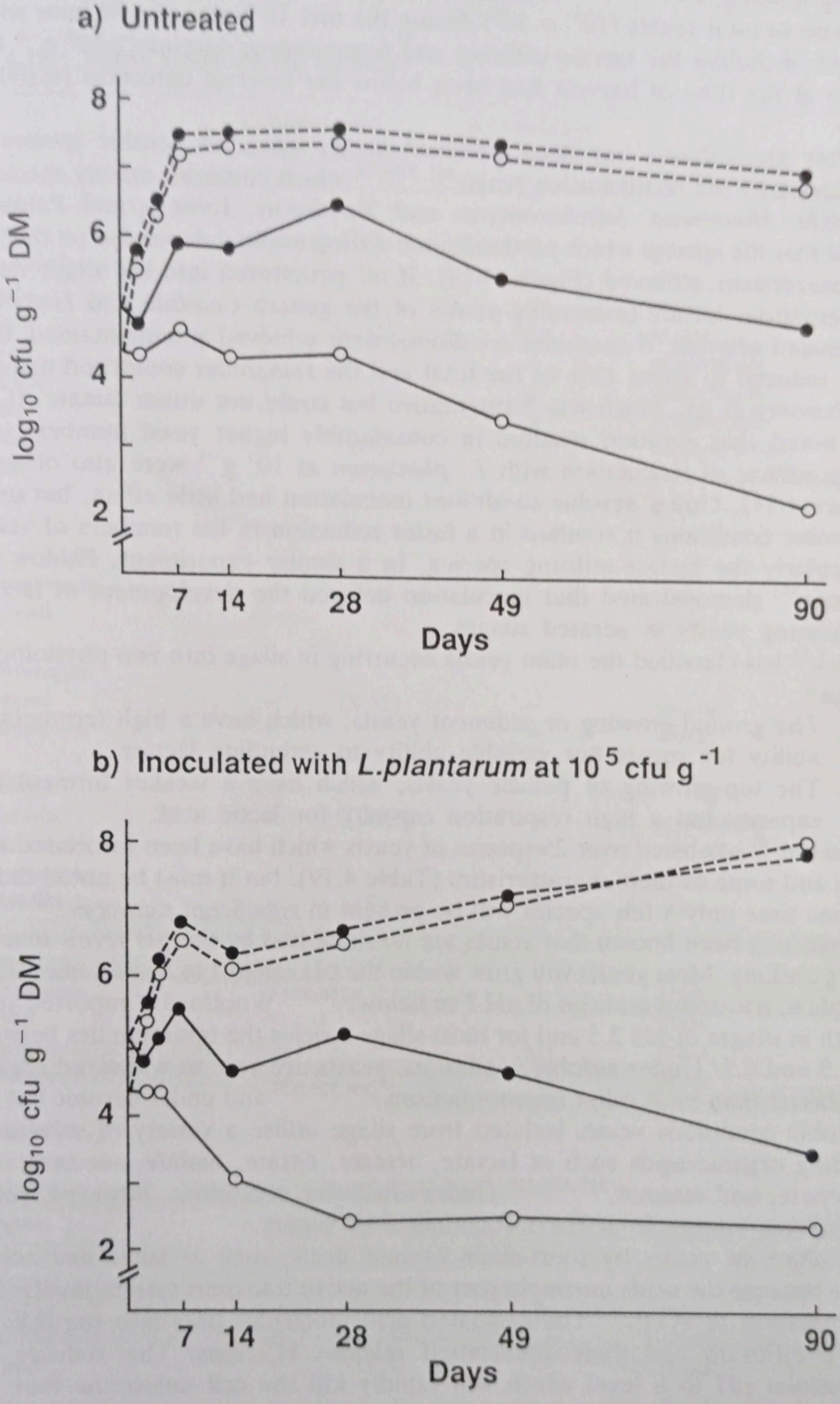


Figure 4.14 Yeast development during fermentation in grass silages under different conditions of aeration; ———— anaerobic; – – – O_2 infused at 220 mg O_2 kg^{-1} DM d^{-1}; ● total yeasts; ○ lactate assimilating yeasts[316]

during tedding, etc.[54,103,321] Jonsson and Pahlow[316] found that there was a rapid increase in total yeasts ($10^3 \rightarrow 10^6$) during the first 12 hours of a 36 hour wilting period, including the lactate-utilising and fermentative species ($>10^4$ g^{-1} DM) which at the time of harvest had been below the level of detection (<100 g^{-1} DM).

After anaerobiosis has been achieved in the silo, the aerobic species are succeeded by the fermentative yeasts[101,317,322] which comprise mainly species of *Candida, Hansenula, Saccharomyces* and *Torulopsis*. Jonsson and Pahlow[316] found that the species which predominated during ensiling depended on the level of anaerobiosis achieved (Figure 4.14). If air penetrated into the silage during fermentation, lactate-fermenting yeasts of the genera *Candida* and *Hansenula* dominated whereas, if anaerobic conditions were achieved and maintained, these were reduced to about 15% of the total and the remainder comprised mainly a *Saccharomyces* sp. which was fermentative but could not utilise lactate. It was also noted that aeration resulted in considerably higher yeast numbers.

The effects of inoculation with *L. plantarum* at 10^5 g^{-1} were also observed (Figure 4.14). Under aerobic conditions inoculation had little effect, but under anaerobic conditions it resulted in a faster reduction in the numbers of yeasts, particularly the lactate-utilising species. In a similar experiment, Pahlow and Zimmer[323] demonstrated that inoculation delayed the development of lactate-assimilating yeasts in aerated silages.

Beck[58] has classified the main yeasts occurring in silage into two physiological groups:

1) The ground-growing or sediment yeasts, which have a high fermentative ability for sugars but variable ability to assimilate lactate.
2) The top-growing or pellicle yeasts, which have a weaker fermentation capacity but a high respiration capacity for lactic acid.

Jonsson[263] has listed over 25 species of yeasts which have been associated with silage and some of their characteristics (Table 4.19), but it must be noted that at any one time only a few species will be present in significant numbers.[263,317,322]

It has long been known that yeasts are not inhibited by the pH levels reached during ensiling. Most yeasts will grow within the pH range 3 to 8 and some strains are able to withstand acidities of pH 2 or below.[258,324] Woolford[324] reported yeast growth in silages of pH 3.5 and for most silage species the optimum lies between pH 3.5 and 6.5. Under aerobic conditions, yeasts are able to withstand organic acids better than most other microorganisms,[266,325,326] and under aerobic but not anaerobic conditions yeasts isolated from silage utilise a variety of substrates, including organic acids such as lactate, acetate, citrate, malate, succinate and propionate, and ethanol.[317,327-329] Under anaerobic conditions, however, yeasts must obtain energy from the fermentation of sugars.

Inhibition of yeasts by short-chain organic acids, such as lactic and acetic, occurs because the acids uncouple part of the active transport system involved in the formation of ATP.[330] Undissociated acid molecules pass into the cells by passive diffusion and their dissociation releases H^+ ions. This reduces the intracellular pH to a level which will rapidly kill the cell unless the ions are extruded again by active transport, a process which requires energy.[331] In anaerobic conditions this energy is supplied by the fermentation of sugars and thus the presence of adequate sugar is essential for the survival of yeasts in acidic, anaerobic environments. This would explain why higher numbers of yeasts are

Table 4.19. Yeasts found during ensiling[263]

	Fermentation Glucose	(sugars)	Assimilation DL-lactate	Crop
Candida				
albican	+	(M)	+/–	grass
bimundalis	(+)		+	grass
famata	+/–		+	maize
holmii	+	(S,G)	+/–	maize
krusei	+		+	grass/maize
lambica	+		+	grass/maize
melinii	–		+	grass/maize
silvicola	+	(G)	–	lucerne/wheat
tenuis	+	(G)	+/–	grass/lucerne/wheat
valida	+		+/–	grass
vini	–		+/–	grass
Debaryomyces				
hansenii	+/–		+	grass
Endomycopsis				
burtonii	+	(S,M)	–	lucerne
selenospora	+		+	wheat
Hansenula				
anomala	+	(G,M,S)	+	grass/maize
canadensis	–		+	wheat
polymorpha	+		–	grass
subpelliculosa	+	(S)	+	grass
Pichia				
fermentans	+		+	grass
media	–		–	grass
membranaefaciens	–/(+)		+/–	grass
polymorpha	(+)		+/–	grass
Saccharomyces				
balii var. *osmophilus*	+	(S,M)	–	grass
diarensis	+	(G)	–	grass
exiguus	+	(G,S)	+	grass/maize
Torulopsis				
candida	+/–		+	maize
glabrata	+		–	grass
holmii	+	(G,S)	+	grass
Geotrichum spp.	(+)		(+)	grass

+ Growth; – No growth; (+) Delayed reaction
M Maltose; S Sucrose; G Galactose.

often found in silages containing residual WSC. The presence of residual sugars may also influence lactate assimilation by yeasts under aerobic conditions because some yeasts, such as *Saccharomyces cerevisiae*, have an active transport system for lactate and other organic acids which is subject to glucose repression.[331]

Woolford[326] demonstrated that a threshold concentration of 250 mM of lactic acid was necessary in order to inhibit yeast growth. In a laboratory study, Warth[330] showed that the addition of glucose to yeast cells under acidic conditions rapidly reduced the intracellular acid concentration and restored their viability. Pahlow and Zimmer[323] demonstrated that inoculation with lactic acid bacteria could improve the aerobic stability of silage. This was suggested to be the result of the synergistic effect of lactic acid and acetic acid, the latter being formed by homofermentative lactic acid bacteria if oxygen is present. As already mentioned, yeasts can detoxify organic acids only if a suitable supply of energy is present, but in silage all the sugars are usually used up at an early stage, especially if the silage is inoculated, and the yeasts can only oxidise the acids if sufficient oxygen is present.

There is a critical threshold between the level of oxygen required to initiate acetate production by the homofermentative lactic acid bacteria and the level which will allow the yeasts to oxidise fermentation acids. Pahlow and Zimmer[323] determined this to be within the range 100 to 150 mg O_2 kg^{-1} DM and suggested that, since such concentrations are likely to occur during unloading, some measure of protection could be provided by the use of an inoculant. On the surface where the oxygen concentration is high, however, inoculation will not inhibit the yeasts as they will be able to oxidise the acids.[332]

Most yeasts grow between temperatures of 0 and 37°C but only a few can grow above 45°C.[333] They are less sensitive to dry conditions than most bacteria, generally growing at a_w levels below 0.9, with osmophilic yeasts growing at levels as low as 0.6.[266,334] Yeasts are encouraged by high DM in silages[103] but, unlike the filamentous fungi, they require a moist surface for growth.[333]

Until fairly recently there was a general lack of interest in yeasts as it was believed that they were consistently a minority group in silage, but under certain conditions this is now known not to be true and counts of yeasts in silages exceeding 10^5 organisms g^{-1} are often found.[103,335,336] High yeast counts are frequently associated with maize silage,[204,337-339] and these organisms have also been found in higher concentrations in grass silages with high residual sugar levels resulting from treatments such as formic acid,[65,97,103,205] as well as in wilted silages[97,246] and silages in which the initial aerobic phase in the silo is prolonged.[203,340,341]

Daniel *et al.*[335] suggested that silages with yeasts in excess of 10^5 g^{-1} were likely to be unstable in air and other investigators also reported that silages with high yeast counts were more prone to deterioration.[263,314,323,342-345] However, Pahlow[343] reported that some silages with more than 10^5 yeasts g^{-1} were stable on exposure to air and this discrepancy can perhaps be explained by looking more closely at the types of yeasts present. It has been proposed that it is the lactate-assimilating yeasts in particular that are responsible for the instability of silages in the presence of air.[332] Based on standard characterisation tests about half of the approximately 500 yeast species currently recognised can utilise lactate as a carbon and energy source.[328,346] However, Middelhoven and Franzen[329] have suggested that all yeasts isolated from silage have this ability if tested under conditions more like those encountered in silage.

In their experiment, Jonsson and Pahlow[316] found that silages with high total yeast counts did not undergo deterioration if lactate-utilising species were absent, for example in the anaerobic control silage (Figure 4.14), whereas there was rapid deterioration in silages where the critical level of lactate-utilising yeasts was exceeded.

Holden[101] found that *S. cerevisiae* and *Candida* spp. (excluding *Candida lambica*) were associated with aerobic deterioration of grass silages. Although *C. lambica* was found to be the dominant species at the end of fermentation in non-acid-treated silages, it did not appear capable of survival during the subsequent aerobic phase. During aerobic deterioration other *Candida* spp. became dominant in untreated and inoculated silages, whereas in formic acid-treated silages *S. cerevisiae* prevailed. Most *Candida* spp. can assimilate lactic acid and, although *S. cerevisiae* is normally thought to have a relatively low capacity for lactic acid assimilation, it was shown to utilise this acid to a greater extent than *Candida* spp. in formic acid-treated silages.

In view of the potential losses due to aerobic deterioration (see p. 247) and the recognised role of yeasts in this process, these organisms now assume an important role in silage research programmes.

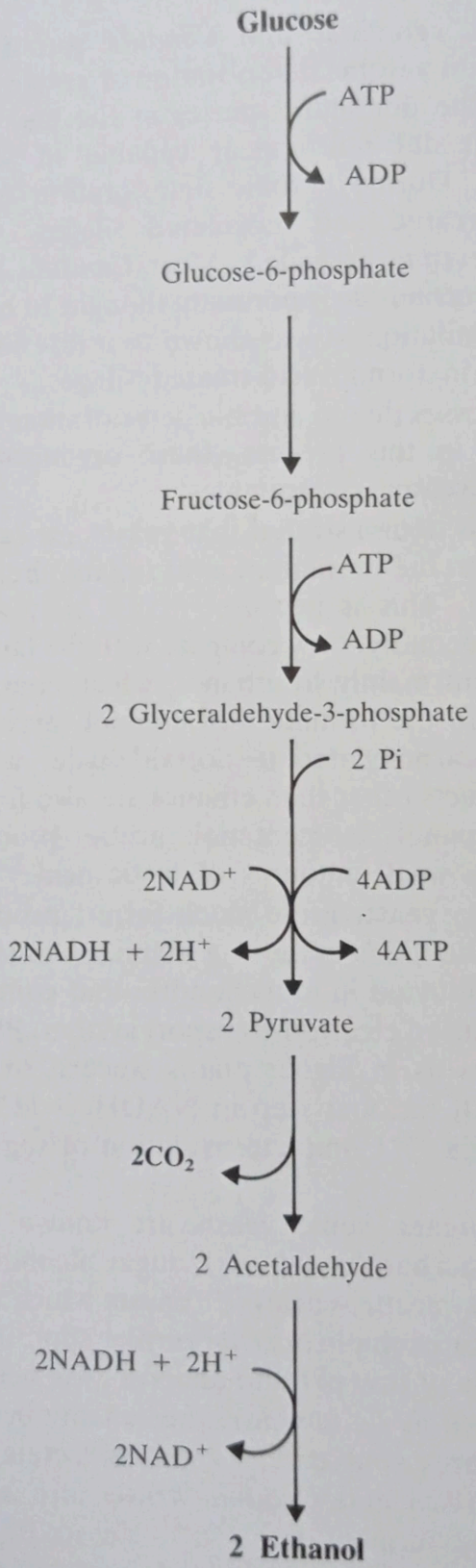
Although Woolford[324] has demonstrated that yeasts are capable of producing both lactate and acetate from the fermentation of sugars, their presence in silage is not considered desirable. This is because, firstly, they are associated with aerobic deterioration and, secondly, they compete with the lactic acid bacteria for substrate which they ferment mainly to ethanol, which contributes little to the preservation of the silage. In the formation of ethanol, pyruvate is first formed from glucose and then decarboxylated to acetaldehyde, which is reduced to ethanol (Figure 4.15). Products other than ethanol are also formed anaerobically by yeasts, including n-propanol, iso-pentanol, acetic, propionic, butyric and iso-butyric acids as well as small amounts of lactic acid.[324,347,348]

Under aerobic conditions, yeasts grow much faster, producing energy from glucose *via* glycolysis and the TCA cycle in a manner similar to that of higher plants.[312] Respiration is conducted in mitochondria that contain enzymes of the citric acid cycle and an organised electron-transport system. Phosphorylation may not be as efficient in yeasts as in higher plants since it seems likely that the coupling site associated with the first step in $NADH + H^+$ oxidation may be lacking in yeast mitochondria.[349] Complete oxidation of sugars by yeasts yields carbon dioxide and water.

In addition to hexose sugars, some yeasts are known to utilise pentoses (D-xylose, D-ribose), polysaccharides (starch), sugar alcohols (mannitol, sorbitol) and organic acids (lactic, acetic, citric).[258] Yeasts which are able to ferment sugars other than glucose, for example *Saccharomyces* spp., appear to be able to withstand the adverse effects of low pH and anaerobiosis better than those only able to ferment glucose, such as *C. lambica*, presumably because this provides them with an additional energy source.[316] As well as lactate and acetate, some yeasts, for example *C. lambica* and *Candida krusei,* are also able to oxidise ethanol produced earlier by fermentation.[316,317] Yeasts obtain their nitrogen supply from organic and inorganic sources and most species can utilise the ammonium ion,[258] but their ability to break down proteins is limited.[350] Under aerobic conditions, appreciable amounts of higher aliphatic alcohols, especially

iso-pentanol, are produced and these are thought to be formed from intermediates in the synthesis of leucine, isoleucine and valine.[347]

Yeasts and moulds are inhibited by fatty acids, the effect being pH-dependent and increasing with increasing chain length; C_{11} to C_{14} acids are particularly



Sum: Glucose + 2ADP + 2Pi → 2 Ethanol + $2CO_2$ + 2ATP + $2H_2O$

Figure 4.15. Fermentation of glucose by yeasts

effective.[325,351-355] Lindgren *et al.*[206] showed that silages made with acetic, formic, propionic or various combinations of these acids were more stable when exposed to air. Propionic acid applied at a rate of 4.8 g kg^{-1} was particularly effective, increasing the time to reach a temperature of 20°C from 9 days in the untreated control to 70 days. Similar results have been obtained by others.[342,356,357] Mann and McDonald[356] suggested that propionic acid acted by inhibiting sugar uptake by specific permeases. Since the rate of sugar uptake by the permeases is already reduced under aerobic conditions, such an inhibition would have a greater effect on cell metabolism under aerobic than under anaerobic conditions.[356] Butyric acid also has antimicrobial properties,[342,351] which explains why clostridial silages usually have increased aerobic stability.[358-360]

Moulds

The conditions in the silo associated with well-preserved silage, i.e. low pH and anaerobiosis, are unfavourable for the growth of moulds and they are generally only associated with those areas of silage, such as on the sides and surface, which are exposed to air. Their presence is undesirable since they break down not only sugars and lactic acid *via* normal respiratory pathways but also hydrolyse and metabolise cellulose and other cell wall components. Furthermore, some moulds produce substances (mycotoxins) such as aflatoxin, patulin and zearalenone which are harmful to animals and humans.[228,361-365]

Although there are over 200,000 recognised species of moulds, decreased feed value and instances of livestock poisoning are usually associated with the growth of species of *Aspergillus, Fusarium* and *Penicillium*. These fungi are widely spread, soil-borne microorganisms which require only water, oxygen and a suitable substrate for growth.[366] Toxin formation is dependent on a wide range of factors, not just the presence of suitable species of mould,[367,368] and it occurs under anaerobic and aerobic conditions.[228] As well as affecting livestock, mycotoxins from mouldy feeds may be passed on to humans through the food chain.[369,370] In addition, mouldy feeds have been responsible for a variety of other health problems in animals and humans, most of which are associated with mould spores. The best known human problems are Farmer's Lung and asthma,[371] both of which result from allergic reactions and are associated with the handling of conserved forages including silage.

Monascus (Xeromyces) bisporus is the most xerophilic fungus known, growing down to an a_w of 0.6, but most fungi will be inhibited by an a_w of less than 0.7.[334,361] Moulds differ widely in their optima and ranges of temperature for growth. Most species found in stored products thrive within the range 10 to 40°C, with optima of 25 to 35°C, but *Aspergillus fumigatus* has an exceptionally wide range of 10 to 55°C.[361] Most species function well in the pH range 3 to 8, with an optimum about pH 5. Changes in pH may alter fungal response to other limiting factors, such as a_w. For instance, decreasing the pH from 6.5 to 4.0 decreases the tolerance to low a_w of a range of *Aspergillus* and *Penicillium* spp..[361]

Moulds, together with yeasts are believed to be responsible for the deterioration of grass silages on exposure to air.[336,353,358,359,370] Holden[101] found that moulds increased at a later stage than yeasts and were associated with the large increase in pH characteristic of the later stages of deterioration. Ohyama *et al.*[353] observed two temperature peaks in their study of the aerobic deterioration of

grass silage and they attributed the first peak to yeast activity and the second peak to moulds. Barry *et al.*[372] also suggested that moulds were a secondary population as a consequence of an increase in pH, but in formaldehyde-treated silage they were preceded by acid-tolerant bacteria rather than yeasts.

Some of the species of mould which have been isolated from silages are as follows: *Arthrinium phaeospermum, Aspergillus fumigatus, Aspergillus niger, Byssochlamys* spp., *Cladosporium herbarum, Fusarium* spp., *Geotrichum candidum, Mucor ramannianus, Mucor pusillus, Mucor miehei, Monilia sitophila, Monascus* spp., *Paecilomyces* spp., *Penicillium* spp., *Petriellidium boydii, Sordaria* spp., *Trichoderma* spp.[263,351,363,373,374] Most mould species require a high oxygen concentration for growth but *Penicillium roquefortii* is unusual in preferring a high carbon dioxide (up to 80%) content and this organism has been reported as dominating in air-tight grain storage systems.[363,375,376]

Although acid-treatments are generally thought to inhibit aerobic deterioration, there are several instances where formic acid has encouraged moulds. Jonsson *et al.*[246] found that moulds were encouraged in big bale silage by the application of formic acid at 4 or 8 litre tonne^{-1}. Formic acid treatment has also been correlated with an increased incidence of aflatoxin detection in stored grain, and its use as a preservative for high moisture grain has now been banned in Sweden.[370]

BACILLI

Members of the genus *Bacillus* resemble those of *Clostridium* in being Gram-positive spore-formers but they differ in that they have the ability to grow under aerobic conditions. The different species carry out a diverse range of biochemical activities but, although some can grow under anaerobic conditions, they are not thought to be of significance during the fermentation process.[12]

Bacillus spp., like *Clostridium* spp., appear to be scarce on fresh plant material.[92,97] In an examination of perennial ryegrass samples harvested from small plots, Gibson *et al.*[12] found that aerobic spore-formers occurred in very low numbers although *Bacillus licheniformis* was isolated from every specimen of grass. In silages made from these grasses, incubated at various temperatures, Gibson *et al.*[12] found *Bacillus coagulans* in every sample examined at 40°C. *Bacillus polymyxa* multiplied in materials richest in nitrogen, becoming prominent after six months storage at 22°C and 30°C. In later studies, these workers[13] found that growth of *Bacillus* spp. was erratic and there was little or no development where lactic acid bacteria multiplied most vigorously.

Woolford[377] examined the activities of *B. coagulans, B. licheniformis* and *B. polymyxa* in a defined environment prepared to simulate grass with low contents of DM and WSC. All of these organisms produced lactic and acetic acids and their growth was not suppressed by these fermentation products, nor by low pH. The acid content of silages made by inoculation with lactic acid bacteria alone and in combinations with each of the bacilli was greater than in silages containing each of the bacilli alone. The acid content of the silages with lactic acid bacteria was not increased by the presence of the bacilli. Of the three species studied, only *B. coagulans* appeared to contribute to a reduction in silage pH and this organism is reputed to ferment sugars largely by means of the homofermentative lactic acid pathway.[92] The main fermentation products of *B. licheniformis* are glycerol and

2,3-butanediol, and those of *B. polymyxa* are ethanol and 2,3-butanediol.[377] Since *Bacillus* spp. are generally less efficient than lactic acid bacteria in producing lactic acid, it is concluded that their growth during ensilage should be discouraged.[377]

Although yeasts are thought to be primarily responsible for aerobic deterioration, bacteria, especially *Bacillus* spp., have been implicated in some circumstances.[54,101,338,355,378-383] Barry *et al.*[373] associated acid-tolerant aerobic bacteria with the initial rise in temperature in silages made with formaldehyde-type additives, with moulds appearing later. In experiments using antibiotics, Woolford and co-workers[344,379-382] found that bacteria are often involved in the initial deterioration of whole-crop cereal silages and maize, whereas in grass silages they were of less importance. The bacteria implicated were mainly species of *Bacillus*, i.e. *Bacillus cereus, Bacillus lentus, Bacillus firmus* and *Bacillus sphaericus*. Although yeasts are generally considered to be responsible for initiating deterioration in grass silages, Jonsson[263] found that in big bale silage yeasts were present in low numbers in the deteriorating parts but *Bacillus* spores and the mould *Monascus ruber* were abundant. The bacilli were identified as *B. licheniformis, B. polymyxa* and *B. sphaericus*, which have previously been associated with deteriorating silages.[12,206,381] Lindgren *et al.*[206] found that high temperatures generated during deterioration caused a shift in the dominant flora from yeasts to *Bacillus* spp.. In studies with gamma-irradiated grass, Holden[101] demonstrated that *Bacillus* spp. were unable to initiate deterioration and suggested that their proliferation depended on the prior development of yeasts to raise the pH to a suitable level.

LISTERIA

The first detailed description of the bacillus which is now known as *Listeria monocytogenes* was in 1926,[384] when it was isolated from laboratory rabbits and guinea pigs. The microorganism was originally named *Bacterium monocytogenes* but this was later changed to its present name.[385]

L. monocytogenes is a Gram-positive, asporogenous, motile, facultatively anaerobic, rod-shaped bacterium which is widely distributed in nature, and found frequently in decaying vegetable matter, sewage, water, faeces, soil and silage,[50,386-388] but generally in relatively low numbers; for example, milk may contain 1 to 30 ml^{-1}, fresh vegetation 10 g^{-1} and sewage 10 to 30 ml^{-1}.[389] It normally lives as a saprophyte[390-392] but is an opportunistic pathogen and causes disease in an extremely wide range of animals.[386] It usually affects individuals whose immune system is suppressed in some way, and in farm animals this usually occurs in pregnant females and neonates. The organism is known to be responsible for a number of diseases in animals, including meningitis, encephalitis, and septicaemia, and to cause abortions. In recent years instances of listeriosis in animals and humans have been on the increase.[393-395] Despite the high level of exposure, only a few individuals develop listeriosis, but unfortunately a high proportion of those affected die.[396]

Sheep are the animals most affected by listeriosis and the number of instances of infection increased from 44 in 1975 to 269 in 1984.[393,397] The pattern of the disease has also changed so that, instead of isolated cases within a flock, whole flock outbreaks are now common.[393,398] The most common form of the disease is

encephalitis, occurring during late pregnancy or soon after lambing, and up to 10% of a flock may be lost. Although cattle are susceptible to abortion and encephalitis caused by listeria, they are inherently more resistant and cattle are more likely to be fed clamp silage than big bale silage.

In many cases of listeriosis the disease has been associated with feeding silage,[399-404] especially poor quality material.[398,405] It has been noted that the increase in listeriosis coincides with the introduction of big bale silage and also with an increase in the use of untreated human sewage sludge and animal slurry on agricultural land.[406] It is known that listeria occur in high numbers in sewage sludge and may survive for at least eight weeks when sprayed on agricultural land.[407]

Big bale silage was first introduced in the late 1970s, and in 1987 accounted for some 16% of the total silage DM conserved in England and Wales.[408] It is a convenient system, especially for smaller farms, and involves less capital investment than a clamp silo. It is particularly popular with sheep farmers. Unfortunately it has several drawbacks. The silage in bales, each of about 500 kg weight, is of a much lower density than clamp silage and undergoes a more limited fermentation, two factors which encourage aerobic deterioration. In addition, big bales have a much greater surface area to volume ratio. Fenlon *et al.*[409] have shown that big bale silage is particularly prone to the growth of listeria, although instances with clamp silage have been reported,[410] as well as some not associated with the feeding of silage.[411]

Grass ensiled under strictly anaerobic conditions ferments well, has a low pH and listeria are absent. In bales where the bags have been damaged, allowing entry of large quantities of air, a general aerobic deterioration occurs which is dominated by more vigorous aerobic species; listeria are outcompeted, with

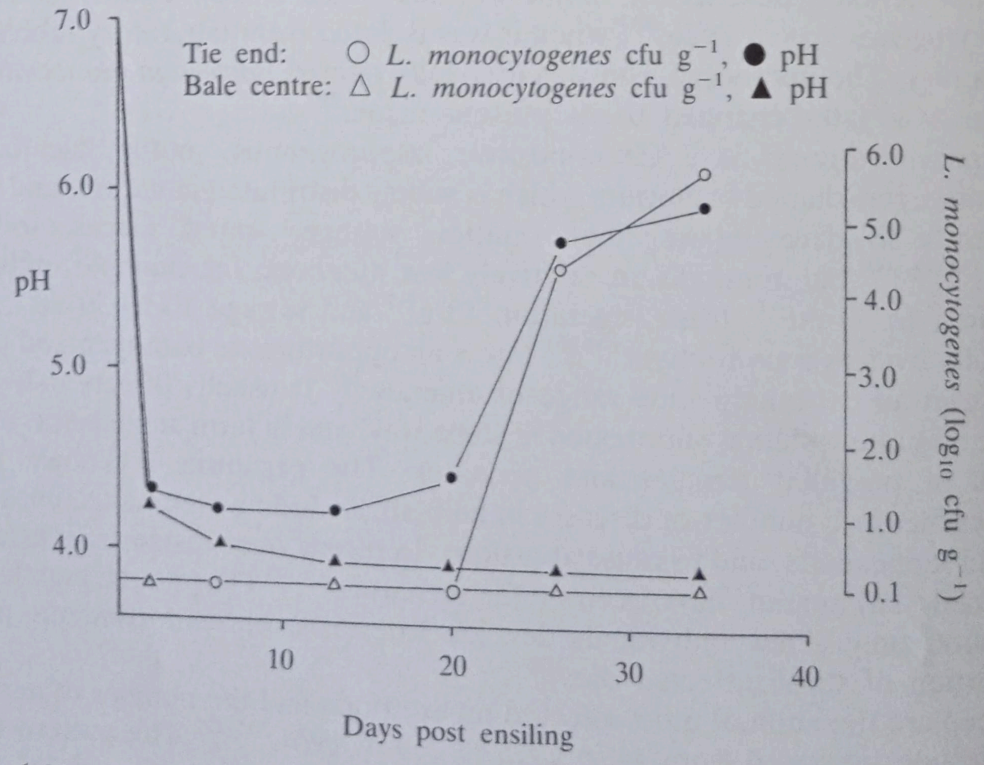


Figure 4.16. The effect of aerobic deterioration on silage pH and on the survival of *L. monocytogenes* in laboratory-scale bagged silage

concomitant low numbers being detected. However, in situations where oxygen filters in slowly over a long period, such as at the tie end of the bag, the relatively low oxygen concentration restricts acidification by lactic acid bacteria and also the growth of aerobes. Thus conditions are favourable for listeria and numbers in excess of 10^6 g^{-1} are common.[387] Such conditions exist only at the surface of the exposed silage and, although there may be high numbers of listeria here, only a few cm into the silage there may be no listeria detected.[387,410] In the example shown in Figure 4.16, a rapid reduction in pH had initially inhibited the growth of any listeria present but air had slowly entered from the tie end, permitting slow growth of aerobic organisms, which had brought about an increase in pH at the surface of the bale, allowing the listeria to grow and to reach high numbers. It is recommended that silage showing signs of aerobic deterioration should be discarded, not fed.[387,412]

Gray and Killinger[386] stated that listeria do not survive below pH 5.6. This was confirmed by Irvin[413] who adjusted the pH of a grass silage medium using hydrochloric acid and sodium hydroxide and incubated it at 4°C. He concluded that the critical pH below which strains failed to multiply was 5.5. George *et al.*[414] investigated the ability of 16 strains of *L. monocytogenes* to grow in a nutrient medium acidified with hydrochloric acid and incubated over a range of temperatures. They found that the minimum pH values at which growth was detected at 30, 20, 10, 7 and 4°C were 4.39, 4.39, 4.62, 4.62 and 5.23 respectively. It must be recognised, however, that the medium was very nutritious and it is probable that in silages, where there are high levels of fermentation acids etc., the limiting pH values are likely to be significantly higher than these. Farber *et al.*[415] found similar results with hydrochloric acid *in vitro* but inhibition was greater with organic acids, especially acetic, the minimum pH for growth at 30 and 40°C being 5.0 and 5.6 respectively. They explained this as being due to the fact that a larger proportion of the acetic acid would be undissociated at these pH levels, which is the form of the acid with the most effective antimicrobial action. Fenlon[416] stated that listeria could not survive at pH levels below 4.5 and at pH 5.0 growth was slow. He also suggested that it was unlikely they would be able to grow well even at higher pH levels unless oxygen was present.

L. monocytogenes is considered unlikely to develop or survive in well-preserved silages of low pH value.[411,413,417,418] However, in a study of listeriosis outbreaks in goats, sheep and cattle fed on silage, Fensterbank *et al.*[419] found listeria in 11 out of 31 well-preserved silages with pH values between 3.6 and 4.0. Recently, Fenlon[389] demonstrated that in fact listeria seemed to be able to survive in silage for long periods at pH levels as low as 3.8, providing oxygen was present, but they were rapidly killed at pH 4.2 if anaerobiosis was maintained. Listeria can survive an alkaline pH of 9.[420]

Listeria are unusual in being able to grow at very low temperatures. They grow well at refrigeration temperatures (4°C)[386] and, according to Bergey's Manual of Systematic Bacteriology,[50] the lower limit for growth is 1°C. Nevertheless, *L. monocytogenes* is not considered to be a psychrophile. Rather, it is considered to be a rapidly growing mesophile with an unusually low minimal temperature and a growth rate at 10°C typical of psychrophiles.[421]

Listeria are capable of surviving in high DM silages[389] which, because of a restricted fermentation, tend to have a higher pH than silages of lower DM. If aerobic deterioration occurs the pH increases and oxidation reactions produce

water, decreasing the DM. Both of these factors encourage subsequent growth of listeria. Later cuts of grass for silage are more susceptible to listeria because the a_w of later cuts is greater even if the DM is not significantly different.[269] This is because, as the season progresses, the proportion of soluble sugars decreases and the proportion of relatively inert fibre increases.

Although other species of listeria occur in silages, *L. monocytogenes* usually predominates.[387] Four other species are known to cause infections in animals and man but the proportion of cases due to species other than *L. monocytogenes* is very low.[395]

L. monocytogenes has also been found in milk.[422] Contamination may be from silage, faeces, or mastitis caused by this organism and it may result in very high numbers of listeria in the milk.[423] Pasteurisation will normally destroy the bacteria,[424,425] but in certain soft cheeses they may remain in high numbers due to the method of cheese production having a similar selective effect to that of ensiling.[422,426-430] In soft cheeses numbers as high as 10^5 have been found,[431] and *L. monocytogenes* has been reported to survive for more than one year in soft cheese stored at 4°C.[432]

Since listeria are widespread in nature, it seems likely that much of the herbage utilised for silage contains small numbers of listeria, which may develop if conditions become suitable. Fenlon[416] has shown that *L. monocytogenes* is present in very small numbers on freshly cut grass. One of the possible sources of these listeria is thought to be seagulls which have been feeding on sewage,[412] since the latter is a source of listeria[407] and most of the sewage in the UK is discharged untreated. The most effective method of preventing growth of *L. monocytogenes*

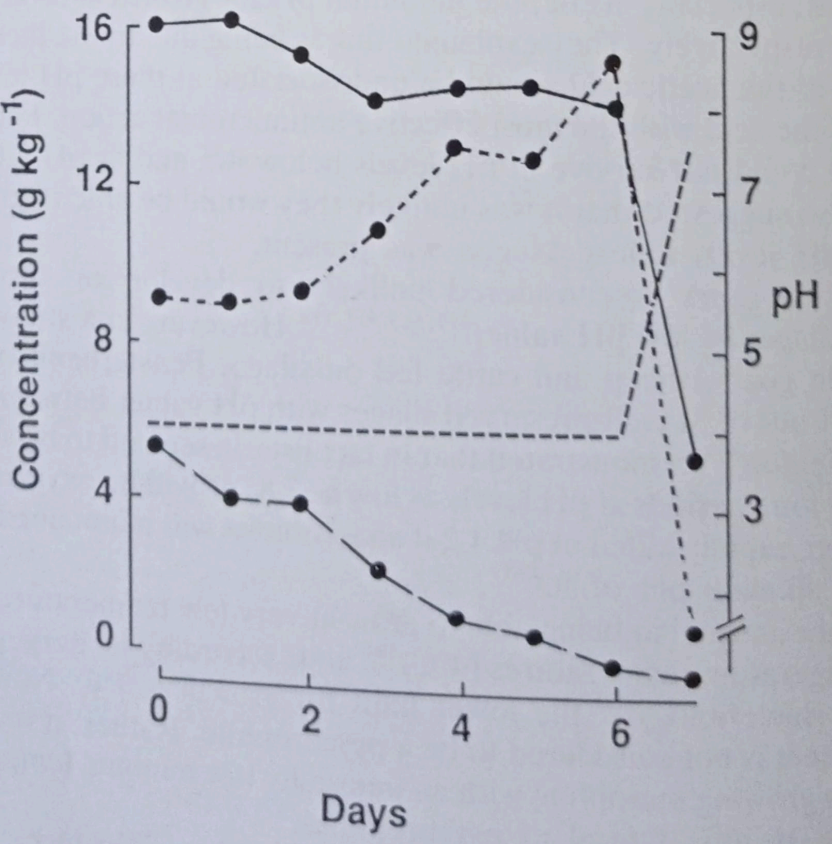
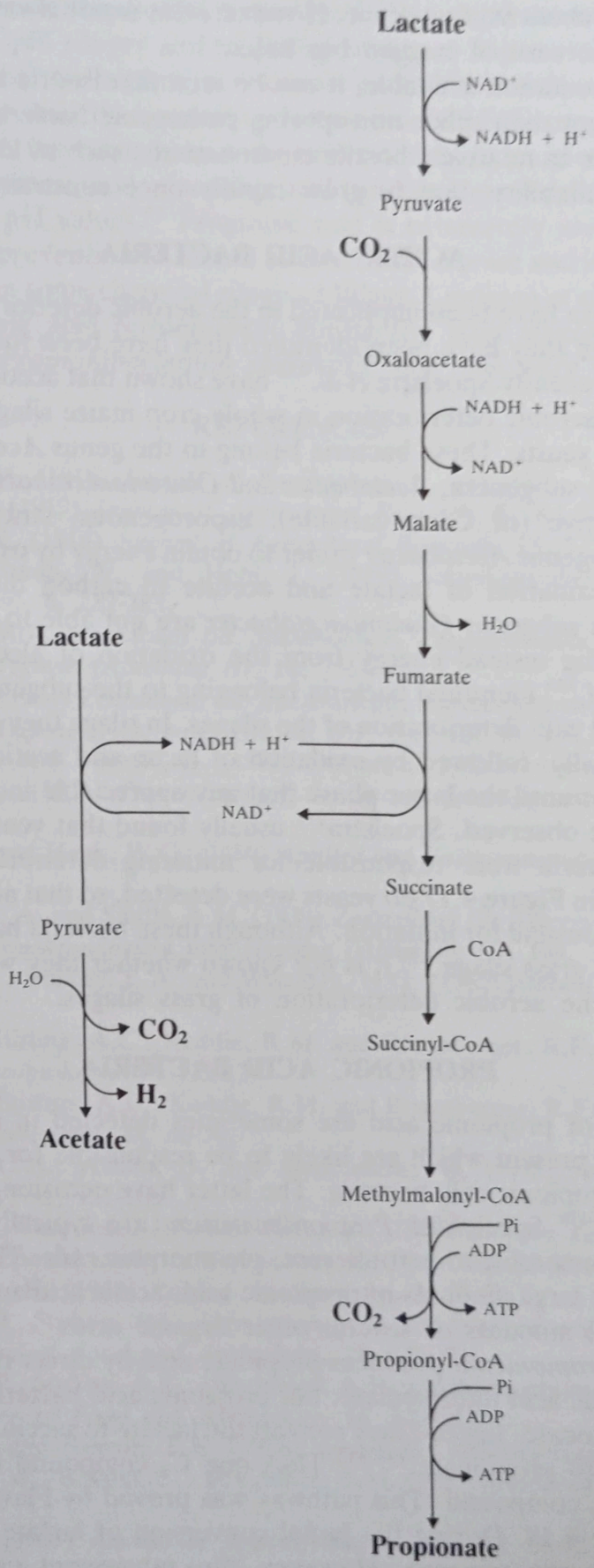


Figure 4.17. Changes in the pH (– – –) and concentration of lactic acid (●——●), acetic acid (●- - -●) and ethanol (●— —●) in maize silage during exposure to air[433]



Sum: 2 Lactate + 2ADP + 2Pi → Propionate + Acetate + CO_2 + H_2 + 2ATP + $2H_2O$

Figure 4.18. Fermentation of lactate by propionic acid bacteria[54]

in silage is to prevent ingress of air. However, this is not always easy to achieve, especially in the case of bagged big bales.

From the information available, it can be seen that listeria are a much greater potential problem than other non-sporing pathogenic bacteria because of their ability to survive in relatively hostile environments, such as low pH, low a_w and low oxygen availability, and to grow rapidly once constraints are removed.

ACETIC ACID BACTERIA

Although bacteria have been implicated in the aerobic deterioration of silages, in most cases where they have been identified they have been found to be *Bacillus* spp. However, recently Spoelstra *et al.*[433] have shown that acetic acid bacteria are able to initiate aerobic deterioration in whole crop maize silage with or without the presence of yeasts. These bacteria belong to the genus *Acetobacter,* which is divided into two subgenera, *Acetobacter* and *Gluconoacetobacter.*[434] *Acetobacter* are Gram-negative (or Gram-variable), asporogenous, strictly aerobic rods. Those in the subgenus *Acetobacter* prefer to obtain energy by oxidation of ethanol to acetate or oxidation of lactate and acetate to carbon dioxide and water. Members of the subgenus *Gluconoacetobacter* are not able to oxidise lactate or acetate, obtaining instead energy from the oxidation of alcohols and sugars.

Spoelstra *et al.*[433] identified bacteria belonging to the subgenus *Acetobacter* as being associated with deterioration of the silages. In silage they oxidise ethanol to acetic acid initially, followed by oxidation of lactic and acetic acids to carbon dioxide. It is not until the latter phase that any appreciable increases in pH and temperature are observed. Spoelstra[211] usually found that yeasts, together with acetic acid bacteria were responsible for initiating deterioration, but in the example shown in Figure 4.17 no yeasts were detected, so that acetic acid bacteria alone were responsible for initiation. Although these bacteria have been detected in effluent from grass silage,[435] it is not known whether they will prove to be of importance in the aerobic deterioration of grass silages.

PROPIONIC ACID BACTERIA

Small amounts of propionic acid are sometimes detected in silages. The only microorganisms present which are likely to be responsible for its formation are clostridia and propionic acid bacteria. The latter have occasionally been isolated from silages.[436-438] Species of *Propionibacterium* are typically Gram-positive, asporogenous, anaerobic to aerotolerant, pleomorphic rods. Their fermentation products include large amounts of propionic acid, acetic acid and carbon dioxide as well as small amounts of several other organic acids.

Clostridium propionicum produces propionic acid by direct reduction of lactic acid *via* an acrylic acid intermediate, but propionic acid bacteria cannot convert acrylate to propionate. Instead they convert the lactate to succinate, which is then decarboxylated to propionate.[439-442] Thus one C_3 compound is converted into another *via* a C_4 compound. This pathway was proved by Flavin *et al.*[443] and is shown in Figure 4.18. During the initial conversion of lactate to pyruvate two hydrogen atoms are released. However, the subsequent carboxylation and reduction of the pyruvate to give succinate requires a total of four hydrogen atoms. The extra two hydrogens are obtained from the conversion of a further molecule of lactate to pyruvate, the latter subsequently being decarboxylated to acetate.

Although species of *Propionibacterium* have been shown to ferment lactate to propionate in low pH silages and to prefer lactate to sugar as a substrate, it is thought unlikely that the activity is intensive enough to result in spoilage of the silage,[437] especially since their action on lactate involves little or no loss of acidity. On the other hand, there has been some interest in using propionic acid bacteria as a source of propionic acid, since this acid (undissociated) prevents the growth of moulds at low pH values.[58] Propionic acid is extensively used as a standard treatment for the prevention of mould in moist stored grain and it is incorporated in small amounts in some chemical silage additives. Lindgren *et al.*[65] attempted to initiate a propionic acid fermentation during the later stages of ensiling by inoculating with *Propionibacterium shermanii*, but they were unsuccessful.

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Chapter 5

Oxygen

The importance of achieving and maintaining anaerobic conditions during the ensilage of forage has been stressed in previous chapters, as it is only under these conditions that lactic acid bacteria can dominate the fermentation. Ideally a crop for silage should be harvested and immediately ensiled in an air-tight environment. This is not always possible in practice because many crops contain too much moisture and have to be wilted in the field prior to ensiling, and most farm silos are of such a size that they cannot be filled in one day. Several days, or in some cases even several weeks, may elapse before the silo is effectively sealed.

Four aerobic phases can be identified in the ensiling process:

1. The field phase;
2. The initial aerobic phase in the silo;
3. The air-infiltration phase;
4. The aerobic deterioration phase after opening.

THE FIELD PHASE

Reference was made in Chapter 3 to the biochemical changes which occur during the wilting of a crop immediately after harvesting. The main nutrients affected as a result of the presence of oxygen are the water soluble carbohydrates which, *in toto*, show only small losses from respiration over a 48 hour wilting period but generally show considerable changes in individual components. For example, sucrose is catabolised to carbon dioxide, fructans decrease and some pentoses are liberated from hemicelluloses (p. 56).

During field wilting, changes in the microbial population of the crop also occur. The population of lactic acid bacteria on the growing crop is often small (p. 88), but these facultative anaerobic organisms can grow in the presence of oxygen and multiply quite dramatically if the herbage has been damaged and the plant sap has been released.[1] The majority of the epiphytic bacteria on the fresh crop are, however, strict aerobes[2] and these can be expected to multiply during this first aerobic phase, the extent depending upon the degree of laceration of the crop and the period of wilting. There is some evidence also to indicate that the yeast population increases during wilting,[3,4] especially if the weather is humid and warm,[5] and this may have an adverse effect on the stability of the silage during the fourth aerobic phase. Development of the enterobacteria is favoured by warm weather and high relative humidity.[5] Wilting is unlikely to affect clostridia because they exist on herbage in the spore form only. If wilting is prolonged and carried out under adverse weather conditions, nutrient losses will be high (see Chapter 8).

THE INITIAL AEROBIC PHASE IN THE SILO

The amount of atmospheric oxygen trapped in a sealed silo is used up very rapidly by the respiratory systems in the plant[6-9] and the quantity of soluble carbohydrate metabolised is negligible.[7,10] Sprague[11] measured the oxygen disappearance in 2 metre diameter plastic silos filled with lucerne under field conditions and found that 90% of the oxygen was lost within 15 minutes and less than 0.5% remained after 30 minutes from the time of sealing. Loss was attributed to a very high rate of respiration continuing in the plant tissue after harvest. The loss of oxygen was more rapid in direct cut forage than in material which had been wilted for 2 hours under good weather conditions.

Studies by Greenhill[12] indicated that plant cell breakdown and the release of plant juices by plasmolysis is a prerequisite for the development of the lactic acid bacteria during the early stages of ensilage. The infiltration of even small amounts of air is thought to delay both plasmolysis and the onset of pH reduction. This is illustrated in the results of a laboratory study by Greenhill, shown in Table 5.1. At a temperature of 25°C the infiltrating air delayed cell breakdown by up to three days or more; at higher temperatures the delay was less, probably because of an increased rate of respiration. The effect of delay in cell breakdown was reflected in the higher pH values after 72 hours. Delaying collapse by only a few hours at the higher temperatures had a marked effect on the pH value, particularly with silage made from legumes.

Several workers have examined the effects of delayed sealing of a silo on the fermentation process. In most cases delayed sealing resulted in a reduction in the amount of lactic acid produced and frequently a normally dominant lactic acid bacterial fermentation was replaced by a clostridial one.[13-19]

Table 5.1. Effect of temperature and access to air on cell breakdown and fermentation[12]

Species	Ensiling temperature (°C)	Time to start of collapse (hours)		pH of silage after 72 hours	
		1*	2*	1*	2*
Ryegrass	25	5.0	>72	4.05	6.85
	30	3.0	72	4.15	6.15
	35	2.5	15	4.25	4.75
	40	2.0	4.5	4.00	4.50
Lucerne	25	5.5	72	5.05	8.15
	30	4.0	25	4.85	7.00
	35	3.0	10	4.80	6.40
	40	2.5	3.5	4.65	6.30
Clover	25	4.0	65	4.60	6.75
	30	2.5	30	4.50	6.15
	35	2.0	12	4.45	5.40
	40	2.0	4.0	4.40	5.40

* 1. Silo sealed immediately.
2. Infiltration of air permitted.

The effect of delaying sealing is to reduce the level of soluble carbohydrates available for fermentation and, unless adequate amounts are present in the original crop, insufficient lactic acid will be produced to inhibit the growth of clostridia.[20,21] With legume crops such as lucerne, which are consistently low in soluble carbohydrate content, a delay in sealing of as little as 12 hours can replace a lactic acid fermentation with a butyric acid one.[22] Similar results have been obtained using laboratory silos in which oxygen:herbage ratios have been altered above normal levels.[9,23-26] In one such study by Ruxton,[27] ryegrass was ensiled in sealed laboratory silos in which the silo atmosphere was replaced either by nitrogen or oxygen to give oxygen:fresh grass ratios (ml g^{-1}) of either 0.26 or 6.59.

The chemical composition of the resultant silages is shown in Table 5.2. At the low oxygen level, which corresponds to an air:herbage ratio of 1.2 (v/v), such as might occur in a well-consolidated silo, the silages were well-preserved and contained no butyric acid. At the high oxygen level (equivalent to an air:herbage ratio of 29.7) the silages produced were of high pH value and contained appreciable amounts of butyric acid and ammonia-N.

In addition to a reduction in the level of fermentable carbohydrates, other effects of aeration have been noted. The presence of oxygen in the silo encourages the development of yeasts[16,24,28-30] and causes an increase in the number of Gram-negative bacteria.[26,31,32] High concentrations of succinate have also been noted in aerated silages, and it is suggested that this acid may be formed from malate and citrate by the lactic acid bacteria at the higher pH levels normally found in such silages.[30,33]

The energy released by the respiration of carbohydrates is responsible for a rise in temperature of the ensiled herbage (p. 48). The final temperature in the silo will be dependent upon the quantity of air present and the insulating properties and specific heat of the herbage mass. In a sealed silo, assuming no heat loss to the environment and a specific heat capacity of 1.89 kJ $kg^{-1}°C^{-1}$ for forage DM, the rise in temperature (T) in °C of the ensiled herbage is given by:

$$T = \frac{1.002Y}{1 - 0.00055D}$$

where Y is the air:herbage (v/v) ratio in the silo and D is the DM content (g kg^{-1}).[10]

Table 5.2. The effect of different oxygen levels on some grass silage characteristics[27]

	Grass	Silages*	
		Low oxygen (0.26)	High oxygen (6.59)
pH	6.08	3.91	5.72
WSC (g kg^{-1} DM)	125	10	13
Acetic acid (g kg^{-1} DM)	—	26	54
Propionic acid (g kg^{-1} DM)	—	0	12
Butyric acid (g kg^{-1} DM)	—	0	32
Lactic acid (g kg^{-1} DM)	—	144	0
Ammonia-N (g kg^{-1} TN)	12	112	308

* After 154 days ensiling period. Figures in parenthesis are oxygen : grass ratios.

From the equation given it can be calculated that when the air:herbage ratio is 2, a value which might occur in a silo containing poorly compacted material, the temperature increase in a crop of DM content 200 g kg^{-1} would be 2.3°C. In a wilted crop of DM content 400 g kg^{-1}, the temperature rise under similar conditions would only be 0.3°C higher. Pitt and co-workers[34] have constructed a quantitative model of the ensilage process and have calculated the duration of respiration in the silo at different air:herbage ratios and different DM concentrations. For example, in ensiled timothy with a DM concentration of 260g kg^{-1} and an air:herbage ratio of 3:1, the duration of respiration in a sealed silo would only be 1.6 hours and the temperature rise would be 3.1°C. These calculated values agree well with those derived by Pitt *et al.*[34] from laboratory experiments. In very dry crops, which are difficult to consolidate, higher air:herbage ratios than those described above may occur, and with these materials it is even more important to fill the silo rapidly and seal immediately.

AIR INFILTRATION AFTER ENSILING

The amount of air which penetrates the silage during the storage period in the silo can have a marked influence on the composition of the final product and on the losses of nutrients. It is common practice with farm bunker silos to cover the surface with plastic sheeting and apply on top of this, consolidation weights consisting of soil, sandbags, tyres or straw bales. If surface consolidation is inadequate, air can penetrate the mass to varying levels depending upon the density of the crop, its physical structure and DM content.[35]

Table 5.3. The effects of different surface pressures on the composition, nutritive value and losses in ryegrass silages[36]

	Surface pressure	
	37 mbar	1.5 mbar
DM (g kg^{-1})	174	148
pH	3.7	4.1
TN (g kg^{-1} DM)	19.3	20.0
Protein-N (g kg^{-1} TN)	456	470
Ammonia-N (g kg^{-1} TN)	73	125
WSC (g kg^{-1} DM)	15	12
Crude fibre (g kg^{-1} DM)	289	299
Lignin (g kg^{-1} DM)	68	75
Lactic acid (g kg^{-1} DM)	115	56
Acetic acid (g kg^{-1} DM)	21	21
Butyric acid (g kg^{-1} DM)	1.8	23
Digestibility of organic matter*	0.74	0.67
Digestibility of nitrogen*	0.66	0.60
Losses of DM (%)	17.4	34.6
Maximum temperature in silo (°C)	26	44

* Using sheep.

The effects of inadequate consolidation were demonstrated by McDonald *et al.*,[36] using small tower silos of 1 tonne capacity in which low (1.5 mbar) and high (37 mbar) pressures were applied to the surface of ensiled unchopped ryegrass.

From the results of this study, summarised in Table 5.3, it can be seen that butyric acid, ammonia-N and cell wall components were higher and lactic acid and crude protein contents and organic matter digestibilities were lower in the badly-consolidated silage than in the well-consolidated material. Most striking of all were the differences in DM losses. About 35% of the DM was lost, mainly as surface waste, from the badly-consolidated material. The effects of this air penetration on temperature changes in the silage are shown in Figure 5.1.

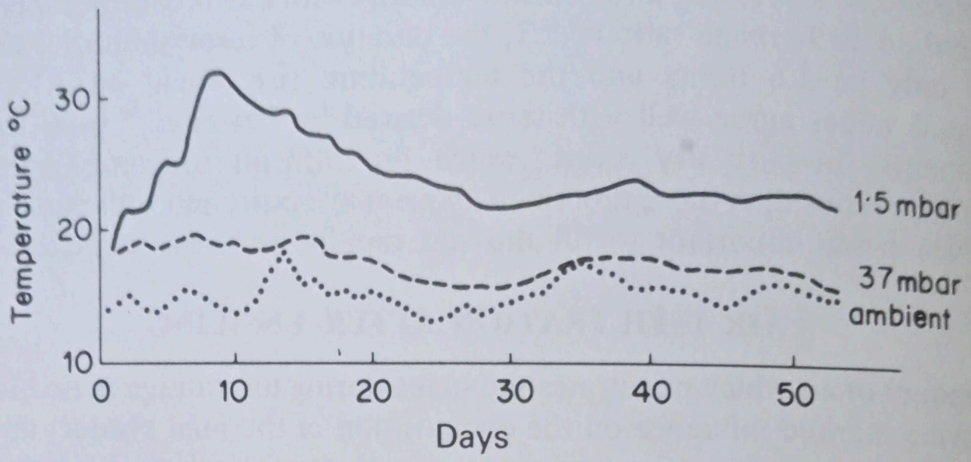


Figure 5.1. The effect of different surface pressures on the temperature changes in ryegrass silages[36]

Entry of air into the silo also renders the silage more susceptible to contamination with *Listeria monocytogenes*.[37] As this organism is an aerobe, it is likely to be found only in the surface layers of the silage clamp. There is some evidence to indicate that big bale silage may be more heavily contaminated with listeria compared to clamp silage because of the larger surface area to volume ratio.[38-40]

Oxidised waste material is more extensive in stack silos than in clamp or bunker silos, as the sides of the former are not usually protected from the atmosphere.[41-43] Air penetration is least in commercial tower silos because of the small area, relative to the whole, which is exposed to the air.[44]

The effect of heat on proteins

If precautions are not taken to prevent infiltration of air after ensiling by effective sealing, then temperatures in the silo may rise to levels which can bring about extensive chemical changes to the proteins.[45] New linkages within and between peptide chains are formed. Some of these linkages resist hydrolysis by proteases of the digestive tract and impede their access to adjacent peptide bonds.[46]

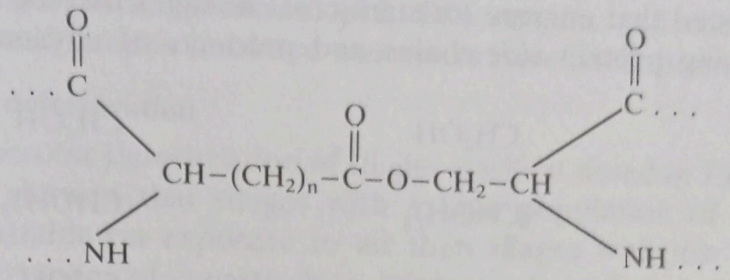
The decreased solubility and digestibility of heated proteins have been attributed to the formation of intramolecular amides and ester linkages. Three examples of possible intramolecular linkages have been suggested:

1. An ester link between the side-chain carboxyl group of aspartic or glutamic acid and the hydroxyl group of a hydroxyamino acid (e.g. serine or threonine).
2. A thioester link between aspartic or glutamic acid and the thiol group of cysteine.

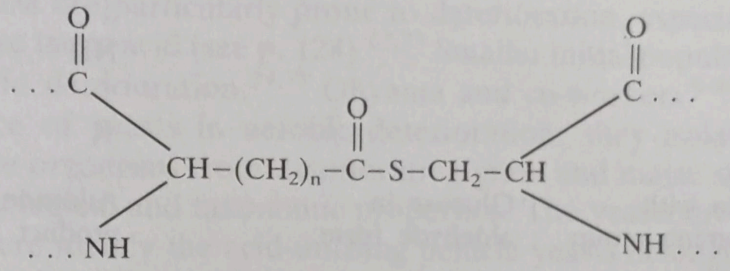
3. An amide link between asparagine or glutamine and aspartic acid or glutamic acid.

These examples are shown in Figure 5.2. Other possible reactions have been discussed by Bjarnason and Carpenter.[47]

(1) Ester link



(2) Thio ester link



(3) Amide link

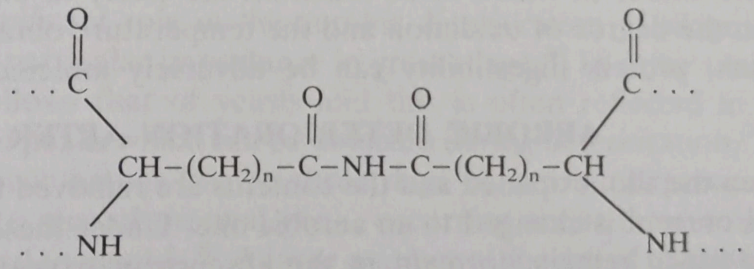


Figure 5.2. Three examples of intramolecular linkages in heated proteins[46]

The specific effect of heating on lysine has received considerable attention, and it has been suggested that a reaction occurs between the ϵ-amino group of this amino acid and carboxyl groups of aspartic and glutamic acids,[48] although Bjarnason and Carpenter[47] concluded that the reaction was more likely to occur with the amide group of asparagine or glutamine.

Susceptibility of proteins to heat damage is greatly enhanced in the presence of various carbohydrates, owing to the occurrence of Maillard reactions which result in the non-enzymic browning of foods during heating and prolonged storage.[49,50] Temperature has an important effect on the reaction rate, the rate being 9,000 times faster at 70°C than at 10°C.[49] Maillard reactions are complex and, as yet, not fully understood. The preliminary steps are shown in simplified form in Figure 5.3.

The first step in the reaction involves a condensation between the carbonyl group of a reducing sugar with the free amino group of an amino acid or protein.

In plant proteins most primary amino groups are represented by the ϵ-amino group of lysine. Under mild conditions of heat there is a fall in the level of available lysine.[51] In this type of reaction little actual browning occurs. With increasing severity of heat treatment, Maillard reactions go past the amino-deoxy-ketose stage shown in Figure 5.3, and amino acids other than lysine, such as arginine, tryptophan, cysteine and histidine, are also affected. It has been suggested that enzyme-resistant cross linkages may be formed through reactions involving protein side-chains and products of advanced Maillard reactions.[52]

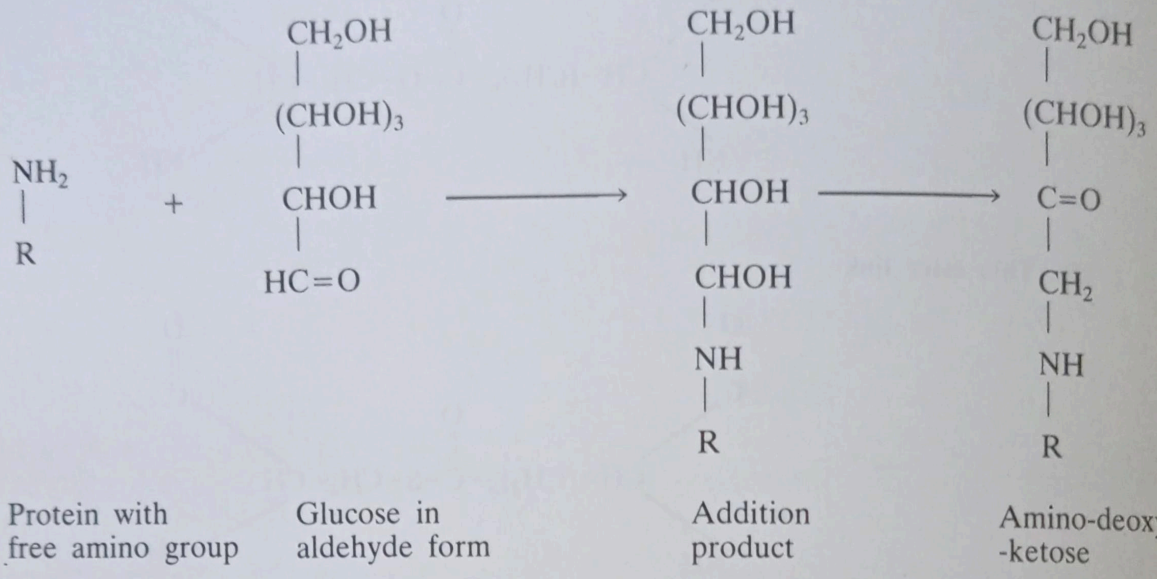


Figure 5.3. Preliminary stages of Maillard reactions

The extent to which these reactions are likely to occur in silage will depend upon the degree of oxidation and the temperature obtained. If excessive heating occurs, protein digestibility can be adversely affected.[41,53-56]

AEROBIC DETERIORATION AFTER OPENING

When the silo is opened and the contents are removed for feeding, the anaerobic environment is changed to an aerobic one. Under these conditions, microorganisms which remain dormant in the absence of oxygen multiply, resulting in a deterioration of the silage. On the farm this deterioration is usually manifested by a rise in temperature and by the appearance of moulds. There is considerable variation among different silages in the rates at which they deteriorate. Some silages show temperature rises a few hours after exposure to air, while other silages remain stable in the presence of oxygen for several days or even weeks. The deterioration process has often been referred to as 'secondary fermentation' or 'after fermentation', but these terms are inappropriate as fermentation, by definition, is an anaerobic process while deterioration of this type is always aerobic. These terms should only be used with reference to clostridial fermentations which generally follow an initial lactate fermentation.

The aerobic deterioration of silage is undesirable because of the high nutrient losses associated with it.[57] Generally, intake of deteriorated material is restricted or it is even rejected completely but, if consumed by animals, it can lead to a wide range of clinical diseases[40,58] and even death.[40,59,60]

Silages reported to be particularly prone to aerobic deterioration are carbohydrate-rich silages such as maize[59,61] and steamed potatoes,[59] those in which fermentation has been restricted by the use of additives,[62,63] and heavily

wilted silages.[9,41,64] During the past few years a considerable amount of research has been carried out in an attempt to identify the microorganisms and the biochemical changes which occur during this type of deterioration. Some sophisticated systems have been devised, consisting of sealed units through which controlled amounts of air can be passed and carbon dioxide production measured.[65,66] However, most studies have been carried out under laboratory conditions in which changes in temperature, pH, DM content and chemical composition in silages placed in open insulated containers have been monitored.

Microbiology of aerobic deterioration

The role of yeasts in the aerobic deterioration of silages was first noted in 1964 by Beck and Gross,[67] who showed that silages with a high population of these organisms were more unstable on exposure to air than silages with low yeast populations. The importance of yeasts has been confirmed by several workers.[37,68-71] The number of yeasts can attain the order of 10^{12} g^{-1} DM within three days of exposure of silage to air.[72] Silages with a yeast population of more than 10^5 organisms g^{-1} DM are particularly prone to deterioration, especially if the types present can utilise lactic acid (see p. 124).[68,73] Smaller initial populations have, however, resulted in deterioration.[74,75] Ohyama and co-workers[76-82] also confirmed the importance of yeasts in aerobic deterioration; they isolated a number of strains of these organisms from deteriorating grass and maize silages and examined their physiological and taxonomic properties. The yeasts involved in deteriorating silages were mainly the acid-utilising pellicle yeasts belonging to the genera *Pichia*, *Hansenula* and *Candida* although the sugar-utilising, sediment yeasts of the genera *Torulopsis* and *Saccharomyces* have also been implicated.[59,83]

Moulds also play a significant role in the aerobic deterioration of silage and have been found to be of particular importance in grass silage.[84] In some silages, the growth of moulds follows that of yeasts and this is often reflected in the appearance of two thermal peaks which can be detected during deterioration. The first peak, which may occur within two to three days of aerobic exposure, is caused by yeasts while the second thermal peak, occurring about three to four days later, can be attributed to moulds.[85] A large number of species of moulds has been isolated from deteriorated silages including members of the genera *Monascus*, *Geotrichum*, *Byssochlamys*, *Mucor*, *Aspergillus*, *Penicillium* and *Fusarium*.[79,86-89]

In early studies it was considered that bacteria played a secondary role to that of yeasts in the aerobic deterioration of silages, but extensive studies by Woolford and co-workers[37,65,90,91] have indicated that bacteria may play a more important part than was at first thought. Those responsible appear to be proteolytic species of *Bacillus*, although lactic acid bacteria may also be involved.[37] More recently, Spoelstra *et al.*[91] have demonstrated that acetic acid bacteria may have a role in the deterioration of maize silages.

Crawshaw *et al.*[92] showed that, in regressions of carbon dioxide production on counts of microorganisms in deteriorating grass silages, bacteria accounted for 48% of the variation in carbon dioxide production, moulds accounted for only 8% and yeasts for virtually none. In contrast, in an examination of 18 commercial grass silages, before and after aerobic deterioration, Henderson *et al.*[75] found that yeast counts increased markedly in 15 silages, whereas bacterial counts showed little or no change in half of the samples studied.

The microorganisms responsible for deterioration appear to be indigenous to the silage and are not aerial-borne invaders. Evidence for this conclusion was obtained in studies in which silage exposed to filter-sterilised air was shown to deteriorate as rapidly as similar material exposed to unfiltered air.[62] However, this study was carried out with silage aseptically removed from the silo and the possibility of contamination of well-preserved silage with surface deteriorated material during unloading cannot be ruled out.[4,93]

Biochemical changes during aerobic deterioration

Lactic acid, acetic acid and WSC are the main sources of energy for the microorganisms involved in silage deterioration. The oxidation of these nutrients results in the production of carbon dioxide and water, with the evolution of heat. With the complete oxidation of glucose, the temperature rise (T) in the silage mass, assuming a specific heat capacity of 1.89 kJ kg^{-1} $°C^{-1}$ for the silage DM and no loss of heat to the atmosphere, is given by the expression:

$$T = \frac{DG}{267.5 - 0.147D}$$

where D is DM content (g kg^{-1}) and G is the amount of glucose (g kg^{-1} DM) oxidised.[10] For example, the temperature rise through the complete oxidation of sugars in an insulated silage of DM content 320 g kg^{-1} and sugar content (as glucose) of 50 g kg^{-1} DM would be 73°C.

In silages in which fermentation in the silo has been restricted by the use of chemical additives or by excessive wilting, glucose, fructose and some pentoses will be the main energy sources, whereas in untreated well-preserved silage the main product oxidised is likely to be lactic acid. The heat produced from the complete oxidation of lactic acid is marginally less (15.16 kJ g^{-1}) than from glucose (15.64 kJ g^{-1}),[94] and therefore the temperature increase from lactic acid oxidation in the above example would be slightly lower, about 70°C. In laboratory studies temperature rises of this magnitude have not been recorded and it is reasonable to assume that many microorganisms would be killed well below such high temperatures, although heat generated in the process could initiate a change to a more thermophilic population.[4] In most practical situations a large part of the heat produced through oxidation will be dissipated into the atmosphere, although temperatures as high as 50°C[59] and DM losses of 30%[95] have been noted in some silage deterioration experiments.

In the early stages of deterioration, crude fibre, crude protein and ash contents are unaffected and therefore tend to increase in the DM, while in the later stages crude fibre and sometimes crude protein decompose.[4] The metabolism of lactic and acetic acids by aerobic microorganisms results in an increase in pH, which is well correlated with temperature rise and also DM loss.[75,81,96] Amino acids are also catabolised[97] and the ammonia liberated after deamination can contribute to the pH rise.[62,74]

Several attempts have been made to identify the chemical characteristics of silages which are particularly prone to aerobic deterioration. The results of a comprehensive study of 50 grass silages carried out by Ohyama *et al.*[81] are shown in Table 5.4. The data suggest that high DM silages tend to have greater yeast activity during deterioration than low DM ones and that silages with high butyric

Table 5.4. Correlation coefficients between the values determined on grass silages immediately after opening and the values during or after a 7-day aerobic exposure period[81]

During or after aerobic exposure	At opening								
	DM	pH	Lactic acid	Acetic acid	Butyric acid	WSC	Yeasts	Moulds	Bacteria
Time for temperature to rise	−0.47**	−0.16	0.17	0.44**	0.36**	0.08	−0.58**	0.29**	0.29
Maximum temperature	−0.07	−0.19	0.25	−0.16	−0.35*	0.24	0.05	0.31*	0.02
Increase in pH	0.25	0.06	0.05	−0.34*	−0.25	−0.22	0.28*	0.13	−0.45**
Decrease in lactic acid	−0.20	−0.30*	0.41**	−0.06	−0.14	−0.19	0.02	0.30*	−0.20
Decrease in acetic acid	0.06	−0.55**	0.33	−0.44**	−0.70**	0.35*	0.25	0.25	−0.02
Decrease in WSC	0.12	0.01	−0.27	−0.05	−0.26	0.62**	0.50**	−0.25	−0.13
Yeast count after 7 days	0.63**	0.10	−0.44**	−0.56**	−0.38**	0.15	0.88**	−0.47**	−0.44**
Mould count after 7 days	0.26	−0.34*	0.13	−0.47**	−0.74**	0.58**	0.35*	0.11	−0.02
Bacteria (aerobic) count after 7 days	−0.17	0.11	0.25	0.27	−0.06	−0.21	−0.24	0.10	0.15

*$p < 0.05$.
**$p < 0.01$.
$n = 50$, except the values concerning WSC and bacteria, where $n = 40$.

acid contents are stable. The latter finding agrees with that of Henderson *et al.*,[75] who showed that the main products of clostridial activity (butyric acid and ammonia-N) were positively correlated with aerobic stability. It is well known that the higher volatile fatty acids have an inhibiting action on the growth of microorganisms[98-101] and, in addition to butyric acid, clostridia also produce isobutyric acid and isovaleric acid from the catabolism of amino acids (p. 115); these acids are known to have a beneficial effect in preserving silages exposed to air.[74] There appear to be very few other relevant relationships shown in Table 5.4. Neither lactic acid nor WSC content seems to be correlated with rate of deterioration, a conclusion also reached by Henderson *et al.*[75] in their study.

Methods of preventing aerobic deterioration

The most obvious and effective method of preventing aerobic deterioration is to ensure that silage is consumed by animals the same day as it is removed from the silo. Frequent removal also limits deterioration from the silo face. Unloading should be carried out without disturbing the layers of material remaining in the silo, and it has been shown that the use of a block-cutter rather than a fore-loader is to be preferred in this respect.[95] The rate of progress along the silo should be 10 to 30 cm day^{-1} and the rate should be greater in summer than in winter.[4,102] In this respect the design of the silo is important. Bunker silos should be as long and as narrow as possible to minimise the surface area of silage exposed to air.[5,88,103]

Treatment of herbage prior to ensiling is an important factor which subsequently influences the aerobic stability of silage. Prolonged wilting, slow filling and a delay in sealing are all procedures which can result in an increase in the population of aerobic microorganisms, which remain dormant until the silo is opened.[4,16,104,105] Continued air infusion into the silage during storage has a similar detrimental effect by encouraging the persistence of aerobic microorganisms.[4,24,90,105]

In studies with grass silages inoculated with and without a lactobacillus culture and perfused with different amounts of oxygen, Pahlow and Zimmer[106] found that oxygen stimulated the multiplication of yeasts. The bacterial additive restricted the development of these organisms and resulted in silages which were aerobically more stable, especially at the low level of oxygen treatment. The improved stability of these silages was attributed to a specific energy-requiring detoxification system of the yeasts against too high intracellular concentrations of organic acids. The energy requirement can be supplied by fermentable sugars, but if only organic acids are available then a sufficient amount of oxygen is also required for the yeasts to metabolise them.

The Dorset-wedge method of ensiling in bunker silos, which is now widely used by farmers in the UK, is an excellent technique of restricting the initial aerobic phase to a minimum.[107] In this system, the silo is filled in a series of wedges, each wedge having a sloping face up which the loading tractor runs. Use is made of plastic sheets to interrupt the circulation of convection currents during breaks in the ensiling operation. The silo is finally sealed with sheets of plastic fixed to the upper edges of the silo sides, and the various sheets are overlapped in such a way as to form a tight seal which sinks with the silage as it consolidates.

Although several workers have indicated that high DM silages are particularly susceptible to aerobic deterioration, there is some evidence[4] to suggest that

deterioration is reduced in silages with DM contents exceeding 500 g kg^{-1}.

Reference was made earlier to the importance of higher volatile fatty acids in inhibiting microbial growth, and several of these have been examined as potential silage additives, in particular isovaleric acid and caproic (hexanoic) acid. Both of these acids have been shown to have a growth inhibitory effect on the organisms responsible for aerobic deterioration of silage in laboratory studies.[74,78,80,84,101,108] The usefulness of these volatile fatty acids and other compounds as potential aerobic deterioration inhibitors is discussed in Chapter 7. Chemical additives, however, are unlikely to prevent completely the aerobic deterioration of silages and can only have a delaying effect on microbial activity.[109] The best methods of prevention lie in efficient management techniques which reduce aerobic exposure of silages to a minimum.

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Chapter 6

Water

The disadvantages of ensiling crops with very high moisture contents have been stressed in previous chapters. Firstly, the critical pH value below which clostridial growth is inhibited varies directly with the moisture content of the plant material and, unless soluble carbohydrate levels are exceptionally high, the ensiling of wet crops will encourage a clostridial fermentation, resulting in high losses and a silage of low nutritional value. Secondly, even if soluble carbohydrate levels are adequate to ensure a lactate fermentation, very wet silages may still be nutritionally undesirable because voluntary DM intakes of these silages are frequently low.[1-5] Thirdly, the ensiling of wet crops results in the production of large volumes of effluent, which are not only difficult to dispose of, but also carry with them, in solution, valuable highly digestible nutrients. Finally, drier crops are preferred because they are easier to handle and a higher quantity of DM can be carried per trailer load.[6]

The main method of reducing the moisture content of crops is by field wilting, although other treatments such as steam heating or spraying with desiccants have also been proposed.

REDUCTION OF MOISTURE CONTENT BY WILTING

Grasses and legumes are the commonest crops which are field wilted and the first stage of this process consists of cutting the crop with a mower or a mower/conditioner, leaving the herbage in the form of a swath. The loss of water by evaporation from the swath is dependent upon a number of factors, which can be considered under three main headings: plant, natural environmental and mechanical treatment.

The **plant factors** include the stomatal behaviour, the nature of the cuticle and the swath characteristics.[7] In the very early stages of wilting, the greater part of the water loss is through the stomata with only a very small loss through the cuticle.[8] The number of stomata present in plants varies with different species; grasses, for example, have fewer stomata on their leaves than lucerne.[9] Loss of water *via* this source is, however, relatively transient as studies have shown that 0.5 to 2 hours after cutting the stomata become tightly closed.[10,11] After this initial period, loss of water from the plant is then mainly through the cuticle.

The cuticle covers the outer walls of the epidermal cells and consists of several layers — wax, cutin, pectin and the cellulose of the periclinal epidermal wall.[9] The functions of the cuticle include the prevention of loss of plant nutrients by leaching, the protection of the plant from physical abrasion, frost and radiation, and the conservation of water.[9] Loss of water *via* the cuticle is controlled by its thickness.[8] Jones and Prickett[12] compared the rates of water loss from tall fescue (*Festuca arundinacea*), timothy, perennial ryegrass and Italian ryegrass and found

that tall fescue dried faster than the other species. They suggested that this species had a lower cuticular resistance to water loss than the other species.

The shape and size of the swath are also important in influencing the rate of moisture loss during wilting, and there is considerable stratification of swath DM content during the drying period. The lower levels of the swath dry much more slowly than the upper ones because of the poor levels of available radiation, the low vapour pressure deficit in the swath interior and the long tortuous resistance pathway through the herbage mass.[7]

The **natural environmental conditions** are of particular importance in controlling the rate of moisture loss.[13-15] In studies with wilting coastal Bermuda grass (*Cynodon dactylon*), Hart and Burton[13] reported relationships between moisture content and weather factors such as solar radiation, vapour pressure deficit, windspeed and rainfall. In other studies with pasture dominated by ryegrass and white clover, the air vapour pressure deficit and intensity of solar radiation were well correlated with the drying rate, but sunshine hours were less well correlated and windspeed had apparently no significant effect.[14]

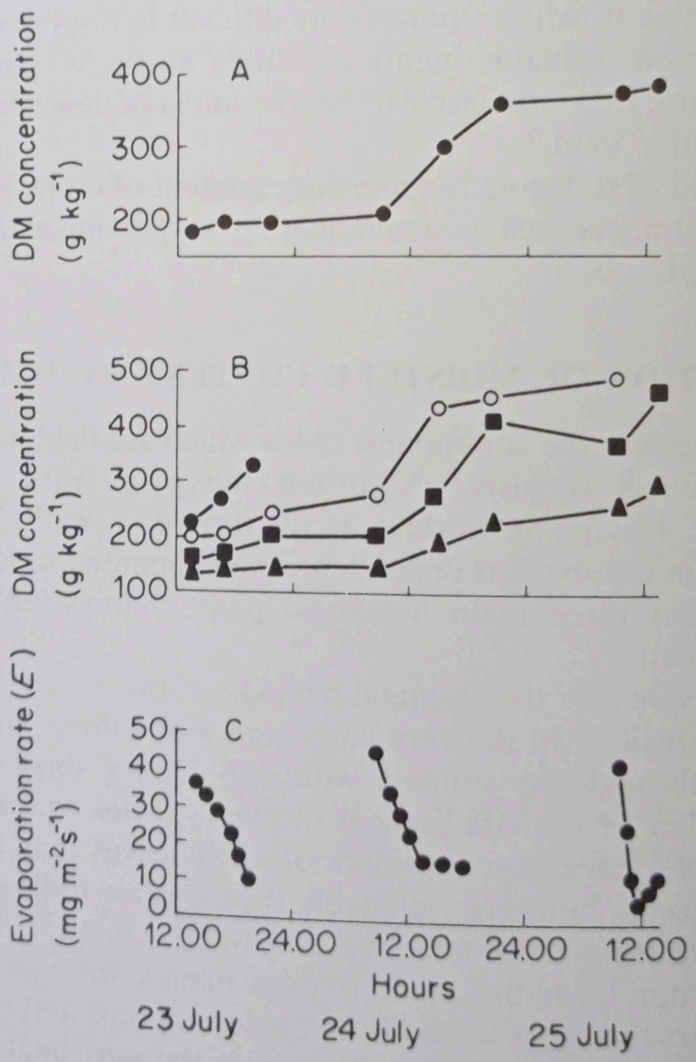


Figure 6.1. Swath drying pattern during a 48 hour wilting period, July 23-25[7]

A. Mean swath DM concentration.

B. Stratified swath DM concentration, ● = 20 + cm layer; ○ = 15-20 cm layer; ■ = 10-15 cm layer; ▲ = 5-10 cm layer of swath above ground level (0-5 cm layer, grass stubble below the swath).

C. Swath evaporation rate, *E*.

It has long been known that **mechanical treatment** such as turning and tedding (shaking-up) the swath increases the drying rate. The sixteenth-century English writer Fitzherbert, in his *Boke of Husbandry* (1523), noted that 'good teddying is the chief poynte to make good hay,'[16] Unfortunately, loss of moisture in crops is not uniform, the leaves tending to lose moisture more rapidly than the stems.[8,17] In this respect the use of conditioners, which rupture the cells, speeds up the rate of drying by increasing the evaporating area.[8,18,19]

The width of the swath is also an important factor influencing the drying rate. In a study by Done and Appleton,[20] two mower/conditioner systems were compared, one selected to leave a normal swath width of 0.9 metre and one modified to leave a swath width of 1.3 metres. After 48 hours of wilting, the mean DM contents of samples from the two swaths were 246 and 290 g kg^{-1} respectively.

Although conditioned crops lose moisture more rapidly than untreated ones, they also absorb it more readily during periods of rain and are more susceptible to loss of soluble nutrients by leaching.[21,22]

A typical pattern of moisture loss from a ryegrass swath over a 48-hour wilting period in the UK is shown in Figure 6.1.[7] The variations in swath evaporation rates throughout the second day (July 24) of wilting can be explained as follows: between sunrise and 09.00 hours, energy from solar radiation is relatively low and is used entirely for sensible heating of the swath mass. Ambient vapour pressure deficit is zero or very low, and swath resistance to evaporation is also low. During this early phase, evaporation of moisture from the swath is limited primarily by the poor evaporating power of the air.

Between 09.00 hours and 12.00 hours, radiation energy increases to a maximum and a high proportion of this is used for latent heat of evaporation, giving a high swath evaporation rate which includes the evaporation of any external surface water (dew or rain) and internal plant water. During this phase, vapour pressure deficit increases to maximum values and swath resistance is low, but begins to increase slowly. During the morning, therefore, the high swath evaporation rate results from the high levels of available energy and ambient vapour pressure deficit and the low resistance of the swath.

Between 12.00 hours and 18.00 hours, the energy available from solar radiation decreases and the proportion used for latent heat of evaporation also decreases markedly, indicating a shift in the swath energy balance in favour of sensible heat. Ambient vapour pressure deficit remains high and swath resistance increases sharply. During the afternoon, therefore, the decrease in swath evaporation rate can be explained by the reduction in available energy and the increased resistance of the swath to evaporation.

Between 18.00 hours and sunset, the swath evaporation rate is near zero, largely because of the very high swath resistances. Overnight, available energy, and hence swath evaporation rate, is zero.

Chemical desiccation and thermal treatment

The loss of moisture from crops by natural wilting is frequently slow in temperate climates because of plant resistance and unfavourable weather conditions. An alternative method of removing moisture is to treat the standing crop with a chemical desiccant.[23-29] The concept of using chemicals to increase the drying rate

of plants is not new. Columella (*c.* AD 60) recommended the use of a lye made from the ash of vine canes with a little olive oil to increase the drying rate of grapes, a preparation that is still used in Greece.[9]

Several workers have investigated the possibility of using herbicides to increase the drying rate of grasses.[22,30-35] Shepherd[32] reported that spraying grasses and legumes with diquat (9,10-dihydro-8a,10a-diazoniaphenanthrene-2A) at the time of cutting resulted in a rapid loss of water from the cut crops. Craven *et al.*[33] and Black *et al.*[34] showed that application of diquat to a standing crop of grass, before harvest, reduced its moisture content. Wilkins and Tetlow[35] examined the effect of diquat and paraquat (1,1'-dimethyl-4, 4'-bipyridylium-2A) on standing crops of maize but obtained only small reductions in moisture content, and desiccant application appeared to reduce crop yield. They concluded that diquat and paraquat had no useful role in the treatment of maize before ensiling.

More encouraging results have been obtained with the lower volatile fatty acids, formic, acetic, and propionic acids.[28,29,36,37] The results of some studies indicate that there is no difference between these acids,[38] although others suggest that formic acid is the most effective desiccant of the three.[27,39] The acids, which are usually applied to the standing crop at a rate of 5 to 10 g kg^{-1},[38] appear to increase transpiration by reacting with the plant cuticular waxes and by damaging the cuticle and deeper tissues.[22] Unfortunately, when applied under poor weather conditions, loss of these acids from the plants can be very rapid.[22,38] Furthermore, removal of the cuticular waxes from the plant allows greater penetration of the tissues by rain, resulting in losses of soluble nutrients, including WSC, in treated herbage.[40]

Another method of increasing moisture loss is to use thermal treatment. In the procedure described by Philipsen,[41] a mixture of hot gases and steam at 300°C is directed under a canopy which is passed over the surface of the standing crop. The herbage is allowed to remain under the canopy long enough to bring the temperature of the aerial parts of the crop up to 70°C. It has been shown that the cuticular wax is removed by this steam treatment, and loss of moisture is thought to be increased by cracking of the cuticle.[24]

Calder[42] compared silage made from steam-treated grass with that made from untreated grass. The steam-treated forage was allowed to stand for 2 hours before being harvested; the untreated grass was cut and ensiled without delay. The silage made from the treated material had a DM content of 293 g kg^{-1} and an NPN content of 21.4 g kg^{-1} TN, compared with corresponding values of 261 g kg^{-1} and 27.5 g kg^{-1} TN for the untreated silage. When the silages were given to cattle, there was an increase of 57% in weight gains resulting from steaming of the standing grass sward prior to ensiling.

In studies with lucerne silages, Charmley and Veira[43] found that heat treatment increased the amount of non-ammonia nitrogen flowing at the duodenum in sheep, but had no effect on nitrogen digestibility.

Comparison of steam treatment with formic acid suggests that the former is more rapid in promoting water loss,[29] probably because the steam penetrates deeper into the plant tissues.[41]

EFFECT OF MOISTURE CONTENT ON FERMENTATION

The amount of moisture present in the ensiled crop affects the total bacterial

count and the rate of fermentation.[44] Wilting delays bacterial multiplication in grass silage,[45,46] while the addition of water to herbage initially stimulates the growth of bacteria, especially the lactobacilli and the Gram-negative organisms.[47] Wilting can also influence the relative growth of homofermentative and heterofermentative lactic acid bacteria during ensiling. Beck[48] studied the qualitative changes in lactobacilli during the fermentation of grass and red clover with low and high DM contents and found that, at the end of a 142-day ensiling period, 75% of the total lactobacilli in silage with a low DM content and 98% of the lactobacilli in silage with a high DM content were heterofermentative species.

If the soluble carbohydrate levels are very high in wet crops, then the lactic acid bacteria will be extremely active and the result will be a low pH silage of high lactic acid content. This is well-illustrated in the study by Morgan *et al.*[49] in which silages were made from freshly cut or wilted ryegrass-clover with a WSC content of 193 g kg^{-1} DM (Table 6.1). In this experiment, wilting the crop from a DM content of 175 g kg^{-1} to about 360 g kg^{-1} decreased the lactic acid content of the silage from 165 g kg^{-1} DM to 34 g kg^{-1} DM; the material preserved well at a pH of 5.09 compared with the unwilted silage which had a pH of 4.00. The effect of decreasing the moisture content on the activity of the lactic acid bacteria and other microorganisms was reflected in the high residual amounts of soluble carbohydrate in the wilted silage. Other fermentation products, especially acetic and butyric acids, were also relatively low in the wilted silage. Similar, but in some instances less extreme, effects of varying the moisture content in silage crops have been noted by other workers.[4,50-59]

In a study by Jackson and Forbes,[54] a mixed sward of timothy-meadow fescue and white clover was ensiled at four different DM levels, after wilting in the field for a maximum period of 48 hours. The results indicated that fermentation was restricted as DM content increased. The decrease in fermentation was reflected in higher pH and WSC values and, generally, in lower levels of lactic, acetic and butyric acids. Wilting had no marked effect on the protein-N contents of the silages, a finding which agrees with that obtained in the study by Morgan *et al.*[49]

Table 6.1. Composition of two grass silages made from unwilted and wilted herbage[49]

	Unwilted silage	Wilted silage
DM (g kg^{-1})	169	359
pH	4.00	5.09
Organic matter (g kg^{-1} DM)	908	907
WSC (g kg^{-1} DM)	11	185
Modified acid detergent fibre (g kg^{-1} DM)	288	293
TN (g kg^{-1} DM)	24.8	22.4
Protein-N (g kg^{-1} TN)	286	286
Ammonia-N (g kg^{-1} TN)	109	80
Nitrate-N (g kg^{-1} TN)	12.1	8.9
Acetic acid (g kg^{-1} DM)	19.0	5.7
Propionic acid (g kg^{-1} DM)	0.54	0.24
Butyric acid (g kg^{-1} DM)	4.7	1.1
Caproic acid (g kg^{-1} DM)	0.21	trace
Lactic acid (g kg^{-1} DM)	165	34
Ethanol (g kg^{-1} DM)	23	10

Table 6.2. Prediction of silage effluent production from DM content of ensiled crop

Regression equation*	DM content at zero effluent production (g kg^{-1})	Reference
1. $Vs = 669.4 - 2.24D$	299	Sutter[60]
2. $Vn = 767 - 5.34D + 0.00936D^2$	290	Bastiman[62]
3. $W = 832.6 - 5.418D + 0.00883D^2$	307	Zimmer[61]

*Vs = Volume of effluent produced (litres $tonne^{-1}$ silage).
Vn = Volume of effluent produced (litres $tonne^{-1}$ herbage).
W = Weight of effluent produced (kg $tonne^{-1}$ herbage).
D = DM content of herbage (g kg^{-1}).

(Table 6.1). However, other studies[58] have indicated that protein-N contents of silages increase with increasing DM contents. Reduction in moisture content appears to inhibit the deamination of amino acids, which is presumably a result of a decrease in the activity of clostridia and enterobacteria.

EFFLUENT

Factors influencing the production of effluent

The volume of effluent produced from a silo is influenced mainly by the DM content of the ensiled crop, and a number of workers have derived regression equations relating effluent production to DM. Examples of these are shown in Table 6.2.[60-62]

The most recent of these equations is that produced by Bastiman,[62] which summarises the results of effluent measurements from 50 silages made in bunker-type silos over a 16-year period. The regression line from Bastiman's data has been plotted in Figure 6.2. It can be seen that very little effluent is produced from herbage ensiled with a DM concentration of 250 g kg^{-1}; the DM content at which it is predicted that no effluent would be formed is about 290 g kg^{-1}, a value which agrees reasonably well with the other predictions shown in Table 6.2.

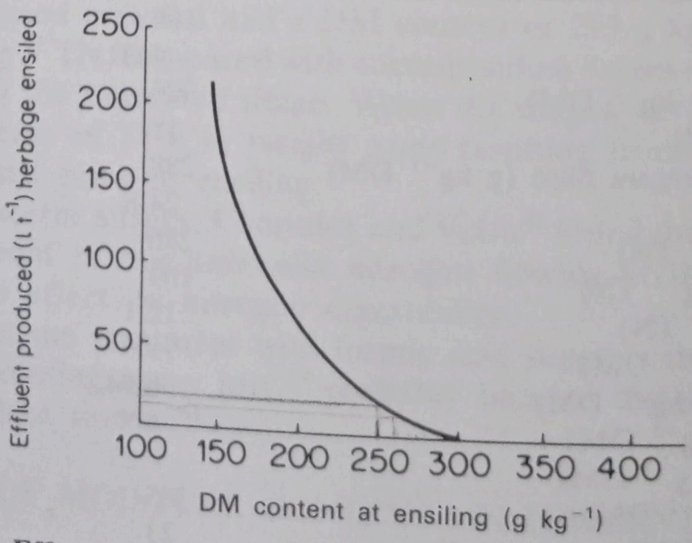


Figure 6.2. Effect of DM content at ensiling on effluent production[62]

The calculation of effluent flow from the DM content of the crop alone ignores several other important factors such as type and dimensions of the silo, degree of consolidation, and nature and pretreatment of the crop. In an attempt to consider some of these factors, Tang and co-workers[63,64] derived a linear relationship between the volume of juice expelled from the silage and the natural logarithm of the DM density of the silage, which they claimed allowed a more realistic prediction of the effluent losses from wet silage.

The amount of pressure applied to the surface of the ensiled crop can influence the amount of seepage from a silo. In one study using 1 tonne capacity tower silos filled with grass (199 g DM kg^{-1}), the effect of increasing the pressure applied to the surface of the herbage from 1.5 mbar to 37 mbar was to increase the total effluent production from 55 g kg^{-1} to 123 g kg^{-1} ensiled grass (Figure 6.3).[65]

Kirsch *et al.*[66] reported that a pressure of 800 g cm^{-2} (785 mbar) resulted in an amount of liquid which was ten times greater than that produced by an application of 80 g cm^{-2} (78.5 mbar); also, material of DM content 400 g kg^{-1} produced effluent when still greater pressures were applied. Waldo[67] stated that maize with a DM content of 300 g kg^{-1} could be ensiled in tower silos up to 12 metres high without the production of effluent, but the DM content had to be increased by 10 g kg^{-1} for every additional 3 metres of height if effluent production was to be avoided. It is clear that the type of silo is an important factor in influencing the amount of effluent produced and that pressures in tall silos will be considerably greater than those normally occurring in bunker silos.

The use of certain silage additives, for example those containing acids, can influence the volume of effluent produced. Pedersen *et al.*[68] found that losses of effluent from silages treated with formic acid, formic acid-ammonium formate, and formic acid-formalin were greater than those from untreated silages. Other workers have obtained similar results with formic acid.[69,70] Pedersen *et al.*[68] attempted to explain the increased effluent flow after the addition of formic acid

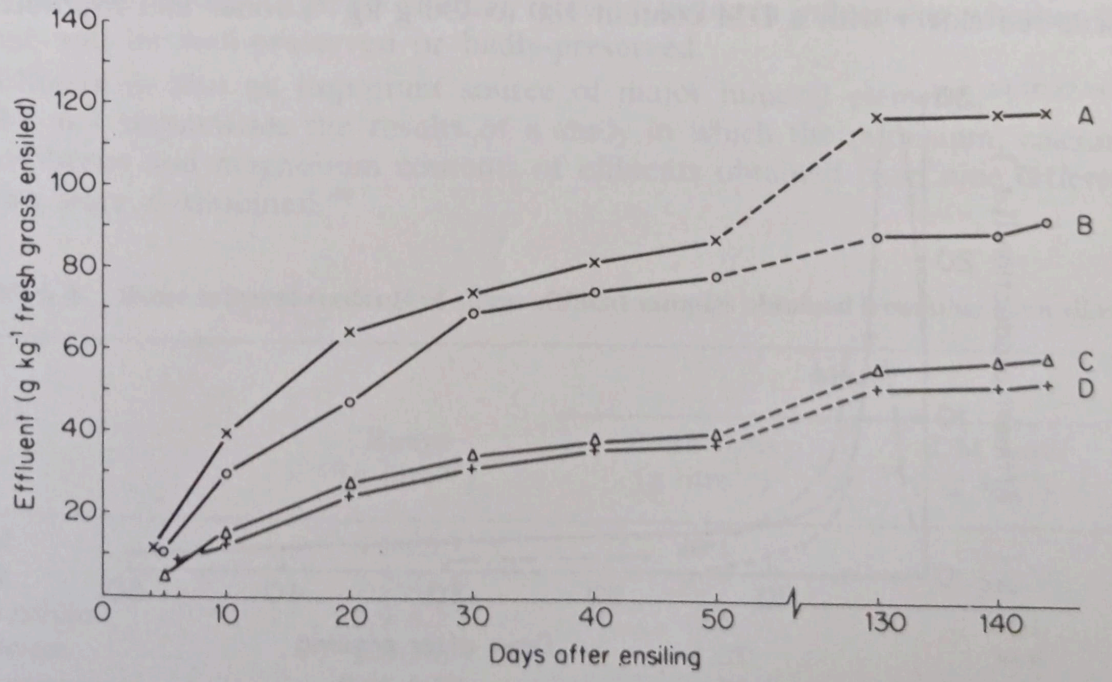


Figure 6.3. Effect of surface pressure on effluent production[65]
Surface pressures (mbar) A. 37; B. 23.7; C. 12.2; D. 1.5

to herbage by suggesting that the pH decrease might affect the lipophilic substances of the plant material. Under normal conditions fatty acids in the intact plant occur almost exclusively in the dissociated form. Addition of formic acid at the usual concentrations will depress the pH below the pK_a value for most fatty acids. The amounts of undissociated fatty acids will increase more than 50 times, and this could induce a general leakage from the cells. It is perhaps relevant, in support of this theory, that formic acid is known to induce a rapid release of cell sap from the fresh plant material.[68]

Most of the effluent flow from a silo occurs in the early part of the ensiling period. In a study of the pattern of effluent flow from 11 silages, Bastiman[62] found that peak flows occurred within the first week (Figure 6.4) and daily rates of up to 29 litres tonne^{-1} herbage ensiled were recorded for material with DM contents below 160 g kg^{-1}. Herbage containing 250 g DM kg^{-1} produced only about 45% of the total effluent in the first 20 days, while this rose to about 90% for material ensiled with a DM content of 160 g kg^{-1}.

Mechanical treatment of the crop prior to ensiling apparently influences effluent production, although the results of different studies are conflicting. In a comparison of bruised (flail harvested) and long cocksfoot grass of low DM content (140 g kg^{-1}), McDonald *et al.*[71] found that bruising increased effluent flow and more than doubled the DM loss in the effluent. Fine chopping has also been found to increase effluent flow.[72,73] Messer and Hawkins[73] examined the effect of chop length on ensiled maize of DM content 200 g kg^{-1} and found that effluent production was 53 litres tonne^{-1} fresh forage when the chop length was 6 mm, and 27 litres tonne^{-1} when the chop length was 32mm. When the DM content of the ensiled maize was 290 g kg^{-1}, effluent production was 7 litres tonne^{-1} irrespective of chop length.

In studies with sugar and fodder beet leaves, Zimmer[74] showed that the DM content of the effluent from the chopped leaves was higher than that from the unchopped ones. In a comparison of ensiled chopped and unchopped meadow fescue-red clover with a DM content 180 to 190 g kg^{-1}, Jones and Murdoch[75]

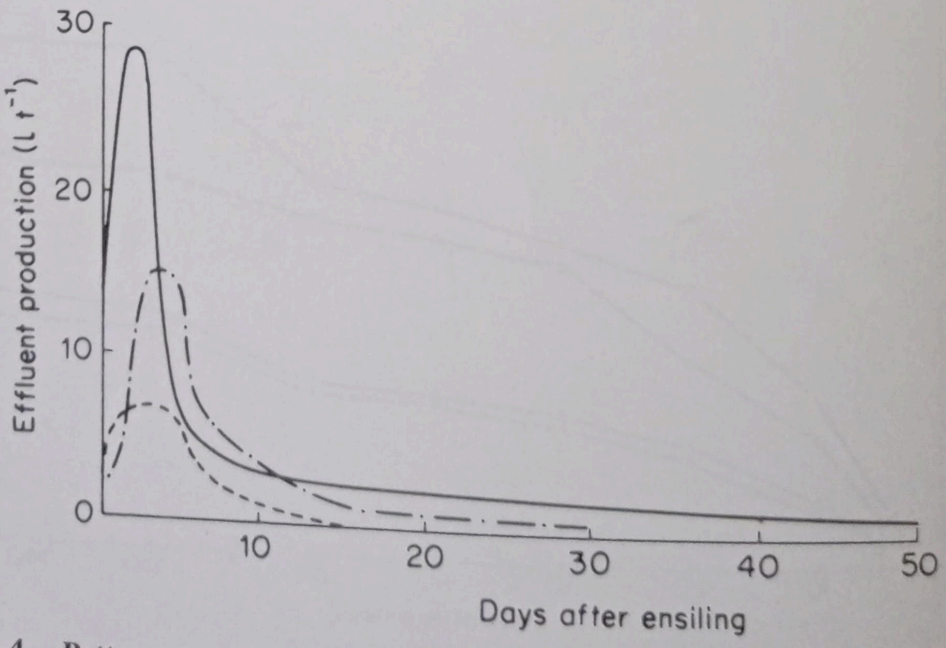


Figure 6.4. Patterns of effluent production[62]
Silage DM content (g kg^{-1}): ——— 158; — · — · — · — 182; - - - - - 219

Table 6.3. Composition of effluent obtained from ensiled Italian ryegrass (192 g DM kg^{-1})[65]

Day	pH	Composition of effluent (g kg^{-1})					
		DM	Total-N	Ammonia-N	Acetic acid	WSC	Ash
3	4.4	52.7	1.87	0.12	1.01	13.4	15.6
5	4.2	64.4	2.91	0.21	1.50	8.2	16.4
7	4.1	67.0	3.20	0.24	1.78	—	16.8
10	4.1	70.0	3.31	0.27	2.18	7.9	16.8
18	4.0	75.6	3.68	0.33	2.75	7.1	16.9
25	4.3	81.3	3.64	0.39	3.67	—	20.9
32	4.1	82.2	4.04	0.40	3.68	4.6	18.3
44	4.0	84.2	4.26	0.59	4.32	—	17.9
63	3.8	89.0	4.45	0.51	4.67	3.3	18.6

found that effluent production was markedly greater in the unchopped herbage than in the chopped material. It would appear from the above studies that the effect of mechanical treatment varies depending upon the DM content and possibly also on the species of crop ensiled.

Composition of silage effluent

The DM content of effluent ranges between 10 g kg^{-1} and 110 g kg^{-1} and increases during the ensiling period.[65,76-78] With the exception of soluble carbohydrates and possibly true protein, all major components of the effluent DM increase in concentration with time (Table 6.3),[65] especially the products of fermentation, such as lactic and acetic acids. Losses of soluble carbohydrates, particularly from wet crops, can be high[65,76] and this depletion of readily fermentable material can be another relevant factor in influencing whether the silage will be well-preserved or badly-preserved.

Effluent is also an important source of major mineral elements.[59,76,77,79,80] Table 6.4 summarises the results of a study in which the potassium, calcium, phosphorus and magnesium contents of effluents obtained from nine different farms were determined.[80]

Table 6.4. Some mineral contents of silage effluent samples obtained from nine farm silos[80]

	Range (g litre^{-1})	Mean	
		Fresh basis (g litre^{-1})	DM basis (g kg^{-1})
DM	6.1–83.7	40.4	—
Ash	2.2–21.5	12.1	299
Potassium	0.9–8.5	3.5	86.1
Calcium	0.2–3.6	1.25	30.9
Phosphorus	0.06–0.72	0.32	7.9
Magnesium	0.05–0.57	0.26	6.4

A similar, but more recent, study was carried out by Kemppainen[81] in which the mineral contents of 19 silage effluent samples were determined. The mean values, expressed in g kg^{-1} DM, were: potassium 118.1, calcium 14.3, phosphorus 10.5 and magnesium 7.1. Patterson and Walker[77] carried out a comprehensive analysis of effluents collected from three farm silages over a period of seven weeks (Table 6.5). In particular, the trace element contents of the effluents showed wide variation.

Invariably the ash content of silage effluent DM is considerably higher than that found in herbage DM. For example, in Table 6.4 the mean ash content of the nine effluents is 299 g kg^{-1} DM, while in Table 2.1 (p. 20) the ash values of silages range from only 31 to 125 g kg^{-1} DM. Because of this disproportionately high loss of mineral matter in the effluent, it might be reasonable to assume that the mineral content of silage DM would be lower than the corresponding value in the original herbage DM. In some studies[82] this has been shown to be the case, although in others the concentration of ash in silage DM is higher than that in the original herbage.[65,71] An increase in the concentration of ash in silage DM is only likely to occur if the combined losses of organic matter from fermentation and oxidation more than offset the mineral losses in the effluent.

In addition to mineral elements, silage effluent is also an important source of amino acids. Table 6.6 lists the amino acid contents of effluents derived after hydrolysis from three different ryegrass silages. In all cases, the dominant amino acids present were alanine, leucine, glutamic acid and aspartic acid, which together comprised about 40% of the total amino acids determined.

Table 6.5. Some mineral contents of effluents sampled up to 7 weeks after ensilage of ryegrasses (kg^{-1} DM)[77]

Mineral	Source of effluent					
	Silage A		Silage B		Silage C	
	Mean	s.e.	Mean	s.e.	Mean	s.e.
Ash (g)	184	19.3	192	9.1	208	6.9
Potassium (g)	29.5	9.62	14.6	2.16	25.8	3.57
Calcium (g)	19.1	2.61	16.1	1.80	21.3	1.19
Phosphorus (g)	11.1	0.67	10.6	1.51	9.8	0.55
Magnesium (g)	4.6	0.38	3.9	0.34	5.9	0.20
Sodium (g)	10.7	1.21	5.7	0.70	6.8	0.29
Iron (g)	1.67	0.20	1.74	0.27	2.65	0.95
Manganese (mg)	442	31.8	340	68.8	334	18.6
Zinc (mg)	239	81.1	1277	1132	546	433
Copper (mg)	3.8	0.92	8.3	4.57	47.1	26.78

Values are the means of 7, 6 or 5 samples respectively of effluents from the following silages:
A treated with 2.3 litres formic acid $tonne^{-1}$
B treated with 2.3 litres sulphuric acid/formalin $tonne^{-1}$
C treated with 2.3 litres sulphuric acid/formalin $tonne^{-1}$.

Microbiology of silage effluent

As the microflora of effluent reflect the fermentation processes occurring in the silage, it has been suggested that a bacteriological examination of the effluent might give a reliable picture of the microbial status of the silage itself.[68,83] Some caution, however, is necessary in adopting this approach for, although the development of lactobacilli in silage and in the corresponding effluent has been found to be parallel, it is possible for a secondary development of yeasts and moulds to occur in the sampling system.[68] Pedersen and Olsen[83] examined the microflora of a number of grass silage effluents and, in a typical example, found that the bacterial count increased from about 10^4 cfu ml^{-1} in the first day's sample to 10^7 ml^{-1} in the fourth day's sample, and finally increased to 10^8 ml^{-1} on the tenth day. The count of fungi in the effluents showed only small variations during the first few days of effluent production (about 10^3 cfu ml^{-1}), but from the sixth day onwards the content of fungi decreased markedly and after the eighth day these organisms could no longer be detected.

Silage effluent is known to be an excellent medium for the growth of aerobic microorganisms, and such growth removes dissolved oxygen from the effluent more rapidly than it can enter into solution from the atmosphere.[76] Consequently, there is depletion of dissolved oxygen, resulting in a biological (or biochemical) oxygen demand (BOD).[76] The BOD of silage effluent is exceptionally high,

Table 6.6. Amino acid contents of effluents sampled up to 7 weeks after ensilage of ryegrasses*[77]

	Source of effluent		
	Silage A	Silage B	Silage C
DM (g kg^{-1})	72.3	105.7	68.7
Crude protein (g kg^{-1} DM)	267	233	303
Amino acid (g kg^{-1} DM)			
Aspartic acid	17.5	12.2	15.7
Threonine	10.9	9.7	11.3
Serine	9.6	7.5	9.0
Glutamic acid	17.5	14.4	16.6
Proline	14.6	12.9	15.4
Glycine	13.3	11.5	12.4
Alanine	22.7	18.6	22.5
Cystine	2.81	1.60	1.98
Valine	15.7	12.8	13.4
Methionine	4.15	3.76	3.54
Isoleucine	11.4	9.9	10.4
Leucine	19.1	16.0	17.2
Tyrosine	4.66	1.72	2.07
Phenylalanine	10.2	8.1	8.6
Lysine	16.5	7.4	9.8
Histidine	4.90	2.63	2.31
Arginine	3.69	2.33	3.49
Total	199.2	153.0	175.7

* For details of silages, see Table 6.5.

Table 6.7. The biological oxygen demands of some pollutants of water courses[84-86]

Pollutant	Biological oxygen demand (mg O_2 litre^{-1})
Silage effluent	12,000 — 90,000
Animal slurries	5,000 — 35,000
Parlour and yard washings	1,000 — 2,000
Domestic sewage	300 — 500

ranging from about 12,000 to 90,000 mg O_2 litre^{-1}, and silage effluent can be as much as 200 times more polluting than raw domestic sewage (Table 6.7).[84-86]

Disposal and methods of reducing the production of effluent

Use as a fertiliser

In most countries the pollution of streams and rivers is illegal and effluents produced from silos must not be allowed to enter these watercourses. One method of preventing this happening is to collect the effluent in a storage container and periodically to spray it onto the fields from which the crop was harvested for silage.[85-89] Silage effluent is an excellent source of fertiliser nutrients, having a similar content of nitrogen to that of liquid manure, but being richer in potassium and phosphorus.[80] As the potassium, calcium and magnesium in effluents are present in about the same proportions as in herbage, an effluent used as a fertiliser will provide a supply of the essential major bases in similar proportions to those taken up by plants.[80] Jansson[90] found that effluent from clover-grass silage applied to soil in pots was decomposed within one week where the soil had a good lime status, but it took two to three weeks where the lime status was low.

The use of effluent as a fertiliser is not recommended in hot dry weather because of its possible scorching effect on growing plants. Scorching can be reduced by applying diluted effluent on days when the weather is overcast and the ground wet.[76] Alternatively, the risk of scorch can be avoided by neutralising the acidity with lime.[84,85]

Precautions are necessary in mixing silage effluent with slurry because of the production of hydrogen sulphide gas, which can be lethal at very low concentrations. Effluent and slurry should never be mixed in enclosed tanks.[85]

Use as a feed for farm animals

Silage effluent is a valuable source of readily digestible nutrients but it is only comparatively recently that its potential importance as a feed for farm animals has been extensively examined. Initial studies concentrated on its use as a pig feed.[91-96] About 75% of the crude protein is in the form of amino acids (Table 6.6). Lysine supply is a guide to the level of 'useful protein' and this amino acid is higher in effluent than in most pig finisher meals.[95]

Silage effluent has also been successfully fed to cattle.[97-100] In one study, Steen[98] found that cattle readily consumed effluent in preference to fresh water and supplementation of a grass silage and cereal-based concentrate diet with

effluent did not affect silage DM intake but increased total DM intake by 10%.

Very few studies appear to have been carried out with sheep, although Par and Přikryl[101] found that diets supplemented with silage effluent, compared with unsupplemented diets, increased significantly body mass gains in sheep.

In all these studies, the authors stress the importance of feeding only well-preserved effluent to farm animals. Unfortunately, because it deteriorates rapidly on exposure to air, effluent must be stored anaerobically or fed to animals within three to four days.[95] An alternative is to treat it with a preservative. Patterson and Walker[102] found that formalin, added at the rate of 3 ml litre^{-1}, preserved silage effluent for 104 days before moulds developed.

Filtration

Barry and co-workers[103-105] have examined the possibility of decreasing the polluting effect of effluent by passing it through an upflow anaerobic filter containing a limestone chip support matrix. They found that such treatment reduced the pollution potential by 85 to 90%, yielding a low strength effluent which could be safely disposed of by land-spraying or by irrigation.

Use of absorbents

The difficulties of collecting and disposing of effluent encourage farmers to seek ways of preventing its production. The most obvious method is to pre-wilt the crop destined for bunker silos to a DM content of 260 to 300 g kg^{-1}, and that for tower silos to about 350 to 500 g kg^{-1}. Weather conditions, however, are not always favourable for adequate wilting and the possibility of retaining the effluent in the silo by the use of suitable absorbents has been considered by several workers.

Absorbents tested include cereal grains,[52,106,107] soyabran flakes,[108] dried sugar beet pulp,[109] straw,[110-117] newspaper[118] and the colloidal clay, bentonite.[119-121] Recently, various commercially manufactured pellets, designed to absorb effluent and to provide an additional supply of nutrients, have become available.[106]

The effectiveness of some of these additives in reducing the flow of effluent is discussed in Chapter 7.

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Chapter 7

Silage Additives

The original objective in using silage additives was to ensure that the lactic acid bacteria dominated the fermentation, resulting in a well-preserved silage. In this respect molasses, which was commercially available, supplied a cheap source of fermentable carbohydrate and was widely used by farmers during the early part of this century. In 1929 Virtanen,[1] working in Finland, adopted a different approach and recommended the rapid acidification of the crop with mineral acids to a pH of about 3.5, which it was originally thought would prevent microbial and plant enzyme activity. This AIV process was widely used in Scandinavian countries until quite recently. In the development of silage additives today, although fermentation control is important, attention is also paid to methods of improving the nutritional value of the product and reducing DM losses.

Silage additives can be classified into five main categories (Table 7.1). Those in the first two groups are concerned with fermentation control and act either by encouraging a lactic acid fermentation (stimulants) or by inhibiting partially, or completely, microbial growth (inhibitors). A third group (aerobic deterioration inhibitors) is aimed primarily at controlling the deterioration of silage on exposure to air. The fourth category (nutrients) is added to crops at the time of ensiling in order to improve the nutritional value of the silage, and the fifth group (absorbents) is added to low DM crops to reduce loss of nutrients and pollution of water courses by effluent.

Many of the silage additives discussed in this chapter have been used only in laboratory experiments. Before an additive can be accepted for commercial use it must be shown to be non-toxic to animals and, in particular, to have no adverse effects on rumen fermentation. Finally, recommendations on the use of silage additives must be based not only on the results of scientific research but also on sound economic advice.

FERMENTATION STIMULANTS

Lactic acid bacteria

The earliest known use of lactic acid bacterial cultures is by French workers on sugar beet pulp, at the beginning of this century.[2] In most early studies the encouraging results of laboratory experiments frequently proved disappointing when scaled up to farm conditions and it was considered by the majority of the early workers that, provided the general principles of silage making were adhered to, the natural lactic acid bacterial population on the ensiled crop would be adequate to ensure a normal fermentation. Understanding of the basic microbiology of silage has advanced since these early experiments were carried out and it is known that growing crops are often poor sources of lactic acid bacteria (p. 88) and that some strains of these organisms are not ideally suited for ensiling purposes.

Table 7.1. Classification of silage additives

Fermentation stimulants		Fermentation inhibitors		Aerobic deterioration inhibitors	Nutrients*	Absorbents
Bacterial cultures	Carbohydrate sources*	Acids	Others			
Lactic acid bacteria	Glucose Sucrose Molasses Cereals Whey Beet pulp Citrus pulp Potatoes Cell wall degrading enzymes	Mineral acids Formic acid Acetic acid Lactic acid Benzoic acid Acrylic acid Glycollic acid Sulphamic acid Citric acid Sorbic acid	Formaldehyde Paraformaldehyde Glutaraldehyde Sodium nitrite Sulphur dioxide Sodium metabisulphite Ammonium bisulphate Sodium chloride Antibiotics Carbon dioxide Carbon bisulphide Hexamethylenetetramine Bronopol Sodium hydroxide	Lactic acid bacteria Propionic acid Caproic acid Sorbic acid Pimaricin Ammonia	Urea Ammonia Biuret Minerals	Barley Straw Sugar beet pulp Polymers Bentonite

* Most substances listed under carbohydrate sources can also be listed under nutrients.

For example, of 81 strains of lactic acid bacteria isolated from silage by Wieringa[3] only one proved to be particularly suitable.

Whittenbury[4] has defined the criteria which a potential organism should satisfy for use in silage:

1. It must grow vigorously and be able to compete with and preferably dominate other organisms.
2. It must possess a homofermentative pathway in order to produce the maximum amount of lactic acid from hexose sugars immediately available.
3. It must be acid tolerant and capable of producing a final pH of at least 4.0. Preferably it should be able to produce this low pH as rapidly as possible in order to inhibit quickly the activities of other microorganisms.
4. It must be able to ferment glucose, fructose, sucrose, fructans and, preferably, pentose sugars.
5. It must not produce dextran from sucrose nor mannitol from fructose.
6. It should have no action on organic acids.
7. It should possess a growth temperature range extending to 50°C.
8. It should be able to grow in material of low moisture content, as might arise when wilted material is ensiled.

Wieringa and Beck[5] suggested that, in addition to the above criteria, the organism should have no proteolytic activity. Whittenbury[4] immediately excluded as being unsuitable the *Leuconostoc* spp. (heterofermentative cocci) and the heterofermentative lactobacilli, leaving a choice between the pediococci and the homofermentative lactobacilli. *Lactobacillus plantarum* has been singled out as being one of the most suitable organisms for inoculation purposes according to Whittenbury's criteria.[5-10] This was borne out in the results of a trial in which Seale and Henderson[11] ensiled *Lolium perenne*, fresh or wilted, and inoculated with *L. plantarum* (10^5 g^{-1}) or a mixture of heterofermentative lactic acid bacteria, *Lactobacillus brevis*, *Lactobacillus buchneri*, *Leuconostoc dextranicum* and *Leuconostoc mesenteroides* (10^5 g^{-1}) (Table 7.2). The major advantage of *L. plantarum* over the indigenous bacteria or the heterofermentative lactic acid bacteria was its ability to produce a rapid drop in pH, and silages with low ammonia-N and acetic acid contents.

Ohyama *et al.*[9] investigated the effect of inoculating Italian ryegrass and cocksfoot with *L. plantarum* organisms (10^6 g^{-1}), with and without the addition of glucose (10 g kg^{-1}), but no beneficial effects of the inoculation treatment could be detected. In a previous study these workers[12] found that, in some cases, inoculation with *L. plantarum* resulted in poor quality silages with low lactate and high butyrate contents compared with the well-preserved control silages. When glucose was added along with *L. plantarum*, however, well-preserved silages of high lactic acid content were obtained. In a later experiment, Ohyama and co-workers[13] demonstrated that treatment with a combination of *L. plantarum* and glucose can have a beneficial effect in preventing clostridial fermentation occurring in grass aerated before ensilage.

Although *L. plantarum* satisfies most of the criteria suggested by Whittenbury[4], some strains are slow to produce lactic acid until the pH falls below 5.0[8]. The ideal inoculum should include lactic acid bacteria which are active within the pH range 5.0 to 6.5. Streptococci and pediococci have been shown to fulfil this role.[6,14] In a mixed inoculum of *Enterococcus (Streptococcus) faecalis* and *L. plantarum*, the former species, which has the advantage that it is aerobically fast growing, should

Table 7.2. Composition of perennial ryegrass as ensiled, and silages treated with and without homofermentative or heterofermentative lactic acid bacteria (10^5 cfu g^{-1})[11]

	Grass		Silages					
	Direct cut	Wilted	Direct cut			Wilted		
			Untreated	+ Homo	+ Hetero	Untreated	+ Homo	+ Hetero
DM (g kg^{-1})	166	346	—	—	—	—	—	—
pH	—	—	4.17	3.84	4.36	4.48	3.82	4.29
TN (g kg^{-1} DM)	28.2	27.0	—	—	—	—	—	—
Ammonia-N (g kg^{-1} TN)	—	—	134	70	122	130	50	88
Water soluble carbohydrates (g kg^{-1} DM)	157	177	11	58	9	24	57	24
Lactic acid (g kg^{-1} DM)	—	—	132	175	78	89	157	96
Acetic acid (g kg^{-1} DM)	—	—	45	16	73	14	9	19
Butyric acid (g kg^{-1} DM)	—	—	0.0	0.8	0.0	3.3	0.7	0.0
Ethanol (g kg^{-1} DM)	—	—	6	4	14	11	5	11

dominate the fermentation in the early stages of ensilage, but not being acid tolerant it should give way to *L. plantarum* as the pH falls below 5.0.

In laboratory studies, Bryan-Jones[6] demonstrated the effectiveness of an inoculum containing these two organisms. In one experiment, he showed that the addition of the bacteria (10^5 g^{-1} DM) to ryegrass resulted in a well-preserved silage of pH 4.1 whereas, in contrast, the untreated material produced a badly-preserved silage of pH 5.1. In a similar experiment with cocksfoot, identical results were obtained when fresh herbage was used, although with wilted material both inoculated and uninoculated silages were well-preserved. On a larger scale, using l tonne capacity silos, the results were inconclusive and no great advantage could be detected from the use of the inoculum, although losses of DM were slightly lower with the inoculated silages. In all the experiments, however, the pH of the ensiled material fell faster in the inoculated treatments than in the controls.

This effect of inoculation on rate of pH fall has been obtained in other laboratory studies.[15-18] Carpintero *et al.*[17] ensiled ryegrass-clover treated with glucose (43 g kg^{-1} DM) and a mixed culture of *E. faecalis, L. plantarum* and *L. mesenteroides* (1.6×10^4 g^{-1} DM) and examined the fermentation changes after four days and 50 days. The results, shown in Table 7.3, indicated that both silages were well-preserved but after four days the pH of the inoculated material had fallen to 3.79 while that of the control silage remained at 4.34. The effects of this rapid fall in pH were reflected in higher protein-N contents and lower ammonia-N levels in the inoculated silages. The possible nutritional advantage of this protein sparing effect has, however, yet to be demonstrated. In the study by Carpintero *et al.*,[17] glucose was added as well as the bacterial culture. Several workers have demonstrated that a combination of inoculum with sugar is more effective in influencing the fermentation than an inoculum used on its own.[3,9,12,19-26]

The commercial exploitation of cultures of lactic acid bacteria as additives for silage came with the development of freeze-drying and encapsulation techniques. Unlike acids, the products are safe to handle and do not corrode farm machinery, and they are therefore attractive to the farmer.

Table 7.3. The effect on silage composition of inoculating ryegrass-clover with a culture of lactic acid bacteria[17]

	Control		Inoculated*	
	4 days	50 days	4 days	50 days
pH	4.34	3.87	3.79	3.64
WSC (g kg^{-1} DM)	71	12	66	63
TN (g kg^{-1} DM)	18.7	18.2	18.2	19.1
Protein-N (g kg^{-1} TN)	338	265	490	409
Ammonia-N (g kg^{-1} TN)	86	95	8	9
Acetic acid (g kg^{-1} DM)	—	28.8	—	17.0
Propionic acid (g kg^{-1} DM)	—	0.18	—	0.25
Butyric acid (g kg^{-1} DM)	—	0.19	—	0.07
Lactic acid (g kg^{-1} DM)	—	122	—	162

* Treated with a mixed culture of *E. faecalis, L. plantarum* and *L. mesenteroides* (2.5×10^4 cfu g^{-1}) and glucose (43 mg g^{-1} DM).

One of the first of the bacterial inoculants to come on the market in the USA contained *Lactobacillus acidophilus*. This organism, which is homofermentative and does not produce ammonia from arginine, was originally isolated from the faeces of infants, but has also been found in silages. Moon *et al.*[27] added *L. acidophilus* and *Candida* spp., each at 10^4 cfu g^{-1}, to ensiled wheat, lucerne and maize, and obtained a more rapid pH decline in the inoculated wheat silage but not in the other two. When *L. acidophilus* was added to grass in the UK, Hopkins *et al.*[28] reported a high concentration of lactic acid in some farm silages, but where the DM was low there appeared to be no real benefit from the use of this additive.

In silage making on the farm it has been assumed that the lactic acid bacteria on the crop will eventually dominate the fermentation. This happens in most situations although there are occasions where this is not so.[27, 29-31] In some silages the development of lactic acid bacteria is very slow[32, 33] and this can lead to the growth of undesirable microorganisms in the early stages of fermentation. Lindgren *et al.*[34] found mostly heterofermentative lactic acid bacteria on fresh crops, with numbers especially low at the beginning and the end of the growing season.[14] Although counts may be low on the standing crop, it is not unusual to obtain a count of 10^5 g^{-1} on the harvested material at the silo.[35] There is therefore a strong case for the use of specific cultures of homofermentative lactic acid bacteria, applied at the rate of at least 10^5 but preferably 10^6 colony forming units (cfu) g^{-1} fresh crop, if silage preserved by natural fermentation is the desired aim.

The efficacy of many of the early commercial bacterial inoculants was poor.[36,37] Data from six experiments conducted at two ADAS Experimental Husbandry Farms from 1980 to 1983 showed that commercial inoculant treatments provided little benefit compared with untreated silage.[38] In a laboratory study over the same period, Henderson and McDonald[39] studied the effect of six commercial inoculants on the fermentation pattern during the ensilage of several grasses. Only one product, a mixture of *L. planatarum* and *Pediococcus acidilactici* applied at 10^6 cfu g^{-1}, had a significant effect on the fermentation. In 1986, Done[40] reviewed the experimental data available on the use of inoculants in Europe and decided that the effectiveness of these products in European conditions was inconclusive.

In recent years there has been an improvement in commercial products in Europe.[41-46] Better strains of bacteria have been found and the viability of the freeze-dried lactic acid bacteria has been increased, allowing higher counts of bacteria to be added to the crop, not less than 10^5 cfu g^{-1}. Seale[47] suggested that the competitiveness of lactic acid bacteria could be increased if they had a faster growth rate and an extended pH range, and had incorporated in their make-up the ability to produce substances antagonistic towards competing microorganisms.

The effect of adding a mixture of *L. plantarum* and *Pediococcus pentosaceus* on the fermentation of perennial ryegrass ensiled in 5 tonne capacity bunker silos was studied by Henderson *et al.*.[48] The bacterial inoculant was applied at the rate of 10^6 cfu g^{-1} and the silage was compared with an untreated silage and one treated with 85% formic acid applied at 3 litre $tonne^{-1}$ (Table 7.4).

The silage treated with the inoculant had the lowest pH value and the lowest ammonia-N content. Formic acid applied at 3 litre $tonne^{-1}$ inhibited the lactic acid bacteria but not the microorganisms responsible for the production of acetic acid and ethanol. Dry matter losses measured by the buried bag technique[49] were

Table 7.4. The composition of silages treated with a bacterial inoculant or formic acid[48]

	Control	Treated	
		L. plantarum + *P. pentosaceus*	Formic acid
pH	4.55	4.09	4.44
DM (g kg^{-1})	168	181	182
TN (g kg^{-1} DM)	33.1	31.9	32.5
Protein-N (g kg^{-1} TN)	386	407	455
Ammonia-N (g kg^{-1} TN)	130	88	109
Acetic acid (g kg^{-1} DM)	46	30	35
Propionic acid (g kg^{-1} DM)	9	7	5
Butyric acid (g kg^{-1} DM)	5	5	2
Lactic acid (g kg^{-1} DM)	59	84	51
Ethanol (g kg^{-1} DM)	13	7	33

lowest in the inoculated silage (136 g kg^{-1} DM ensiled) and higher in the control and formic acid-treated silages (178 and 183 g kg^{-1} DM ensiled).

In North America grass, alfalfa (lucerne), corn (maize), sorghum and wheat have been ensiled with bacterial inoculants, with varying results.[50–55] Ely *et al.*[51] found that inoculation of lucerne and wheat with *L. plantarum* (10^7 g^{-1}) had a positive beneficial effect on nutrient recovery, but when Shockey *et al.*[55] inoculated poor quality alfalfa with a mixed inoculum (10^4 g^{-1}) of homofermentative lactic acid bacteria they had no influence on the composition of the silage although there were differences in microbial growth trends.

Other crops which have benefited from inoculation with lactic acid bacteria include steamed potatoes,[56] wastes from vegetable processing[57] and pressed beet pulp.[58]

Ideally, an inoculum of active homofermentative lactic acid bacteria would also include a supply of carbohydrate material, which would serve as an immediate substrate for the added microorganisms, or a combination of enzymes which would produce additional fermentable sugars from the cell walls or cell contents.[59]

Carbohydrate sources

Carbohydrate-rich materials are added to silage crops in order to increase the supply of available energy for the growth of the lactic acid bacteria, and they are of particular importance in crops such as legumes which are deficient in soluble carbohydrate content. Materials which have been used for this purpose include sugars, molasses, cereals, whey, beet pulp, citrus pulp and potatoes.

Sugars

Several workers have demonstrated the advantages of glucose or sucrose as silage additives.[9,20,60-67] Weise[62] added sucrose (10 g kg^{-1}) to grass containing 150 g DM kg^{-1} and 100 g WSC kg^{-1} DM and showed that the addition of the sugar caused

an initial increase in the number of lactic acid bacteria, especially those with a high capacity for producing lactic acid. In the untreated grass, the lactic acid bacteria did not begin to develop until the second week of ensilage. Ohyama and co-workers[12,13] have demonstrated the advantages of adding glucose to herbage to improve the fermentation. In one experiment,[12] using Italian ryegrass with a WSC content of 41 g kg^{-1} DM, the addition of glucose at a rate of 20 g kg^{-1} resulted in a silage of pH 3.69 and a lactic acid content of 20 g kg^{-1}, compared with the untreated silage which had a pH of 5.71 and contained no lactic acid. Similar patterns of fermentation were repeated in other experiments using ryegrass and cocksfoot.[12] The addition of glucose to lucerne at the time of ensiling has been shown to reduce proteolysis[68] and to improve the stability of amino acids.[69]

A few studies using radioactively-labelled sugars have been carried out in order to follow their fate during fermentation. Hartfiel and Marquering[70] ensiled ryegrass-clover with [^{14}C] sucrose (10 g kg^{-1}) and found that, after 47 days, 20% of the labelled carbon was present as carbon dioxide, 25% as fermentation acids in the Lepper-Flieg distillate, and the fate of the remaining 55% was undetermined. In studies in which molasses and [^{14}C] sucrose were added to young grass, Brown[71] found that 5.5% of the added radioactivity was recovered in the silage effluent and 88.1% in the silage. In an examination of the silage fermentation acids, Lessard[72] found that 13.9% of the activity was in acetic acid, 10.4% in lactic acid, and 4.7% in butyric acid.[72]

It is clear from the many experiments carried out that the addition of either glucose or sucrose to a crop at the time of ensiling is likely to be beneficial in improving the fermentation. Of the two sugars, glucose and sucrose, the former is preferable as some of the fructose component of sucrose is likely to be converted by indigenous heterofermentative lactic acid bacteria to the neutral product mannitol. Seale *et al.*[73] added either glucose or fructose with an inoculum of homofermentative lactic acid bacteria to lucerne (Table 7.5). There were no significant differences in the compositions of the silages with added glucose or fructose.

In practice, sucrose, glucose and fructose are not normally used because of their high cost and the cheaper product, molasses, is preferred.

Molasses

This by-product of the sugar-beet and sugar-cane industries has a DM content of 700 to 750 g kg^{-1} and a soluble carbohydrate content of about 650 g kg^{-1} DM,[74] of which sucrose is the main component.[75] A large number of silage experiments

Table 7.5. Composition of inoculated lucerne silage with added glucose or fructose[73]

	pH	Protein-N (g kg^{-1} TN)	Ammonia-N	Acetic acid	Butyric acid (g kg^{-1} DM)	Lactic acid
Inoculated	5.00	257	116	27	12	85
Inoculated + glucose	4.29	358	64	18	1	126
Inoculated + fructose	4.22	337	61	16	0	133

has been carried out using molasses,[62,76-86] and this additive has been shown to increase the DM and lactic acid contents and to reduce the pH and ammonia-N levels in treated silages.[75,79,80,82,87-89] Several workers have found that the addition of molasses to ensiled crops increases effluent losses,[76,82,89,91] although its use may result in a reduction in overall DM losses.[92,93] Castle and Watson[94] compared the application of molasses with formic acid (2.0 litre tonne^{-1}) and concluded that the rate of application of molasses which equated with formic acid was approximately 20 litre tonne^{-1} for fermentation pattern, approximately 30 litre tonne^{-1} for intake and 20 to 30 litre tonne^{-1} for milk production. Molasses is of particular benefit when applied to crops low in soluble carbohydrates, such as legumes and tropical grasses,[89,95-98] although in order to obtain maximum benefit it must be used in relatively high concentrations, about 40 to 50 g kg^{-1}.[77]

In a study using lucerne, the results of which are summarised in Table 7.6, Carpintero *et al.*[99] added molasses (40 g kg^{-1}) to the crop and raised the WSC content from 70 g kg^{-1} DM to 190 g kg^{-1} DM. The molasses silage had a lower pH value, lower acetate and ammonia-N contents, and higher lactate and residual WSC levels than sodium metabisulphite-treated, wilted and control silages.

An additive consisting of four parts molasses to one part peat was tested by Orth and Rohr.[100] Treated clover-grass silages were found to contain more lactate and less acetate and ammonia-N than untreated controls. Several commercial products supply lactic acid bacteria with molasses. Equipment specially developed for spraying molasses on the swath is described by Bosma and Cornelissen.[101]

Cereals

Maize, barley, oats, wheat and milo have all been used as additives in an attempt to improve both the fermentation quality and the nutritional value of silages.[34,76,87,102-108] When added in large quantities, cereal grains have also been shown to reduce effluent production.[109,110] Maize has been added, whole, ground, cracked, as corn and cobmeal, or as hominy meal.[76] Allen and Porter[103] added ground maize (54.5 kg tonne^{-1}) to lucerne-bromegrass and obtained increases in silage DM, total acids and lactic acid. Allen and Foreman[104] found no differences in contents of ammonia-N and reducing sugars between wilted lucerne and lucerne containing ground maize (45.4 kg tonne^{-1}). In studies with ensiled lucerne-timothy containing barley meal (45.4 kg tonne^{-1}), Murdoch *et al.*[87] found that the meal was effective in preserving the crop but was no better than wilting or the addition of molasses or acid. Jones[110] concluded that the soluble carbohydrate in the cereal (30 to 40 g kg^{-1}) and the increase in DM with the addition of cereal may be significant factors in improving the fermentation in grass low in WSC.

As the main carbohydrate in cereals is starch, a polysaccharide not available to the majority of the lactic acid bacteria,[111,112] it has been suggested that cereals would be more effective as fermentable carbohydrate sources if amylase, or amylase-rich materials such as malt, were added along with cereal to ensiled crops.[113-116] The addition of malt-cereal mixtures to grass, clover and lucerne has resulted in the production of well-preserved silages. De Vuyst *et al.*,[102] however, stated that the cost of supplementing cereals with malt would be too high to be of practical value and they concluded that cereals alone are suitable additives for silage and that ground barley, for example, can give a yield of lactic acid of about 150 g kg^{-1}.

Table 7.6. The effect of four different treatments on the composition of lucerne silage[99]

	Lucerne	Silage				
		Control	Molasses*	Formic acid†	Sodium metabisulphite‡	Wilted§
DM (g kg^{-1})	170	175	195	186	173	353
pH	5.9	4.8	4.1	4.2	5.0	5.9
Buffering capacity (mE kg^{-1} DM)	470	1150	1050	590	1130	930
WSC (g kg^{-1} DM)	70	13.9	21.1	93.8	13.5	15.5
Formic acid (g kg^{-1} DM)	1.0	2.9	1.9	15.4	1.2	2.7
Acetic acid (g kg^{-1} DM)	4.8	17.1	20.4	4.8	27.6	25.9
Propionic acid (g kg^{-1} DM)	—	1.4	0	0	0	0
Butyric acid (g kg^{-1} DM)	—	0	0	0	0	0
Succinic acid (g kg^{-1} DM)	1.2	14.0	3.5	15.4	15.2	8.2
Lactic acid (g kg^{-1} DM)	—	51.2	93.3	0	22.7	27.8
TN (g kg^{-1} DM)	29.8	28.6	25.7	27.6	28.9	31.0
Ammonia-N (g kg^{-1} TN)	81	203	78	18	215	148
Amide-N (g kg^{-1} TN)	0.8	0.7	1.7	2.1	1.0	0.5

* 40 g molasses kg^{-1}.
† 8.5 g formic acid kg^{-1}.
‡ 2.3 g sodium metabisulphite kg^{-1}.
§ 24 hour wilting.

Whey

Whey, the by-product of cheesemaking, has a DM value of 66 g kg^{-1} and a lactose content of about 44 g kg^{-1} [117] and, although this disaccharide can be fermented by a number of lactic acid bacteria, the by-product is regarded by many as being a too dilute source of the sugar to be satisfactory as a silage additive.[2,75,111,117,118] Dried whey, however, has been used successfully in a number of silage studies.[2,75,76,79,119-121] Axelsson and Eriksson[122] found that dried whey added to a range of different crops increased the DM and lactic acid contents of the silages but they concluded that the additive was not effective enough to merit the high costs of drying and that molasses was preferable.

Several workers[123-125] have applied an inoculum of lactic acid bacteria with whey. In general, inoculation increased the lactic acid content of the silage.

Dried beet pulp, citrus pulp and potatoes

Dried beet and citrus pulp have been used as silage additives mainly to increase the DM content of high moisture crops.[76,78,126,127] Molassed beet pulp is preferable to ordinary pulp because of the additional sugar content, and this material has proved successful as a silage additive for legume crops.[2]

Although starch is the main component of the potato, this tuber also contains some soluble sugars but these rarely exceed 50 g kg^{-1} DM.[128] Potatoes have produced satisfactory silage when ensiled, both fresh and steamed, mixed with legumes and other crops.[2,129]

Cell wall degrading enzymes

The use of cellulolytic and hemicellulolytic enzymes as silage additives has been considered from two points of view; firstly, as a means of increasing the content of fermentable sugars and, secondly, as a method of improving the digestibility of organic matter.[130-137] The ensiling process is known to have an effect on the cell wall carbohydrates, in particular the hemicelluloses.[138] Morrison[139] has demonstrated that losses of 10 to 20% of the hemicellulose fraction can occur during the ensilage of perennial ryegrass, the extent of the loss differing with the type of chemical additive applied to the grass. In the same experiment the cellulose content decreased only by up to 5%.

Henderson and McDonald[140] ensiled ryegrass treated with a commercial cellulase obtained from *Aspergillus niger* at an application rate of 4 g kg^{-1}. After 133 days, 361 g kg^{-1} ensiled cellulose had been hydrolysed in the treated silage compared with 157 g kg^{-1} in the untreated material. The pH values of the two silages were 4.87 and 5.61 respectively. In a comparison of commercial cellulases prepared from *A. niger* and *Trichoderma viride (reesei)* added to formic acid-treated ryegrass-clover, these workers found that the *T. viride* was the most active in hydrolysing cellulose. When the same workers[141] treated perennial ryegrass, lucerne and clover with formic acid (4.5 litre $tonne^{-1}$) and a commercial cellulase preparation derived from *T. viride* (4 g kg^{-1}), the greatest losses of cellulose occurred when the herbage was minced and the silos were held at 15° and 35°C. *T. viride* is known to produce a complete enzyme system capable of hydrolysing cellulose to glucose, and in these studies the optimum pH for the activity of the enzyme was achieved by adding formic acid.

No *et al.*[142] applied a commercial enzyme alone to lucerne and to timothy (0 to 8 g kg^{-1}) and concluded that this enzyme should be used to improve the fermentation when herbage with a low WSC content and high fibre content is ensiled. When Rauramaa *et al.*[143] applied a cellulase preparation to grass with a high WSC content, however, the cellulase-treated silages had the highest yeast counts and the highest ethanol contents, indicating an inefficient fermentation of the monosaccharides released by the enzyme.

Many silage additives now contain enzymes along with an inoculum of homofermentative lactic acid bacteria,[144] added to convert the glucose to lactic acid only.[145,146] An alternative way of producing a combined inoculant/enzyme additive would be to incorporate cellulase activity into a lactic acid bacterium, but this involves a substantial number of genes if a complete enzyme system is required.[147]

Most commercial cellulases contain hemicellulases[140] and the combined action of these enzyme systems on plant polysaccharides will result in a mixture of hexose and pentose sugars. Cellulose is a similar polysaccharide irrespective of its source, but hemicelluloses exist in a variety of forms. Hemicelluloses of grasses are mainly arabinoxylans with small amounts of β-glucans. The arabinose side chains are more easily hydrolysed than the main xylan backbone.[138] As this hydrolysis occurs naturally during the ensilage process,[139] the application of hemicellulases may speed up the process, but have little effect on the extent of the hydrolysis. Most of the additional sugar is therefore likely to come from the cellulose fraction. The effect of cell wall degrading enzymes on the chemical composition of grass silage harvested at three different maturities and ensiled directly or after wilting was investigated by van Vuuren *et al.*.[148] They found that the enzymes were most effective when added to young herbage ensiled at a low DM content but that the breakdown of the hemicellulose was not affected by enzyme treatment. Huhtanen *et al.*,[149] however, did observe some hydrolysis of hemicellulose in more mature grass with higher levels of application of a cellulolytic preparation.

Although cellulase enzymes undoubtedly increase the soluble carbohydrate content of herbage, experiments to demonstrate possible increases in digestibility have found them to be less effective. In one study using pigs, Whittemore and Henderson[135] found that in silage made from young grass treated with *T. viride* cellulase and formic acid, although the fibre content decreased by 38 g kg^{-1} DM and the WSC content increased by 154 g kg^{-1} DM, the digestibility of the enzyme-treated silage did not differ from that of the control silage. McCullough[130] added cellulase with a calcium carbonate buffer to sorghum prior to ensiling and found that the treated silage had 20% less cellulose than the untreated material and the digestibility of the former was slightly increased. Olsen and Voelker[132] and Owen[133] added enzymes produced by *Aspergillus oryzea* to maize but failed to improve the digestibility of the silage by ruminants, although the cellulase-treated silages had pH values lower than those of the untreated silages. Autrey *et al.*[134] added a cellulase, derived from *T. viride*, to maize at various rates (0, 0.5, 1.0 and 2.0 g kg^{-1}) and obtained significant reductions (about 13%) in cellulose content after an ensiling period of one year. In this study there was some indication of improved digestibility of the silages by cows at the higher levels of enzyme treatment, although differences were not significant.

McHan[150] treated Coastal bermudagrass of 32 g DM kg^{-1} with cellulase

(1 g kg^{-1}). Water soluble carbohydrates and acids were higher in the treated silage after 60 days and there was a small increase in digestibility *in vitro* from 59 to 62%. Baintner *et al.*[151] examined the effect of urea on the activity of a commercial cellulase applied to maize and maize-sorghum (150 g $tonne^{-1}$) and found that urea slowed down the drop in pH, increased the activity of the cellulase and increased the digestibility of the silage by 12%. In the experiments carried out by van Vuuren *et al.*[148] with grass, however, the application of a cellulase enhanced the fermentation, increased the instantly degradable fraction of organic matter but did not alter the *in vitro* digestible organic matter content.

FERMENTATION INHIBITORS

Mineral acids

Although Virtanen developed a method of preserving crops using mineral acids (the AIV process) in the late 1920s,[1] the original proposal to use these acids as silage additives dated back to 1885.[2] It was Virtanen, however, who worked out scientifically the details of the system and his proposals were widely accepted and carried out in practice by many farmers, especially in Scandinavia. In the UK the AIV process was not popular, mainly because of difficulties in handling the corrosive acids.[2,112,118]

Virtanen's main objective was to calculate the amount of acid needed to lower the pH of forage to a level at which plant and microbial enzymes would be inhibited completely. Ideally, in order to achieve this objective, the pH of the crop should be reduced to below 3.0 but at this level silage becomes unpalatable to animals.[1] Virtanen found that when the pH of the material was reduced to 3.6 by hydrochloric acid no free mineral acid remained in the fodder and he concluded that: 'The acidity was not due to the presence of free hydrochloric acid but to protein bodies of an acid nature and the weaker organic acids such as oxalic, malic and citric which are liberated by the stronger mineral acids reacting with the basic constituents of the fodder'. In feeding trials silage produced in this way, at pH 3.6, was found to be acceptable to ruminants, although in later studies it was concluded that neutralisation of AIV silage prior to feeding resulted in better performance.[152,153] While it was realised that the achievement of pH 3.6 does not inhibit completely biological activity, for example yeasts can still grow and plant respiration and proteolysis can continue at this pH, it is considerably reduced.[1,2]

Virtanen also stressed the importance of preventing proteolysis during ensiling and, as the results of one of his experiments demonstrate (Table 7.7), the rapid attainment of about pH 3.6 virtually prevents the breakdown of protein to NPN (soluble-N) compounds. However, many proteases have pH optima below 3.6 (Chapter 3, p. 70) and other workers[17] have found extensive proteolysis in silage with an initial pH as low as this.

The acid solution originally proposed by Virtanen for commercial use was composed of a mixture of hydrochloric and sulphuric acids which was diluted with water before application. Later, other acids were included in the mixture and one product marketed in the 1930s included phosphoric acid instead of sulphuric acid.

In the Penthesta process[2] phosphorus pentachloride was added to water, giving rise to a mixture of hydrochloric and phosphoric acids. Results of successful experiments using the Penthesta method have been described by Watson and

Table 7.7. Effect of hydrochloric acid on the breakdown of protein in clover aftermath after four months[1]

		Hydrochloric acid added			No acid added
	Fresh crop	Initial pH	3.7	4.1	4.5	Final
		Final pH	3.6	4.3	4.6	pH 4.5
Soluble-N (g kg^{-1} TN)	260		280	440	600	650
Ammonia-N (g kg^{-1} TN)	15		20	120	210	220

Nash.[2] Phosphoric acid itself has been used successfully as a silage additive. In studies by Hellberg, quoted by Barnett,[117] there was very little difference in silage characteristics between phosphoric acid-treated material and that treated with either AIV solution or hydrochloric acid. In a more recent study using a product containing equal quantities of peat and phosphoric acids, the additive improved the preservation quality of ensiled Italian ryegrass and red clover.[154,155]

With the rise in the cost of production of silage in the 1980s there has been renewed interest in sulphuric acid as a silage additive. Recently 42 to 45% w/w sulphuric acid has been made available and, as it is applied at a much lower rate than that previously adopted for AIV acid, it is cheaper than most other commercial silage additives.[144] The current objective in using the acid is not to suppress the fermentation but to supplement the natural fermentation process by the addition of moderate amounts of acid.[156-158] Sulphuric acid-treated silage has been associated with a reduction in the liver copper status of cattle,[159] but this can be overcome if the sulphuric acid is fortified with added copper.[156]

Formic acid

The use of formic acid as a silage additive was originally advocated by Dirks in 1926[2] and a few years later Von Kapff[2] described the 'silo acid' process, in which a mixture of formic and hydrochloric acids was sprinkled over the ensiled crop. However, early studies with formic acid as a silage additive were disappointing, partly because of difficulty of distribution, but also because it was not very effective when applied to long herbage.[160] With the introduction of the flail-type forage harvester, however, and the development by Aas and Naerland[161] of a suitable container which could be attached to the harvesting machine and which effectively sprayed the concentrated formic acid directly onto the herbage immediately after cutting, the use of formic acid as a commercial silage additive became widespread.

The normal commercial product has a formic acid concentration of 850 g kg^{-1}. In the UK it is usually applied undiluted to herbage at a rate of 2.3 litre $tonne^{-1}$ (2.8 kg $tonne^{-1}$), equivalent to 2 litre $tonne^{-1}$ (2.4 kg $tonne^{-1}$) of pure formic acid, although higher levels are advocated for difficult crops such as legumes or to restrict the fermentation in grass silages. In Scandinavia, higher rates are applied to all crops.

Effect of formic acid on pH

Although formic acid is the strongest of the fatty acid series, it is considerably

weaker than the mineral acids used in the AIV process and no attempt is made in using it commercially to reduce the pH of the crop to below 4.0. The relationship between level of acid applied and pH of macerates of ryegrass harvested at 50% ear emergence is shown in Figure 7.1.[162] When applied to such a crop at the commercial level of 2.8 g kg^{-1} and assuming a DM content of the herbage of 200 g kg^{-1}, the pH would be immediately reduced to about 4.9. With wetter ryegrass crops of DM content 170 g kg^{-1}, a similar application rate would result in a pH fall to about 4.6. Grasses harvested at early stages of growth are more highly buffered than those cut late, and higher levels of formic acid are required with these materials to effect a similar fall in pH.[162]

Legumes, which are even more highly buffered than grasses (p. 32), need larger amounts of acid to reduce the pH value to a satisfactory level. Lancaster and Brunswick[163] recommended an optimum level of 5 to 6 litre $tonne^{-1}$ for lucerne.

Effect of formic acid on microflora

The antibacterial action of formic acid, and other fatty acids, is due both to a hydrogen ion concentration effect and to a selective bactericidal action of the undissociated acid.[160,164,165] Within the fatty acid homologous series, the hydrogen ion concentration effect decreases with increasing molecular weight while the antibacterial effect increases, at least up to the C_{12} acid.[166,167]

Woolford[166] examined the antimicrobial action of the first twelve acids in the fatty acid series, with particular reference to those microorganisms associated with silage. In general, the minimum inhibitory concentration decreases with increasing chain length, although butyric acid is an exception in that bacteria, especially clostridia, tolerate this acid better than the lower molecular weight

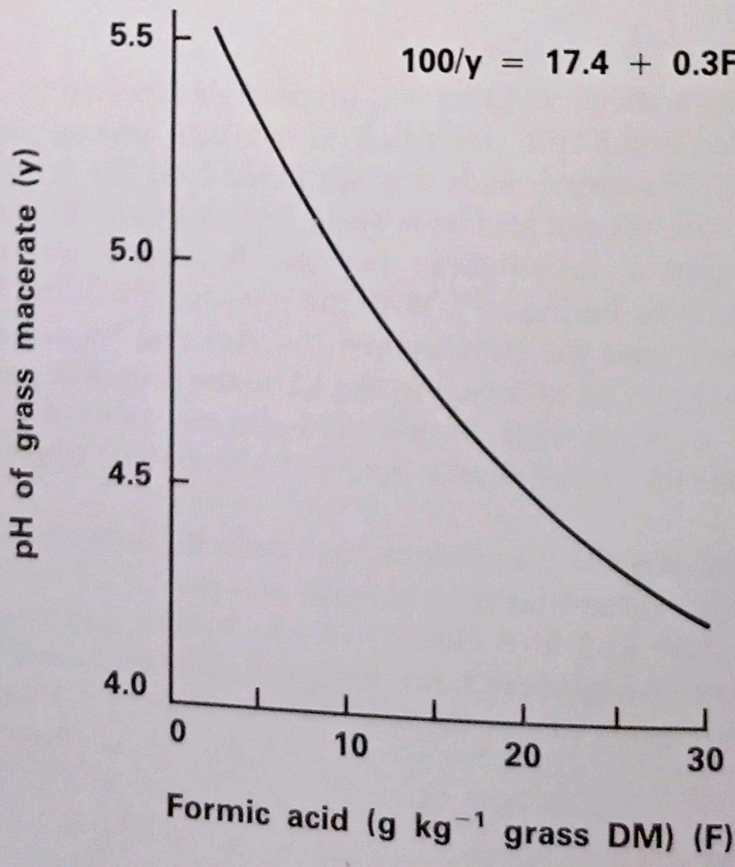


Figure 7.1. The effect of level of formic acid on the pH of ryegrass macerates[162]

acids. Some results from Woolford's studies relating to formic, acetic and propionic acids are shown in Table 7.8. Of the three acids, formic appeared to be the most effective in inhibiting bacterial growth at pH 4, although within the pH range 5 to 6 propionic acid inhibited more effectively the growth of clostridia, *Bacillus* spp., and the Gram-negative bacteria.

Mann[168] also examined the antimicrobial activity of these three volatile fatty acids, using a gradient plate technique, and found that at a formic acid level of 4.5 ml kg^{-1} selected strains of *Leuconostoc, Pediococcus* and *Enterococcus* were all inhibited while *Lactobacillus* strains (*L. buchneri, L. casei* and *L. plantarum*) were not completely inhibited. At this concentration, neither acetic acid nor propionic acid was effective in preventing the growth of lactic acid bacteria. The *Bacillus* spp., *B. subtilis, B. pumilis* and *B. brevis,* grew in the presence of 4.5 ml kg^{-1} formic, acetic and propionic acids. Chamberlain and Quig[157] ensiled perennial ryegrass with 0, 2, 4 and 6 litre formic acid $tonne^{-1}$ and found that rates of application greater than 2 litre $tonne^{-1}$ inhibited the increase in numbers of lactic acid bacteria during the early stages of ensilage. In a study with wilted perennial ryegrass of DM content 360 g kg^{-1}, Henderson *et al.*[169] found that formic acid (3.3 g kg^{-1}) reduced the total count of microorganisms but had only a marginal effect on the activities of the lactic acid bacteria.

Yeasts have been found to be particularly tolerant of formic acid, and high counts of these organisms leading to high ethanol contents have been noted in silages treated with this additive.[169] In a study with formic acid-treated (2.5 g kg^{-1}) timothy-meadow fescue, Pedersen *et al.*[170] found that the enterobacteria were inhibited in the early stages of ensiling but the lactic acid bacteria were active and lowered the pH of the silage to 4.0. Beck[171] found that formic acid had a pronounced inhibitory effect on the clostridia and the enterobacteria, but the effect was strongly dependent on the concentration of the acid used. At minute concentrations the growth of undesirable organisms was actually promoted by formic acid.

In the study of Chamberlain and Quig,[157] yeast counts varied greatly both with time of sampling and with treatment. Although the numbers of enterobacteria were reduced progressively by increasing levels of acid addition, the addition of acid did not prevent a rise in population as ensilage proceeded. By day 90 the highest numbers were present in the silages prepared with formic acid at 4 and 6 litre $tonne^{-1}$. As enterobacteria are known to generate formic acid, this acid probably has less effect on reducing their growth[34] and it is the rapid proliferation of lactic acid bacteria rather than the decrease in pH associated with the application of formic acid which is important in reducing numbers of enterobacteria.[157] Chamberlain and Quig produced well-preserved silage with the application of 2 and 6 litres formic acid $tonne^{-1}$ grass but with 4 litres $tonne^{-1}$ the fermentation was poor. The low level of formic acid aided the natural fermentation and the high level severely restricted the fermentation, but the intermediate level inhibited the lactic acid bacteria to a greater extent than the enterobacteria.

Effect of formic acid on silage composition

The effect of formic acid application on the chemical composition of silage varies with the level applied, the DM content of the ensiled material and the species of

Table 7.8. Minimum inhibitory concentrations (mmol litre^{-1}) of formic, acetic, and propionic acids against various groups of microorganisms at three pH levels[166]

	Formic acid			Acetic acid			Propionic acid		
	pH			pH			pH		
	4	5	6	4	5	6	4	5	6
Lactic acid bacteria (homo)	13	150	>200	94	188	375	63	250	375
Lactic acid bacteria (hetero)	P	150	>200	94	379	375	31	250	250
Clostridia	P	50	200	P	47	188	P	<8	<8
Bacillus spp.	P	19	>200	P	<12	47	P	<8	31
Yeasts	75	>200	>200	94	281	375	63	125	375
Moulds	100	200	>200	94	188	375	63	63	250
Gram-negative bacteria	P	38	200	P	23	94	P	31	31

P = Antimicrobial effect due to pH.

Table 7.9. The effect of formic acid and formalin additives on the composition of lucerne silages harvested by two methods[172]

	pH	DM (g kg^{-1})	Organic matter (g kg^{-1} DM)	TN (g kg^{-1} OM)	Protein-N (g kg^{-1} TN)	Ammonia-N (g kg^{-1} TN)	WSC (g kg^{-1} OM)	Lactic acid (g kg^{-1} OM)	Acetic acid (g kg^{-1} OM)	Propionic acid (g kg^{-1} OM)	Butyric acid (g kg^{-1} OM)
Flail harvested											
Untreated	5.53	176	839	37.7	312	209	7	18	115	13	4
Formic acid†											
Low (1.5 litre tonne^{-1})	5.35	201	768	37.2	326	194	6	41	88	7	5
Medium (3.0 litre tonne^{-1})	4.62	220	784	33.3	403	143	6	64	56	4	1
High (6.0 litre tonne^{-1})	4.20	229	789	33.4	489	93	20	51	33	2	1
Formalin‡ (8 litre tonne^{-1})	4.92	189	831	36.6	635	107	35	57	46	3	7
Precision harvested											
Untreated	4.74	191	882	34.4	371	129	6	42	92	7	1
Formic acid†											
Low (1.5 litre tonne^{-1})	4.19	190	894	34.3	425	83	8	56	38	6	1
Medium (3.0 litre tonne^{-1})	3.96	200	898	33.1	505	42	35	39	29	1	0
High (6.0 litre tonne^{-1})	4.25	198	901	34.2	543	45	60	21	17	1	1
Formalin‡ (8 litre tonne^{-1})	5.16	199	890	35.1	727	72	102	16	19	1	1

† Containing 850 g formic acid kg^{-1}.
‡ Containing 350 g formaldehyde kg^{-1}.

crop. Typical effects of formic acid on a crop low in soluble carbohydrates are shown in Table 7.9. In this study by Barry *et al.*[172] the acid was applied at low, medium and high levels to lucerne, which was harvested by two different methods. A formalin treatment was included for comparison. In the flail-harvested material, the low formic acid treatment had virtually no effect in preventing a poor fermentation and it was only at the high level of acid application that effective preservation occurred. With precision chopped material, all formic acid-treated silages were well-preserved. Increasing the concentrations of formic acid resulted in decreasing levels of lactic and acetic acids and increasing concentrations of soluble carbohydrates and protein-N in the silages. In further studies by these workers[173] it was shown that changes to the amino acids, particularly in the precision chopped lucerne, were considerably restricted by the use of formic acid.

The general patterns of fermentation obtained by Barry *et al.*[172] with formic acid-treated lucerne have been repeated by other workers using a range of crops of low soluble carbohydrate content.[163,174-179]

When formic acid is applied to crops rich in soluble carbohydrates, which normally preserve satisfactorily as a result of a dominant lactate fermentation, the additive has the effect of restricting the production of lactic and acetic acids and conserving the soluble carbohydrate components. This is illustrated in the results of an experiment, shown in Table 7.10, by Carpintero *et al.*[17] in which a ryegrass-clover crop containing 203 g WSC kg^{-1} DM was ensiled in laboratory silos with varying levels of formic acid. When high levels of formic acid are used, the soluble carbohydrate content of the resultant silages may exceed the levels present in the original herbage, presumably because of hydrolysis of polysaccharides occurring during the storage period.

The effects of formic acid on the nitrogenous components of herbage are also interesting and it can be seen in Table 7.10 that increasing formic acid levels result in decreasing rates of proteolysis and deamination, effects also noted by other workers.[160, 175, 180-188] On the other hand, Chamberlain and Quig[157] and Wilson[189]

Table 7.10. The effect of different levels of formic acid (g kg^{-1}) on the composition of ryegrass-clover silages after a 50-day ensiling period*[17]

	Formic acid+					
	0	0.4	1.0	2.0	4.1	7.7
pH	3.87	3.77	3.67	3.81	3.88	3.80
WSC (g kg^{-1} DM)	12	33	72	124	211	250
TN (g kg^{-1} DM)	18.2	17.8	18.5	19.3	19.2	18.6
Protein-N (g kg^{-1} TN)	265	285	325	358	401	462
Ammonia-N (g kg^{-1} TN)	95	79	59	46	12	12
Acetic acid (g kg^{-1} DM)	28.8	24.1	18.9	13.3	4.5	3.1
Propionic acid (g kg^{-1} DM)	0.18	0.27	0.22	0.36	0.28	0.19
Butyric acid (g kg^{-1} DM)	0.19	0.04	0.04	0.16	0.23	0.03
Lactic acid (g kg^{-1} DM)	122	153	115	117	66	5

* Original composition of herbage: DM = 199 g kg^{-1}; WSC = 203 g kg^{-1} DM; TN = 19.3 g kg^{-1} DM; protein-N = 819 g kg^{-1} TN.
\+ Containing 850 g formic acid kg^{-1}.

Table 7.11. The composition of Italian ryegrass silages treated with a constant level of formic acid (11.5 g kg^{-1} DM) at different dry matter levels*[162]

DM (g kg^{-1})	pH		WSC	
	Control	Acid-treated	Control	Acid-treated
150	3.7	3.8	6	53
200	4.0	3.9	10	69
250	4.2	4.1	16	90
300	4.3	4.3	22	115
350	4.4	4.5	31	148

* Ryegrass harvested at 50% ear emergence and with a WSC content of 151 g kg^{-1} DM. Data calculated from regression equations.

have reported adverse effects on the fermentation in response to increases in the rate of application of formic acid from 2 litre tonne^{-1} to 4 litre tonne^{-1}.

The preserving effect of formic acid is enhanced with increasing DM levels[162,190,191] and this is particularly noticeable in the soluble carbohydrate fraction, as seen in Table 7.11. In this experiment the rate of application of formic acid in terms of fresh weight increased as the DM increased. Formic acid has also been shown to have an inhibiting effect on plant respiration and this is reflected in lower silage temperatures occurring during the storage period.[169,192,193]

Salts of formic acid

Calcium and sodium formates have both been used as silage additives,[2,118,194] the former often being applied with sodium nitrite which, according to Pfeiffer,[195] produces nitric oxide which, in the early stages, protects silage from the action of clostridia (Chapter 4, p. 113). Experiments using calcium formate-sodium nitrite mixtures have shown improvements in silage fermentation quality,[2,152,196] although results with this additive have been variable.[75,118] In a survey of silages from commercial farms, Haigh[197] found that the application of calcium formate-sodium nitrite mixtures provided no benefit in fermentation, as measured by ammonia-N concentration and pH, when compared with untreated herbage.

To overcome the corrosion and handling problems associated with the use of formic acid a complex acid salt, ammonium tetraformate, was developed for commercial use.[198] It has a lower acid content than straight formic acid and this must be rectified by a higher rate of application. This is illustrated by the results of a laboratory study in 1980 in which Henderson and McDonald (unpublished data) treated ryegrass, wilted under poor conditions, with formic acid (850 g kg^{-1}) at 3 litre tonne^{-1} and ammonium tetraformate at 3 litre tonne^{-1} (Table 7.12). At this rate of application, formic acid had a greater inhibitory effect than ammonium tetraformate on the lactic acid bacteria, as seen from the lower lactate production, but it did not prevent the fermentation of sugars to ethanol.

When Santi *et al.*[199] applied ammonium tetraformate to white clover-cocksfoot (3.4 g kg^{-1}) it was as effective as formic acid (2.5 g kg^{-1}), but when they were applied at the same rates to lucerne the fermentation was poorer with the salt than with the acid.

Table 7.12. The composition of ryegrass silages untreated, treated with formic acid (3 litre tonne^{-1}) or treated with ammonium tetraformate (3 litre tonne^{-1})

		Silage							
				(g kg^{-1} DM)					
Treatment	Grass pH	pH	Ammonia-N (g kg^{-1} TN)	WSC	Acetic acid	Propionic acid	Butyric acid	Lactic acid	Ethanol
Untreated	6.35	3.82	153	0	26	0.7	0.2	150	4
Formic acid	4.25	4.02	117**	67***	16	0.6	0.6	96***	13***
Ammonium tetraformate	4.72	3.83	162	49***	17	0.6	0.1	112**	4

* = Significantly different from untreated ($p<0.05$).
** = Significantly different from untreated ($p<0.01$).
*** = Significantly different from untreated ($p<0.001$).

Acetic and propionic acids

The possibility of using acetic acid as a silage additive has been dismissed by many workers because its presence in silage in high concentrations has been associated with poor animal performance resulting from low voluntary DM intakes.[200,201] However, recent studies by Deswysen[202] have shown that acetate *per se* induces only a very small reduction in the voluntary DM intake of silage by sheep, and any effect of high acetate levels in silage on animal performance is likely to arise from factors associated with the acetate production and not with the acid itself. Several workers[164,203,204] have found that acetic acid was less effective than formic acid in restricting fermentation, although De Vuyst *et al.*[205] in their studies concluded that, with application rates of 3 litre tonne^{-1} for ryegrass and 4 litre tonne^{-1} for lucerne, acetic acid was almost as effective as formic acid.

The action of propionic acid on microorganisms has long been appreciated in bakery technology and it is widely used in the preservation of moist grain.[206] Of the lower volatile fatty acids, propionic acid is the most effective antimycotic agent[166] and its role as a potential additive in inhibiting aerobic deterioration will be discussed in a later section of this chapter. Propionic acid also has a beneficial effect in controlling silage fermentation and has been found to reduce ammonia-N formation[203,206,207] and temperature in ensiled grasses,[203] legumes[208] and haylage,[209,210] to stimulate the growth of lactic acid bacteria[211] and to improve DM intake of maize silages.[212] However, as a silage additive used to restrict fermentation, propionic acid is less effective than formic acid and is unlikely to replace the stronger acid on economic grounds.[164,203,213]

Other organic acids

Reference was made earlier to the increased antimicrobial activity of fatty acids with increasing chain length. A number of higher fatty acids, including decanoic, dodecanoic, tetradecanoic, hexadecanoic, octadecanoic, octadecenoic, and octadecadienoic acids, have been examined in silage studies (Table 7.13), but results have proved disappointing and it is generally concluded that these acids cannot be recommended as fermentation inhibitors.[164,214]

Lactic acid is an obvious choice as a silage additive, as it is known to restrict the activities of clostridia and the Gram-negative bacteria. Watson and Nash[2] commented on the early studies carried out using this acid and, although it is effective in inhibiting undesirable fermentations, these authors concluded that lactic acid was unlikely to be used commercially as a silage additive because of its high cost. However, Woolford[215] found that although lactic acid was an effective inhibitor of spore-bearing bacteria it was ineffective against yeasts and moulds, and when Byers *et al.*[216] applied it to maize, at 47 g kg^{-1} DM, gas and ethanol production increased but there was a 41% reduction in acetic acid production.

Several workers have examined the usefulness of benzoic acid and benzoates as silage additives.[91,215,217-221] Sodium benzoate was found to possess antimicrobial properties and to be most effective at low pH values. The acid salt displayed a marked discrimination towards heterofermentative lactic acid bacteria.[215]

Initial laboratory screening trials by Woolford[222] indicated that acrylic acid might have a potential use as a silage additive. This acid was found to inhibit all groups of bacteria, particularly spore-bearing and Gram-negative organisms at pH 5 and 6, and this activity appeared to be about ten times as strong as that of

Table 7.13. Chemical formulae of some organic acids tested as silage additives

Acid	Formula
Formic (methanoic)	HCOOH
Acetic (ethanoic)	CH_3COOH
Propionic (propanoic)	C_2H_5COOH
Caproic (hexanoic)	$C_5H_{11}COOH$
Heptylic (heptanoic)	$C_6H_{13}COOH$
Caprylic (octanoic)	$C_7H_{15}COOH$
Pelargonic (nonanoic)	$C_8H_{17}COOH$
Capric (decanoic)	$C_9H_{19}COOH$
Lauric (dodecanoic)	$C_{11}H_{23}COOH$
Myristic (tetradecanoic)	$C_{13}H_{27}COOH$
Palmitic (hexadecanoic)	$C_{15}H_{31}COOH$
Stearic (octadecanoic)	$C_{17}H_{35}COOH$
Oleic (octadecenoic)	$C_{17}H_{33}COOH$
Linoleic (octadecadienoic)	$C_{17}H_{31}COOH$
Lactic (hydroxypropanoic)	$CH_3CHOHCOOH$
Benzoic (benzenecarboxylic)	C_6H_5COOH
Acrylic (propenoic)	$CH_2CHCOOH$
Glycollic (hydroxyethanoic)	$HOCH_2COOH$
Sulphamic (amidosulphonic)	H_2NSO_3H
Citric (hydroxypropanetricarboxylic)	$HOOCCH_2COHCOOHCH_2COOH$
Sorbic (hexadienoic)	C_5H_7COOH

formic acid. Although acrylic or propenoic acid ($CH_2 = CHCOOH$) is closely related structurally to propionic acid (CH_3CH_2COOH), it is a stronger acid, having a pK value of 4.25 compared with 4.87 for propionic acid.[223] In a comparison of acrylic acid with formic and propionic acids applied at similar rates (2.5 g kg^{-1}) to ryegrass, Wilson *et al.*[223] found that the acrylic acid-treated silage contained less lactic and acetic acids, and more soluble carbohydrates than the other two additive-treated silages (Table 7.14). Sodium acrylate applied at a rate of 3.3 g kg^{-1} was only slightly less effective as a fermentation inhibitor. In eight laboratory experiments with cocksfoot, oats, Italian ryegrass and red clover (DM < 200 g kg^{-1}), Kalač[224] found that acrylic acid was more effective than formic acid against clostridia and other putrefactive microorganisms and Baintner *et al.*[225] demonstrated that sodium and calcium acrylate inhibited heterofermentative lactic acid bacteria at a lower concentration than that at which they inhibited homofermentative lactic acid bacteria.

Other organic acids which have been laboratory-tested as potential silage fermentation inhibitors include glycollic acid,[2,87,222] sulphamic acid,[222] citric acid,[226,227] sorbic acid,[165,215,228] salicylic acid[226] and maleic acid.[229]

Formaldehyde

Formaldehyde is a well-known sterilising agent and is commercially available as formalin, which contains 40% of the gas in aqueous solution. Watson[230] reported that formalin was used successfully as a bacteriostat in silage making by Kuchler and Von Wachter in 1931, and in 1932 it was included in a commercial mixture of

Table 7.14. Composition of silages made from ryegrass treated with formic acid, propionic acid, acrylic acid and sodium acrylate[223]

	No additive	Formic acid (2.5 g kg^{-1})	Propionic acid (2.5 g kg^{-1})	Acrylic acid (2.5 g kg^{-1})	Sodium acrylate (3.3 g kg^{-1})
pH	3.93	3.87	3.88	4.21	4.30
Lactic acid (g kg^{-1} DM)	69.6	71.4	134.1	16.6	19.1
Acetic acid (g kg^{-1} DM)	42.2	25.3	28.9	12.2	12.2
Ethanol (g kg^{-1} DM)	4.5	7.6	8.8	29.2	22.6
WSC (g kg^{-1} DM)	13.7	70.9	21.9	107.4	74.5
Protein-N (g kg^{-1} TN)	288	455	368	328	351
Ammonia-N (g kg^{-1} TN)	81.5	47.6	83.7	55.8	65.6

chemicals used in the toro-silon process. Renewed interest in the use of formalin as a silage preservative occurred some 40 years later as a result of the studies of Brown and Valentine.[231]

These workers were interested in the additive from two points of view, firstly because of its bacteriostatic properties and, secondly, because of its known property of protecting plant protein from microbial degradation in the rumen. In their original study with lucerne, Brown and Valentine[231] applied formaldehyde at the rates of 166, 249 and 331 g kg^{-1} crude protein (equivalent to a formalin application of 22.6, 33.9 and 45 g kg^{-1} fresh herbage respectively) and they found that, at these rates of application, fermentation in the silo was inhibited and plant proteins were protected, but voluntary DM intake and digestibility were markedly reduced when the silages were offered to sheep. These workers concluded that the amounts of formalin applied to the lucerne were too high, and in a later experiment[232] formalin applied to the same crop species at a level of 6.7 kg tonne^{-1} produced a silage in which fermentation had occurred but which contained lower concentrations of ammonia-N, lactic acid, propionic acid and butyric acid than the untreated silage. In this second study, treatment with formaldehyde significantly reduced the digestibility *in vivo* of nitrogen, produced a non-significant increase in DM intake and significantly increased wool growth. In an additional treatment, a combination of formalin (6.7 kg tonne^{-1}) and formic acid (5 kg tonne^{-1}) was more effective in inhibiting silage fermentation than when either additive was used alone.

Wilkins *et al.*[233] examined in detail the effects of different levels of formalin on the composition and nutritive value of ryegrass silages. Their results, summarised in Table 7.15, demonstrated that fermentation in silages made with formalin applied at a level of 8 litre tonne^{-1} and above was negligible. The addition of formalin at 12.3 litre tonne^{-1} and 17.2 litre tonne^{-1} depressed DM intake to very low levels and decreased significantly the DM and cellulose digestibilities. At the lowest level of formalin application (2.8 litre tonne^{-1}), butyrate and ammonia-N contents of the silage were higher than in the untreated silage. There is some evidence from other studies[234-237] that low levels of formalin application tend to encourage growth of clostridia, and it has been suggested that these organisms may be more resistant than lactic acid bacteria to low concentrations of formaldehyde.[238]

Effect of formaldehyde on silage proteins

The general effect of formaldehyde on proteins has been reviewed by Barry.[239] In most cases the initial step appears to be the rapid formation of a methylol compound:

$$R-XH + HCHO \rightleftarrows R-X-CH_2OH$$

XH in the above formula can be a terminal amino group, the ε-amino group of lysine, the primary amide groups of asparagine and glutamine, the guanidyl group of arginine, the hydroxy groups of threonine and serine, the sulphydryl group of cysteine, the phenol group of tyrosine, the phenyl group of phenylalanine, the indole group of tryptophan, or the imadazole group of histidine. Condensation reactions then occur slowly over a period of time, resulting in the formation of stable methylene cross linkages between and within protein chains:

$$R-X-CH_2OH + R^{\alpha}-NH_2 \rightarrow R-X-CH_2-NH-R^{\alpha} + H_2O$$

$$R-XH + R^{\alpha}-NH-CH_2OH \rightarrow R-X-CH_2-NH-R^{\alpha} + H_2O$$

The methylene linkages are considered to be hydrolysed under the acid-pepsin conditions in the abomasum, the liberated proteins are subsequently digested and the products are absorbed from the small intestine.

Because of these important reactions of formaldehyde with proteins, the level of additive which should be applied to harvested crops is critical and it should be closely related to the protein content of the ensiled material. If too much formalin is applied to herbage, it is possible for the rumen microorganisms to be starved of a supply of degradable nitrogen,[240-242] and if free formaldehyde (ie, not bound to

Table 7.15. The effects of different levels of formalin on the composition and nutritional value of ryegrass silage[233]

	Formalin added (litre tonne^{-1})					
	0	2.8	5.0	8.0	12.3	17.2
DM (g kg^{-1})	183	171	151	180	164	169
pH	4.2	4.6	5.1	5.4	5.4	5.3
WSC (g kg^{-1} DM)	11	14	131	217	222	235
Ash (g kg^{-1} DM)	91	91	89	93	81	85
Lactic acid (g kg^{-1} DM)	110	86	104	16	10	9
Acetic acid (g kg^{-1} DM)	58	34	14	6	6	5
Propionic acid (g kg^{-1} DM)	1	2	1	0	0	0
Butyric acid (g kg^{-1} DM)	3	14	4	0	0	0
Ammonia-N (g kg^{-1} TN)	67	84	47	20	16	8
Cellulose (g kg^{-1} DM)	274	293	306	252	256	241
DM intake* (g (kg LW$^{0.75}$)$^{-1}$ day^{-1})	60.2	56.0	56.7	57.2	23.9	13.9
DM digestibility*	0.801	0.767	0.729	0.746	0.646	0.602
Cellulose digestibility*	0.862	0.851	0.840	0.824	0.677	0.586

* Using sheep.

the nitrogenous components) is present, this could have additional effects of inhibiting the activity of the rumen microorganisms[233,243] or, more probably, changing their composition or numbers.[244,245] The presence of free formaldehyde in the silage could, if given to cows, result in some of the formaldehyde being transferred to the milk.[246] It has also been suggested that, if the level of formaldehyde applied to crops is too high, irreversible bonding can occur and protein digestibility in the small intestine will be reduced.[241]

Wilkinson *et al.*[247] suggested that a proposed safe and effective working range for the application of formaldehyde as a silage additive for grass should be 30 to 50 g formaldehyde kg^{-1} crude protein, and for unwilted lucerne about 100 to 150 g kg^{-1} crude protein. In the case of grasses, assuming an average DM content of 170 g kg^{-1} and a crude protein content of 140 g kg^{-1} DM, the recommended level of formalin application would be equivalent to 2.0 to 3.4 litre $tonne^{-1}$. At this concentration, however, the additive would have very little effect as a preservative (Table 7.15) and it would be possible for a clostridial fermentation to occur. In order to prevent this, formalin can be mixed with an acid prior to application.

Formalin and acid mixtures

The two most common acids used in commercial products are sulphuric and formic acids and a large number of experiments have been carried out using different combinations of formalin with these two acids.[35,197,235,245,248-259] Propionic acid has also been used in several experiments.[260,261] Because acids are liable to polymerise formaldehyde, an anti-polymerisation agent is usually added to commercial mixtures containing these compounds.[262]

Table 7.16 shows the results of one study by Barry[263] using herbage consisting predominantly of ryegrass and white clover, to which formalin, formalin-formic acid, or formalin-sulphuric acid had been added. In all three treatments the level of formalin applied was 4.8 litre $tonne^{-1}$ and the quantity of acid used in the two formalin-acid treatments was 1 litre $tonne^{-1}$. Although all the additive-treated silages were well preserved, the presence of acetic, butyric and lactic acids in measurable amounts indicated that some fermentation had occurred. The additives were effective in restricting proteolysis and also in reducing the deamination of amino acids, although ammonia concentrations were relatively high, especially in the formalin and the formalin-sulphuric acid-treated silages. The additives had no adverse effect in reducing the digestibility of the DM but the digestibility of the nitrogen was significantly decreased. Most striking of all were the increases in voluntary DM intake on the three additive-treated silage diets compared with the control.

In the above study by Barry, a relatively low concentration of formic acid was used with formalin compared with the normal recommended level of 2.3 litre $tonne^{-1}$ when used on its own. In a study by Hinks *et al.*,[264] formic acid was applied at a rate of 2.8 litre $tonne^{-1}$ with three different levels of formalin (1.40, 2.7 or 5.2 litre $tonne^{-1}$) on a fresh basis to wilted Italian ryegrass and the resulting silages were fed to cattle. Fermentation was inhibited in the three treated silages, increasing levels of formalin being associated with higher concentrations of soluble carbohydrates and lower concentrations of fermentation acids. Voluntary DM intake and animal performances on the treated silages were similar but

significantly higher than on the untreated control silage. It was suggested that an additive mixture containing about 2.8 litre tonne^{-1} formic acid and a low level of formaldehyde (about 19 g kg^{-1} crude protein) was probably the best combination for use with grass crops.

Kaiser *et al.*[258] treated red clover with formic acid (2 litre tonne^{-1}) together with formaldehyde at 0, 16, 34, 52, 77 or 117 g kg^{-1} crude protein, but when the silages were offered to young growing cattle the results of the trial demonstrated little advantage in favour of formaldehyde and serious disadvantages when large quantities of formaldehyde were applied. However, when Thomson *et al.*[245] applied formic acid (4 litre tonne^{-1}) with formaldehyde (50 g kg^{-1} crude protein) to perennial ryegrass, the amount of food protein escaping degradation in the rumen was increased and the flow of total amino acids at the duodenum was 33% higher in cattle offered formic acid-formaldehyde diets than in those offered the control silage. The results of a later experiment carried out by Kaiser *et al.*[262] were in general agreement with those of the previous experiments.[245,258]

Although formalin-acid mixtures are very effective on grass, their use has been banned in some countries. The potential health risks of exposure to formaldehyde are now recognised[265] and effort should be put into the containment of formaldehyde vapour where it is used.

Losses of formaldehyde during ensiling

The proportion of the formaldehyde applied to harvested crops which can be recovered in the silage progressively declines with time, and after 100 days may be

Table 7.16. The effect of formalin and formalin-acid mixtures* on the composition and nutritive value of ryegrass-clover silages[263]

	Untreated	Formalin	Formalin + formic acid	Formalin + sulphuric acid
DM (g kg^{-1})	197	216	194	215
pH	4.61	4.71	4.52	4.50
Buffering capacity (mE kg^{-1} DM)	899	698	728	768
Organic matter (g kg^{-1} DM)	879	865	869	865
TN (g kg^{-1} DM)	24.2	23.7	22.4	21.9
Protein-N (g kg^{-1} TN)	472	713	806	750
Ammonia-N (g kg^{-1} TN)	162	90	65	95
Acetic acid (g kg^{-1} DM)	36.1	23.1	24.2	22.7
Propionic acid (g kg^{-1} DM)	5.6	4.9	3.7	4.5
Butyric acid (g kg^{-1} DM)	28.8	26.8	22.1	24.2
Isobutyric acid (g kg^{-1} DM)	—	5.2	6.1	3.9
Lactic acid (g kg^{-1} DM)	31.4	17.6	24.7	24.3
2,3-butanediol (g kg^{-1} DM)	6.6	21.1	17.7	22.8
DM intake† (g (kg LW$^{0.75}$)$^{-1}$ day^{-1})	29.9	43.0	50.4	44.2
DM digestibility†	0.640	0.626	0.625	0.640
N digestibility†	0.618	0.537	0.499	0.529

* Application rates: formalin, 4.76 litre tonne^{-1}; formic acid, 1.00 litre tonne^{-1}; sulphuric acid, 1.00 litre tonne^{-1}.

† Using sheep.

as low as 20%.[239] It is suggested that losses of the additive during field application can be high.[239,266] In studies using [^{14}C] formaldehyde, Kreula and Rauramaa[267] found that 84% of the added radioactivity was present in the silage and only 12% was lost through evaporation. When the silage was treated with various solvents, it was found that 40 to 45% of the formaldehyde was tightly bound to the silage.

Aerobic stability of formaldehyde-treated silages

There are several reports in the literature which suggest that treatment of herbage with formalin-based additives produces silages which are aerobically more unstable than untreated ones,[203,254,268,269] although studies by Van Bockstaele *et al.*[254] suggest that this may only arise when low formalin levels are used.

Other aldehydes

Because of the high volatility of formalin, application losses can occur, leading to a wastage of the chemical and uncertainty about the amount of formaldehyde included with the ensiled crop.[238] Paraformaldehyde, a solid polymer containing about 82 to 99% formaldehyde, has a much lower volatility and hence should give rise to lower additive losses during ensiling. In a comparison of paraformaldehyde with formic acid, applied to cocksfoot-1ucerne at levels of 1.0 kg tonne^{-1} and 5.4 kg tonne^{-1} respectively, Waldo *et al.*[270] found that although paraformaldehyde was less effective than formic acid in inhibiting undesirable fermentation changes, daily liveweight gains in dairy heifers fed the two treated silages were nearly identical and considerably better than those obtained with the untreated silage (Table 7.17).

Table 7.17. The composition and nutritional value of cocksfoot-1ucerne silages treated with and without formic acid or paraformaldehyde[270]

	Untreated	Formic acid*	Paraform-aldehyde†
DM (g kg^{-1})	229	257	224
pH	5.13	4.42	4.85
TN (g kg^{-1} DM)	26.6	26.0	27.9
Ammonia-N (g kg^{-1} TN)	176	86	122
Protein-N (g kg^{-1} TN)	449	509	493
WSC (g kg^{-1} DM)	5	34	8
Acetic acid (g kg^{-1} DM)	45.8	18.7	48.3
Propionic acid (g kg^{-1} DM)	7.6	1.1	5.3
Butyric acid (g kg^{-1} DM)	12.7	0.7	5.6
Lactic acid (g kg^{-1} DM)	16.2	47.8	27.6
DM intake (g kg^{-1} LW day^{-1})‡	21.2	24.7	24.6
DM digestibility‡	0.553	0.614	0.576
N digestibility‡	0.578	0.609	0.554
Liveweight gain (g day^{-1})‡	258	736	690

* Formic acid (90%) 5.4 kg tonne^{-1}.
† Paraformaldehyde 1.0 kg tonne^{-1}.
‡ Using cattle.

When Cook *et al.*[271] added paraformaldehyde to perennial ryegrass and lucerne to provide about 0.1, 0.2 or 0.4% formaldehyde on a fresh weight basis, the paraformaldehyde addition restricted the fermentation in both crops and led to an increase in the proportion of TN which was insoluble in hot water. The fall in digestibility was similar in magnitude to that recorded for ryegrass and lucerne treated with similar levels of formaldehyde applied as formalin.[233] The results of these trials suggested that there is no justification for applying paraformaldehyde or formalin to ryegrass or lucerne at rates in excess of 0.2% formaldehyde tonne^{-1} crop fresh weight (50 to 60 g HCHO kg^{-1} crude protein). Santi *et al.*[199] also found paraformaldehyde to be an effective additive for lucerne silage.

The studies of Mangan *et al.*[272] suggested that glutaraldehyde may offer certain advantages over formaldehyde as a silage additive. Siddons *et al.*[273] compared the effects of formaldehyde or glutaraldehyde application to lucerne before ensiling on silage fermentation and silage-N digestion in sheep. Formaldehyde was more effective in restricting carbohydrate fermentation but the decrease in N degradability in the rumen and the increase in non-ammonia-N absorption from the small intestine were greater with glutaraldehyde.

Sulphur dioxide and sodium metabisulphite

Sulphur dioxide and sodium metabisulphite ($Na_2S_2O_5$) have been extensively investigated in silage studies, with improvements being obtained, but insufficient to compete with other additives.[75] Ensiling experiments using sulphur dioxide were carried out as early as 1885.[2] Silages treated with this gas have been shown to have lower lactic acid, volatile fatty acids and ammonia-N levels than untreated silages.[274] Mathison and co-workers[275,276] concluded from their studies with legumes, lucerne-grass and cereals that the use of sulphur dioxide as a preservative would be economically feasible under some circumstances for legume and lucerne-grass silages but they could not recommend its use for cereal silages.

Kroulik *et al.*[277] found that the addition of sulphur dioxide decreased the overall bacterial count in silage but not before more selective acid-forming microflora had become dominant.

The use of gases as silage additives is unlikely to prove attractive in practice because of difficulties in applying them to crops. An alternative approach is to use the solid, sodium metabisulphite, which contains 65 to 67% by weight of sulphur dioxide.[194] This additive, which dissolves in water to form sodium bisulphite and sulphur dioxide, is a strong reducing agent capable of using up large amounts of oxygen and it is considered to be of particular value in encouraging rapid anaerobic conditions in the silo.[2]

A large number of experiments was carried out in the 1950s using this material[2,194] and, in general, it was found that the additive reduced total acidity and contents of lactic, acetic and butyric acids, and ammonia-N in silages, although results have been conflicting.[105,123,278-280] In the study by Carpintero *et al.*[99] reported earlier (Table 7.6), sodium metabisulphite applied to lucerne at a level of 2.3 g kg^{-1} showed no improvement in fermentation over that of the control. When it was applied at a higher concentration (4 g kg^{-1}) to ryegrass, Macpherson *et al.*[281] found that the additive was an effective bacteriostatic agent but it did not inhibit proteolysis, a conclusion also reached by De Vuyst *et al.*[282]

When it was applied to red clover at a level of 5 g kg^{-1}, Kibe[283] found that the additive reduced the amounts of lactic, acetic, propionic, butyric and isobutyric acids in silage but increased the ammonia-N level compared with that in the control material. Several workers[2,194,284] have stressed the importance of adequately mixing the metabisulphite with the ensiled crop and some of the contradictory results obtained with this chemical may have resulted from different ensiling techniques used. Lusk[194] pointed out that slow filling and packing results in gradual oxidation of the metabisulphite and it becomes ineffective. Lanigan[77] indicated that, even though the additive inhibits undesirable fermentation when applied in adequate amounts, its activity is considerably reduced if temperatures in the silo exceed 30°C.

Ammonium bisulphate

This compound was tested as a potential silage additive by McCarrick and co-workers.[285-287] In a comparison with molasses, the bisulphate treatment produced silages which, when fed to cattle, caused acidosis and a reduced voluntary DM intake.

Sodium chloride

Common salt has long been used for the pickling and preserving of foods for human consumption, but early studies carried out in the 1930s and 1940s using salt as a silage additive gave disappointing results.[2] In a more recent study by Gouet *et al.*,[288] salt was added (10 g kg^{-1}) to fresh and wilted lucerne prior to ensiling but, when compared with untreated silage, no significant effects on conservation losses, fermentation quality and silage DM intake by sheep as a result of salt addition could be detected. Using a similar application rate, Schukking[289] found that DM intake by dairy cattle was significantly lower in treated silages compared with untreated material. In a laboratory study, Goering and Gordon[290] added sodium chloride (2 to 10 g kg^{-1}) to wilted cocksfoot, but found that mould was present in all treatments by the third day after ensiling. In a study using iodised salt, Voelker and Bartle[291] found that the consumption of DM was less than that of metabisulphite-treated and untreated silages.

Sodium hydroxide

Sodium hydroxide (NaOH) is the most effective alkali for the improvement of the digestibility of straws[292] and cereal grains.[293] In the 1980s there was some interest in the ensilage of whole-crop cereals treated with sodium hydroxide.[294-297] Whole-crop cereal silage is normally prepared when the crop is at an immature stage and when the digestibility is high. Cutting later, at the hard-dough stage, results in maximum yield but a lower digestibility.

Tetlow and Mason[295] concluded from their studies that whole-crop cereals directly harvested at the hard-dough stage, at approximately 600g DM kg^{-1}, can be effectively preserved and upgraded by ensilage with 50g NaOH kg^{-1} DM. At this maturity, crop yields are close to maximum and the potential nutritive value of the upgraded material is high. The same workers[296] found that, at this DM, 50 to 70g NaOH kg^{-1} DM not only increased the digestibility but also reduced the ammonia-N content of the silage and enhanced its aerobic stability. On low DM

crops, however, treatment with sodium hydroxide may not be beneficial.[294,297,298] When Sneddon *et al.*[294] treated sunflowers and lucerne-grass with sodium hydroxide, the silages had high pH values and contained more butyric acid than the untreated silages.

Antibiotics

A large number of antibiotics have been examined as potential silage additives, including achromycin,[299] aureomycin,[300] chloromycetin,[299] monensin,[301-303] neomycin,[300,304] nisin,[305,306] oleandomycin,[307] penicillin,[299,300,305,307] pimaricin,[308] streptomycin,[299] terramycin,[309] tylosin[308,310] and zinc bacitracin.[280,300,311–316] Because of the widespread use of antibiotics in the control of diseases in man, in most countries the use of antibiotics is strictly controlled and relatively few are classified as 'feed antibiotics' which can be added to foods intended for certain classes of farm animals.

Among these feed antibiotics, monensin and zinc bacitracin have been singled out for most study. McHan *et al.*[301] treated Coastal bermudagrass with 0 to 375 mg kg^{-1} of monensin and found that monensin inhibited the fermentation in the silage. Digestibility *in vitro* of monensin-treated silage decreased as monensin level increased. Owens and Prigge[302] showed an increase in lactic acid production for monensin-treated corn silage up to 33 mg kg^{-1}, but a drop in lactic acid production at higher concentrations. Results of experiments using zinc bacitracin have been conflicting. In some studies zinc bacitracin delayed fermentation,[300] improved fermentation quality,[311] produced silages with increased voluntary DM intake potential[312] and increased DM digestibility. In other studies no difference in fermentation quality,[315] DM intake,[280] nor DM digestibility[304] could be detected. There is some evidence to indicate that certain strains of lactic acid bacteria are inhibited by zinc bacitracin to a greater extent than are clostridia.[317]

Other fermentation inhibitors

Other fermentation inhibitors which have been tested as potential silage preservatives include carbon dioxide,[2,318] carbon bisulphide,[2] hexamethylene-tetramine,[319] Bronopol (2-bromo-2-nitropropane-1,3-diol)[320] and sodium chlorite.[321]

AEROBIC DETERIORATION INHIBITORS

The major organisms responsible for the aerobic deterioration of silages have been identified in Chapter 4. These organisms, which include yeasts, bacteria and moulds, are thought to be mainly indigenous to the silage and are not aerial-borne invaders.[322] Woolford *et al.*[323] treated grass silages with antibiotics which were specifically antimycotic or antibacterial and demonstrated that yeasts are instrumental in the aerobic deterioration of both direct-cut and wilted silage, particularly if air has been permitted to enter the silo during the fermentation. Potential deterioration inhibitors for grass silage must therefore inhibit the growth of yeasts. Another type of inhibitor is required in silages where different microorganisms, for example moulds, aerobic bacteria or acetic acid bacteria, are responsible for aerobic deterioration.

Lactic acid bacteria

Pahlow and Zimmer[324] ensiled prewilted grass with and without an inoculum of a *Lactobacillus* culture. The bacterial inoculant restricted the development of yeasts and the treated silages remained stable for at least two days after unloading. Untreated silages had yeast counts up to 10^7 g^{-1} silage DM and started to deteriorate immediately on exposure to air. Pahlow and Honig[325] claimed that inoculants of lactic acid bacteria can prolong the stability of silages if the crop to be ensiled contains a sufficient amount of sugars. Within the range of unavoidable air infusion, inoculation can inhibit the silage yeasts which otherwise would proliferate in the presence of oxygen. Under aerobic conditions lactic acid bacteria will produce acetate and lactate. At low oxygen concentrations the acetate will inhibit the yeasts, but at higher oxygen concentrations the yeasts are able to detoxify the acetate and use it as an energy source.[324] Even under suboptimal ensiling conditions, added lactic acid bacteria will increase the content of lactic acid, lower the pH quickly and reduce the viable counts of yeasts and moulds, thus increasing the aerobic stability of the silage.

Propionic acid

Propionic acid has been widely used as a microbial inhibitor in the preservation of stored grain, and several workers have shown that this acid can also have a beneficial effect in inhibiting the aerobic deterioration of silage.[203,206,212,326-334] In a study by Woolford and Cook,[334] propionic acid applied to maize at a rate of 12.5 g kg^{-1} DM reduced deterioration in low DM silage and prevented it in high DM silage over a period of 19 days (Table 7.18).

Daniel *et al.*[206] ensiled a grass-clover mixture with two levels of propionic acid treatment (3 g kg^{-1} and 10 g kg^{-1}) at three different DM contents (300, 440 and 500 g kg^{-1}). When the silages were exposed to air over a nine-day period, the untreated silages were all unstable while the lowest level of propionic acid application delayed the deterioration. At the highest level of acid addition, the silages were all aerobically stable over the exposure period. The highest DM losses during this period occurred in the high DM (500 g kg^{-1}) untreated silages (8.4%), with corresponding losses of 3.3% and 1.1% for the low and high propionic acid silages respectively.

The effect of different levels of sodium propionate on the growth of moulds isolated from silages has been studied by Barry *et al.*[335] and their results are summarised in Table 7.19. The addition of sodium propionate to potato dextrose

Table 7.18. The losses of DM (%) in propionic acid treated maize silages during exposure to air for 19 days at an ambient temperature of 15°C[334]

	Silage DM (g kg^{-1})		
Propionic acid (g kg^{-1} DM)	200	280	340
0	17.5	18.9	20.5
12.5	9.5	2.7	0
25.0	6.0	0.1	0

Table 7.19. Influence of sodium propionate on growth of isolated moulds in the laboratory*[335]

Mould	Sodium propionate (g kg^{-1})†				
	0.0	0.5	1.0	5.0	10.0
Byssochlamys sp.	1.5	1.0	0.5	0	0
Fusarium sp.	1.5	1.0	0.5	0	0
Geotrichum candidum	2.3	2.0	2.0	2.0	1.5
Humicola sp.	3.5	3.0	1.7	0.5	0
Petriellidium boydii	1.3	1.3	1.0	0.7	0.7
Scopulariopsis brevicaulis	1.0	1.0	1.0	0	0
Trichoderma viride	5.0	4.5	3.7	1.5	1.0

* Values represent colony diameter (cm) after three days' growth on potato dextrose agar.
† pH of media = 6.5.

at a concentration of 1 g kg^{-1} resulted in some reduction in growth of most of the moulds, while at 5 g kg^{-1} the additive was even more effective. At the highest level of application (10 g kg^{-1}) most moulds were completely or severely restricted, with the exception of *Geotrichum candidum* which was the most resistant of the moulds tested.

Hara and Ohyama[329] added propionic acid to maize silages (7.4 g kg^{-1}) and found that all untreated silages deteriorated in three days, while three out of five treated silages remained stable for seven days. Application of propionate reduced the growth of *Saccharomyces exiguus* but the growth of *Pichia membranaefaciens* was not affected, suggesting that this yeast organism was tolerant to propionic acid. In another study these workers[331] demonstrated that, to prevent aerobic deterioration, wet silages required higher levels of propionic acid than did high DM silages.

It would appear that propionic acid inhibits most, but not all, organisms responsible for silage deterioration, but only when it is applied to crops in relatively high concentrations.[328,336] Woolford[166] has shown that the antimicrobial effect of propionic acid increases with decreasing pH, but even with the synergistic effect on aerobic deterioration derived from a mixture of formic and propionic acids a high level of application was required to obtain a substantial effect.[332]

Higher volatile fatty acids

Several workers have demonstrated that clostridial silages are more stable in the presence of oxygen than are well-preserved lactate silages,[322,333,337,338] and this increased stability can be partly attributed to the presence in these silages of higher volatile fatty acids such as butyric, isovaleric and caproic[339] acids. Caproic acid (hexanoic acid) has been singled out for special study.[340-344] In general, caproic acid applied at the time of ensiling in concentrations of 4 to 6 g kg^{-1} will effectively inhibit the aerobic deterioration of silages. The application of a lower quantity of caproic acid is desirable from the economic point of view. As the

inhibitory effect of caproic acid is greater at a low pH, Ohyama *et al.*[342] added hydrochloric acid with the caproic acid. The combined acids were more effective than caproic acid alone at inhibiting the fermentation, but not the aerobic deterioration.

Most studies using caproic acid have been confined to laboratory experiments. There is little information to indicate how this acid may influence the nutritional value of treated silages, although when silages treated with a commercial product containing a mixture of hexanoic and heptanoic acids were given to sheep voluntary DM intake was depressed.[345]

Other aerobic deterioration inhibitors

Gross and Beck[326] drew attention to the importance of sorbic acid as a potential deterioration inhibitor, and Woolford[215] confirmed that this acid had a strong inhibiting effect on the growth of yeasts and moulds. Acrylic acid, on the other hand, is effective against bacteria and therefore has a place in ensiling whole-crop maize,[218,346] but Woolford[222] found that it had little effect on yeasts and moulds.

Wilson *et al.*[223] found that acrylic acid and its sodium salt, applied at a rate of 2.5 g kg^{-1} and 3.3 g kg^{-1} respectively to maize, were effective in preventing deterioration of silages over a 13-day exposure period. However, this result with maize was in contrast to that occurring in ryegrass, where these additive treatments produced silages which were less stable than those made without the additive. This difference in stability between the silages from the two crops indicated that, while effective against bacteria in maize silage, acrylic acid and sodium acrylate had little effect on the ryegrass silages, where yeasts were probably the major degradative agents. Uchida *et al.*,[347] however, did find acrylic acid and sodium acrylate effective at preventing post-opening deterioration of high moisture ryegrass silage.

There are several reports indicating that the addition of non-protein nitrogenous compounds to maize tends to stabilise the silages under aerobic conditions.[348-351] Both urea and ammonia appear to have this effect, although in the studies carried out by Honig[349] ammonia was more effective than urea in reducing DM losses during deterioration.

Ammonium isobutyrate has been shown to have a desirable antimicrobial spectrum, as it discriminates against the spore-bearing bacteria and to some extent moulds.[217] Yu and Thomas[209] showed that silage surface temperatures during storage and feeding were decreased in ammonium isobutyrate-treated lucerne haylage compared with untreated materials, and Koželuhová *et al.*[352] noted an inhibitory action of both isobutyric acid and ammonium isobutyrate on the development of yeasts and yeast-like fungi when maize silage was exposed to the air for 72 hours.

The antibiotics pimaricin[308,353,354] and Mylone[355] have been shown to have an inhibitory effect on silage deterioration. Woolford *et al.*[354] found that pimaricin had no apparent effect on the course of the fermentation nor on the composition of the silages, but the beneficial effect on the aerobic deterioration occurred even though only from 1 to 66% of the pimaricin initially applied was recovered after anaerobic storage in the silos. However, the high cost of pimaricin relative to other silage additives would preclude its commercial use.

NUTRIENTS

Nutrient additives are defined as substances which, when added to ensiled materials, contribute significantly to the nutritional needs of animals consuming the silage.[118] Many of the additives described earlier under fermentation stimulants, such as molasses, cereals, whey, beet pulp and citrus pulp, can also be included under the heading of nutrients. However, in this final section attention is concentrated on two categories of additives which are used primarily to improve the nutritional value of silage and normally have no beneficial role in improving the fermentation.

Nitrogenous compounds

Certain crops, such as maize, are nutritionally deficient in nitrogen and, when fed to ruminants as silage, require supplementing with a protein-rich food. An alternative method is to improve the crude protein content of the silage by adding urea to the crop at the ensiling stage.

When applied to maize, urea produces silages with higher pH values and fermentation acid contents than those found in untreated silages.[356-361] This is illustrated by the results shown in Table 7.20, which relate to a study by Lessard *et al.*[362] in which the changes in composition of ensiled Canadian grown maize treated with and without urea were followed over a 20-day period. In spite of the higher content of fermentation acids, the pH values of the treated silages were higher than those of the untreated materials, a result which can be explained by the increased buffering capacity resulting from ammonia released from the urea.

Urea addition also had a marked effect on the nitrogenous components of the silages, resulting in increases in crude protein, true protein, free amino acids and ammonia. These results indicate that not only was there a sparing effect on true protein due to the addition of urea, but there was also microbial synthesis of protein. Other workers have also found increases in true protein in silages treated with urea[363] and isotope studies using [^{15}N] have confirmed that the added urea-N is the origin of this synthesised protein-N.[364-366]

Lessard *et al.*[362] also found that treatment with urea resulted in an increase in many of the individual amino acids, including in particular alanine, aspartic acid, glutamic acid, isoleucine, lysine, threonine and valine. In a study by Thomas *et al.*,[367] maize grown in the UK was ensiled at three DM contents (226, 270, and 416 g kg^{-1}) with four levels of urea (5, 10, 15, and 20 g kg^{-1} DM). Although pH values increased and total fermentation acids fell with increasing DM content of the crop, addition of urea had little effect on these parameters. These results differ from those of Lessard *et al.*[362] and other workers and it was suggested that the lower soluble carbohydrate content of maize crops grown in the UK compared with those grown in America could have been a contributory factor in causing similar fermentation patterns in urea-treated and untreated silages.

Ammonia has also been used as an additive in silage making, mainly to improve the nitrogen content of the product. It can be added as anhydrous ammonia, aqueous ammonia solution or ammonia-mineral solution. Many commercial additives contain molasses and minerals.[368-372] The molasses ensures that additional fermentation acids are produced to offset the increased buffering effect of the ammonia. In addition to increasing the insoluble nitrogen content of maize silage, ammonia has been shown to reduce proteolysis during ensilage[373-376] and

Table 7.20. Changes in the composition (g kg^{-1} DM) of maize silages treated with (+) and without (−) urea (6 g kg^{-1}) at the time of ensiling[362]

Days	pH		Acetic acid		Lactic acid		Crude protein		True protein		Free amino acids		Ammonia		Urea	
	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
0	5.90	5.88	2.7	4.8	0.2	0.1	89	145	50	50	3	4	0.37	1.53	0.25	12.00
2	3.91	3.56	9.5	14.3	16.8	27.8	81	195	37	39	9	10	0.78	5.03	0.04	17.39
5	3.44	3.46	12.9	21.5	27.4	44.7	109	182	42	59	7	11	0.87	5.72	0	15.66
10	3.40	3.51	13.0	20.1	99.5	53.4	92	190	43	55	9	16	0.66	5.18	0	17.14
15	3.22	3.47	13.8	20.0	61.7	70.3	93	182	40	47	12	21	0.79	5.84	0	15.75
20	3.18	3.58	13.2	26.5	85.1	154.7	84	207	37	51	10	25	0.72	7.23	0	18.48

to increase the stability of the silage.[377] Kung *et al.*[378] found that ammonia treatment increased the lactic acid content in lucerne silages ranging in DM content from 300 g kg^{-1} to 600 g kg^{-1}, but when Deschard *et al.*[379] ensiled whole-crop winter wheat, of DM 410 g kg^{-1}, with ammonia or urea the lactic acid content was reduced and the silage was pungent.

Ely[76] has reviewed the large number of feeding trials carried out on silages treated with additives containing ammonia and the results of these studies have been shown to be very variable. In some experiments animal performance was improved on treated silages compared with controls, whereas in other studies no improvements were noted.

In studies with biuret, Condon *et al.*[380] and Pereira and da Silva[381] found that this additive caused smaller increases in silage fermentation acids and pH than urea. Other nitrogenous compounds which have been tested as silage additives include ammonium carbonate, ammonium bicarbonate, ammonium sulphate and ammonium nitrate.[118,382]

Minerals

In addition to being nutritionally deficient in nitrogen maize silage is also a poor source of calcium, and improvements in animal performance have been noted on silages treated with limestone and urea compared with silages treated with urea only.[76,383]

Provided the ensiled crops contain adequate levels of soluble carbohydrates, sufficient amounts of lactic acid can normally be produced to counteract the alkaline nature of the calcium salt.[384] In some studies with maize the addition of limestone has, however, resulted in clostridial-type silages containing high concentrations of butyric acid.[385,386]

Magnesium compounds are sometimes added to silage as a means of preventing hypomagnesaemia.[387-389] In one study involving outwintered cattle,[387] the addition of kieserite (magnesium sulphate) to harvested grass (2.3 kg $tonne^{-1}$) raised the magnesium content of the silage from 1.9 to 3.2 g kg^{-1} DM. When the treated silage was fed to the cattle, blood levels were markedly increased above those of the animals given untreated silage.

ABSORBENTS

Ensiling grass without wilting and the use of acid additives have exacerbated the problem of pollution from silage effluent.[390] Absorbents have the potential for reducing effluent production, but the efficiency of absorption is influenced by the physical characteristics of the crop, method of application, silo design and drainage. Several materials used to absorb effluent have been mentioned in Chapter 6.

Barley

Rolled barley incorporated in layers in the grass at the rate of 50 kg $tonne^{-1}$ grass has been shown by Jones and Jones[391] to reduce effluent flow in the silo by up to 50%. These workers reported that the silage had a higher DM content, a higher metabolisable energy (ME) content, and contained less ammonia-N than the untreated silage. When the same workers[392] incorporated whole barley in the

silage the results were disappointing. The whole barley was less effective than rolled barley at absorbing effluent, and when steers were offered the silage approximately 20% of the whole grains appeared in the faeces. This was reflected in the liveweight gains, 0.77 kg and 0.90 kg per head per day for the whole barley and rolled barley inclusion respectively.

Straw

Mixing straw with grass is an accepted technique for reducing effluent loss but a number of disadvantages exist in practice, including the effects on the fermentation and on silage quality. Jakhmola *et al.*[393] added barley straw to grass (DM 228 g kg^{-1}) in the ratio of 60% grass to 40% straw on a DM basis, to evaluate these effects. The addition of straw did not adversely affect the pH value nor ammonia-N content but lactate and acetate levels were markedly lower in the mixed silage. Overall, the composition of the silages closely reflected the difference between the grass and grass plus straw at the outset. Over two seasons, Offer and Al-Rwidah[394,395] compared the absorptive capacities of chopped, baled, or alkali-treated and pelleted straw (Table 7.21). Perennial ryegrass, with DM contents of 148 g kg^{-1} and 122 g kg^{-1} fresh matter in years one and two respectively, was treated with 4.5 litre 85% formic acid $tonne^{-1}$, and straw at the rate of 60 kg $tonne^{-1}$ and 75 kg $tonne^{-1}$ in years one and two respectively. Straw bales at the base of the silo improved the drainage and increased the effluent flow. Chopped straw in layers throughout the grass decreased effluent production by over 50% but it reduced the quantity of grass which could be stored in the silo by 45% and reduced the nutritive value of the silage.

Sugar beet pulp

Absorbent-type products based on sugar beet pulp are now available commercially. These come in the form of nuts, shreds, or pellets with or without added molasses. They may also contain an inoculum of lactic acid bacteria. The incorporation of molassed sugar beet shreds has been shown to reduce effluent production and to improve fermentation quality.[392,394-398] Jones[392] found that the maximum effect was obtained when the sugar beet pulp was intimately mixed by hand with the grass in the silo. When a fertiliser spreader was used to apply the sugar beet pulp in layers, it created lateral drainage channels across the silo which increased the drainage and reduced the absorption of effluent. Offer and Al-Rwidah[396] stated that beet shreds must be used in sufficient quantity for the total prevention of effluent, since any effluent which is produced will be more concentrated. Kennedy[398] found that the inclusion of sugar beet shreds at the rate of 30 kg $tonne^{-1}$ on grass with a DM content of 190 g kg^{-1} had little effect on the ME content of the silage but DM intake and liveweight gain were significantly higher when the silages were offered to mature beef cattle.

Acrylamide-based polymers

Although most acrylamide-based polymers are sensitive to low pH and high osmotic pressure and shrink, partially or completely losing their fluid-imbibing capacity, some of these polymers appear to have a potential application in reducing silage effluent loss.[399,400] In a laboratory experiment, Thomas *et al.*[399]

Table 7.21. Silage composition* and effluent losses from 10 tonne silos[395]

Treatment		Effluent			Silage			
		Volume (litre tonne^{-1})	Organic matter (g kg^{-1})	(kg tonne^{-1} silage)	DM (g kg^{-1})	Ammonia-N (g kg^{-1} TN)	ME (MJ kg^{-1} DM)	Density (tonne metre^{-3})
No absorbent		137	29	4.0	156	70	10.8	1.18
Alkali-treated	L†	114	41	4.7	201	81	9.9	1.32
straw pellets	B‡	130	47	6.1	155	80	10.6	1.43
Chopped straw	L†	53	25	1.3	164	61	9.8	1.90
Straw bales	B‡	146	25	3.6	168	79	10.4	2.20
Beet shreds	L†	71	42	3.0	169	69	11.1	1.53

* Second cut perennial ryegrass (DM 148 g kg^{-1}) treated with 4.5 litre 85% formic acid tonne^{-1}; absorbents added at 60 kg tonne^{-1} of fresh grass.
† = Absorbent in layers.
‡ = Absorbent at bottom.

demonstrated that some of these polymers can be used with no apparent adverse effects on silage fermentation. Effluent loss was reduced progressively as the rate of application of the polymer was increased from 0 to 10 g kg^{-1} grass. When Mayne[400] treated a perennial ryegrass sward with an inoculum of *L. plantarum* (10^6 cfu g^{-1}) with or without an absorbent polymer (ammonium polyacrylamide) at the rate of 1 kg $tonne^{-1}$ the effluent output was reduced by 20% relative to the inoculant treatment, but the DM loss as effluent was reduced by only 14%.

Bentonite

The colloidal clay bentonite has been found to be an extremely effective absorbent.[401] However, Woolford and co-workers concluded that it would reduce the nutritional value of the silage.[402,403]

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Chapter 8

Losses During Ensilage

The efficiency of any conservation system must take into account not only the nutritional value of the product but also the losses which occur between the harvesting and feeding stages. In a survey of about 800 experiments carried out between 1938 and 1960, Watson and Nash[1] quoted mean DM losses during ensiling of 16.1%. In 1967, Zimmer[2] analysed the DM losses from 504 experiments involving herbage with a range of DM contents and found DM losses varied from 0.8% to 71%, with a mean value of 19.4%. From these data, Zimmer devised a regression equation which related %DM loss to type of silo and herbage DM content. In 1985 Bastiman and Altman[3] published the results of 205 silage experiments carried out on ADAS Experimental Husbandry Farms. Total DM losses averaged 24.7% for unwilted silages and 31.9% for wilted silages.

About the same time the results of a co-ordinated experiment involving eight European countries were published.[4] This study was designed to compare silage made from unwilted grass ensiled with an additive and wilted grass ensiled without an additive. The total losses of DM in 23 experiments averaged 18.6% for unwilted silages and 16.5% for wilted silages. A model of the pattern of losses,

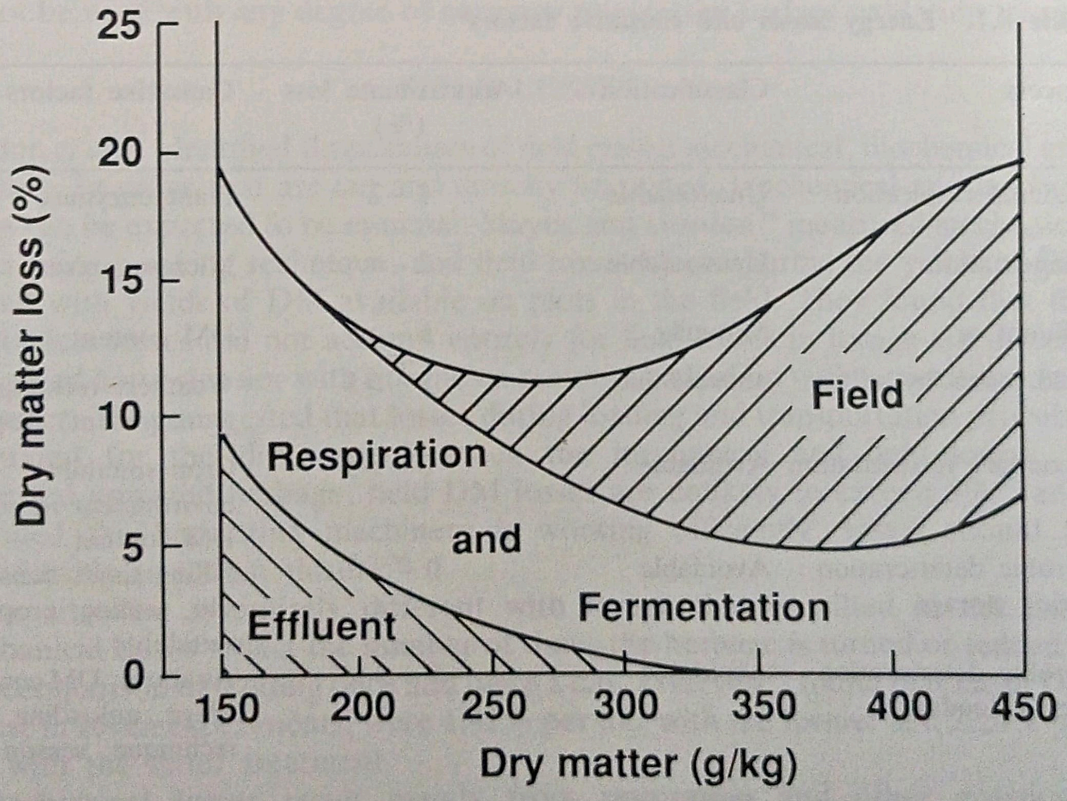


Figure 8.1. Model of dry matter losses in well managed silage systems[4,5]

derived by Wilkinson[5] from the data obtained in this European study, is shown in Figure 8.1.

Zimmer and co-workers[6-9] categorised the losses during the ensiling process and a classification of these, together with the causative factors, are shown in Table 8.1. Zimmer concluded that unavoidable energy losses during ensilage need be no higher than 7%.

MEASUREMENT OF LOSSES

The measurement of true losses of DM and nutrients during ensilage is extremely difficult. In order to simulate field conditions it is necessary to use large silos, preferably farm scale, but this is extremely laborious, involving weighing and sampling each load of material entering and leaving the silo. In order to simplify this procedure, many workers have constructed scaled-down tower or bunker silos designed specifically for measuring losses; some of these have already been described in Chapter 1.

The accurate measurement of DM changes depends upon obtaining a representative sample of material for DM determination. Gross and Averdunk[10] concluded that the main error occurring in measurements of losses was caused by inaccurate sampling of the ensiled herbage. Furthermore, the determination of DM itself presents difficulties because of losses of volatiles such as lower fatty acids and alcohols on oven drying.[11-17] Several alternative methods to oven drying have been proposed including freeze drying,[18] the use of desiccants,[19] the Karl Fischer titration technique[20] and methods involving gas chromatography.[18,21-23] Probably the commonest method in current use is the direct assessment of

Table 8.1. Energy losses and causative factors[6]

Process	Classification	Approximate loss (%)	Causative factors
Residual respiration	Unavoidable	1 – 2	Plant enzymes
Fermentation	Unavoidable	2 – 4	Microorganisms
Effluent or	Mutually	5 – >7	DM content
Field losses by wilting	unavoidable	or 2 – >5	Weather, technique, management, crop
Secondary fermentation	Avoidable	0 – >5	Crop suitability, environment in silo, DM content
Aerobic deterioration during storage	Avoidable	0 – >10	Filling time, density, silo, sealing, crop suitability
Aerobic deterioration after unloading	Avoidable	0 – >15	As above, DM content, silage, unloading technique, season
Total		7 – >40	

moisture content using a toluene distillation technique involving corrections in the distillate for fermentation acids[24] and alcohols.[25] If volatile losses on drying are not taken into account the errors in DM determinations may, in some cases, exceed 20%.[17,26]

The assessment of the amount of 'waste' material is another source of error, since this is invariably determined on the basis of visual appearance. Serious underestimates of surface waste material can arise if the waste is simply weighed and sampled for DM analysis. During the formation of this material a large amount of DM is lost in gaseous form, and this must be taken into account in addition to the weight of waste remaining. The only certain technique of measuring total loss in waste formation is that derived by Wittwer *et al.*.[27] This involves placing a marker sheet made of rot-proof netting on the surface of the herbage when the silo is about two-thirds to three-quarters full. The amount of herbage placed above the sheet is weighed and this weight is compared with that of the resulting silage after subtraction of the waste material. The weight of well-preserved silage above the marker sheet is corrected for fermentation losses by reference to DM changes in rot-proof mesh bags containing herbage buried in the well-preserved region immediately above the marker sheet.

Buried bags have also been used successfully by a number of workers to assess overall losses during ensiling.[27-32] Wittwer *et al.*[27] used 100 paired bag samples of 1 kg herbage and distributed one of each pair throughout full-scale tower silos. The other bags were used for a DM determination of each paired sample. Hagemeister[30] used a similar method but increased the size of the sample to 4 kg. Ruxton[31] also used bags of 4 kg capacity and found that the buried bag technique compared well with the input-output balance method, provided a large enough number of bags was used. A disadvantage of the buried bag method is that it cannot be used with any degree of accuracy to measure surface oxidation losses.

FIELD LOSSES

Gordon *et al.*[33] identified three causes of field losses: mechanical, biochemical and leaching. In crops that are cut and directly harvested, biochemical and leaching losses can be expected to be minimal. Mayne and Gordon[34] measured mechanical losses using a raking technique, and field losses by comparing the yields of DM ensiled with yields of DM available on plots in the field. They found that the mechanical losses did not account entirely for field losses in forage cut directly with a flail harvester nor with pre-mown material picked up with a precision-chop harvester. They suggested that losses during loading and transportation probably accounted for the difference between the mechanical and field losses. In harvested unwilted herbage, field DM losses are unlikely to exceed 5%[35] and, provided the harvesting machinery is working efficiently, losses should be considerably less than this.[4,5,36]

The situation is entirely different with forage that is wilted in the field. Mechanical losses reflect the number of times the herbage is turned or tedded.[37] In a comparison of tedding once and twice a day, Overvest[38] found that mean DM losses, in seven experiments, were 1.41% per day with the former and 2.24% per day with the latter treatment.

Biochemical losses result mainly from respiration and other enzymatic processes occurring in the plant after harvesting. The nutrients affected and the

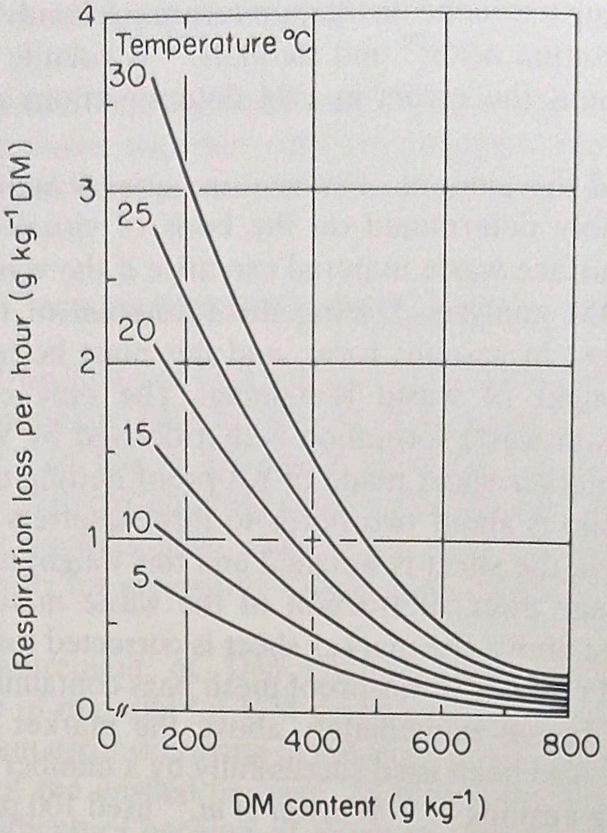


Figure 8.2. DM loss by respiration in grass[37]

factors controlling the rate of activity of these processes have been described in Chapter 3. Wilkinson and Hall[39] wilted lucerne in respiration chambers at various temperatures and found that maximum DM loss was 4% per day, which was obtained in immature herbage maintained at a temperature of 27°C. Honig[37] demonstrated that respiration intensity (respiration loss per hour) increased according to quadratic functions with decreasing DM content and increasing temperature (Figure 8.2). If drying is prolonged, especially in rainy weather, microbial growth on the harvested crop can increase the losses of nutrients quite dramatically.

The effect of rain on losses during wilting was studied by Kormos and Chestnutt[40] in experiments using simulated rain. Increasing amounts of rain resulted in increasing DM losses. There was an interaction between the effect of mechanical treatment and rainfall on losses; bruising of the grass prior to wilting increased the ill effects of the rain. Van Bockstaele *et al.*[41] measured the field

Table 8.2. Influence of weather conditions on average field period, increase in dry matter content and dry matter losses during the wilting period (manual tedding)[41]

Weather conditions	No. of experiments	Field period (days)	DM content increase (g kg^{-1} DM)	% DM losses	
				Total	Per unit DM increase
Without rain	6	2.3	292	3.9	0.014
Rain only on one day	4	4.5	251	4.0	0.015
More than one day's rain	9	6.7	223	9.8	0.044

losses during wilting grass in 20 experiments carried out in Belgium over a period of years, and found that a wilting period of two or three days with good drying conditions was sufficient to obtain a DM content of 350 to 450 g kg^{-1} and resulted in low DM losses (3 to 4% for manual tedding and 5 to 7% for mechanical tedding). Rain on partially wilted material lengthened the wilting period and doubled the DM loss (Table 8.2).

In the Netherlands it is common practice to ensile heavily-wilted grass (approximately 500 g kg^{-1}) and the average length of the wilting period is four to five days. Schukking and Overvest[42] found that in 20 different trials with pure grass swards, carried out over several years, losses of DM during a short period of wilting (two to four days) were only 1.2% per day, a figure which agrees well with that reported by other Dutch workers.[43,44] Under very prolonged periods of wilting (9 to 14 days) Schukking and Overvest reported DM losses of 1.7% per day.

FERMENTATION LOSSES

The losses arising from fermentation depend upon the nutrients fermented and the organisms responsible. Because several of the products of fermentation are of higher gross energy value than the substrates (Table 8.3), losses of DM are greater than energy losses during fermentation.

Table 8.3. Gross energy values (kJ g^{-1}) of some forage and silage constituents

Glucose	15.64	Acetic acid	14.60	Crude fibre	17.45
Fructose	15.70	Butyric acid	24.93	Ether extract	38.53
Sucrose	16.51	Lactic acid	15.16	Protein	24.60
Starch	17.70	Ethanol	29.80	Citric acid	10.33
Cellulose	17.49	Mannitol	16.73	Malic acid	10.00

From a consideration of the biochemical pathways detailed in Chapter 4, it can be calculated that energy losses during the fermentation of sugars and organic acids by lactic acid bacteria are virtually nil, while DM losses range from nil to 33% (Table 8.4). Using the derived values shown in Table 8.4, it is possible to calculate the DM and gross energy (GE) changes in a theoretical silage model system.[45]

In the example shown in Table 8.5, which is typical of an unwilted ryegrass of high soluble carbohydrate content (200 g kg^{-1} DM), it is assumed that the fermentation is dominated by the heterofermentative lactic acid bacteria. The calculated GE values of the grass and silage are 17.74 MJ kg^{-1} DM and 18.42 MJ kg^{-1} DM respectively, representing a GE concentration increase during ensiling of 3.8% The DM loss is 4.2% while GE loss is only 0.5% Silage studies using laboratory silos have confirmed that energy is almost completely recoverable in a closed fermentation system, in which lactic acid bacteria have dominated the fermentation,[45] and many workers have noted considerable increases in GE concentration during ensilage (p. 258).

The theoretical example given in Table 8.5 is a simplified picture of the changes which would occur in practice. In the calculations no account has been taken of the fermentation of the amino acids, serine and arginine, by lactic acid bacteria

Table 8.4 Dry matter and gross energy losses calculated from some important fermentation pathways

	Loss (%)	
	DM	Energy
A. Lactic acid bacteria		
Homofermentative		
glucose (or fructose) + 2 ADP + 2 Pi → 2 lactate + 2 ATP + $2H_2O$	0	0.7
2 citrate + ADP + Pi → lactate + 3 acetate + 3 CO_2 + ATP	29.7	+1.5
malate → lactate + CO_2	32.8	+1.8
*Heterofermentative**		
glucose + ADP + Pi → lactate + ethanol + CO_2 + ATP + H_2O	24.0	1.7
3 fructose + 2 ADP + 2 Pi → lactate + acetate + 2 mannitol + CO_2 + 2 ATP + H_2O	4.8	1.0
B. Clostridia		
2 lactate + ADP + Pi → butyrate + 2 CO_2 + 2 H_2 + ATP + H_2O	51.1	18.4
C. Enterobacteria		
glucose + 3 ADP + 3 Pi → acetate + ethanol + 2 CO_2 + 2 H_2 + 3 ATP + 2 H_2O	41.1	16.6
D. Yeasts		
glucose + 2 ADP + 2 Pi → 2 ethanol + 2 CO_2 + 2 ATP + 2 H_2O	48.9	0.2

* Citrate and malate fermentations are the same as for the homofermentative lactic acid bacteria.

which could produce a further DM loss of about 0.5% to 1%,[46] resulting in a total DM loss of about 5%. In the above calculation, it has been assumed that the fermentation was primarily of the heterofermentative type. If the homofermentative lactic acid bacteria had been dominant, DM losses would have been lower.

Confirmation of these calculated fermentation losses from practical silage studies is difficult because of the problem of separating these from initial oxidation losses. The most reliable results are those derived from experiments in which herbage is ensiled in small silos which have been rapidly filled and sealed. Anderson and Jackson,[47] using airtight metal containers of 1 metre^3 capacity, obtained losses of 2.7% to 10% for heavily-wilted herbage of DM content 380 to 480 g kg^{-1}. Brown and Kerr,[48] using plastic-lined trench silos, obtained losses of 5% and 8% for heavily-wilted herbage. Shepherd *et al.*[49] indicated a minimum loss of 1% from wilted lucerne/grass silage stored in a gas-tight silo. More recently Honig and Zimmer,[50] using gaseous exchange measurements, found that DM losses of 2% occurred in gas-tight silos in the first 10 days. After that no additional loss occurred over a 95-day ensiling period.

In a summary of results obtained at Edinburgh for well-preserved silages, using 1 tonne capacity tower silos, fermentation plus initial oxidation losses in 22 experiments averaged 3.4%.[46] Using larger silos of 100 tonne capacity, Mayne

Table 8.5. Energy and dry matter changes in a theoretical silage model[45]

A. Composition of grass (g kg^{-1} DM)	
Fructan	90
Sucrose	30
Glucose	40
Fructose	40
Citrate	15
Malate	15
Crude protein	180
Ether extract	35
Cell wall polysaccharides	48
B. Total substrates available (after hydrolysis of WSC) and type of fermentation (g kg^{-1} DM)	
Fructose (yeast)	55.9
Fructose (heterolactic)	89.5
Fructose (homolactic)	11.2
Glucose (heterolactic)	56.5
Citrate (heterolactic)	15
Malate (heterolactic)	15
C. Energy balance	
Energy ensiled in 1 kg grass DM (MJ)	17.74
Energy removed in 0.958 kg silage (MJ)	17.65
Energy loss (MJ)	0.09
Energy loss as % energy ensiled	0.51
DM loss as % DM ensiled	4.20
GE content of grass DM (MJ kg^{-1} DM)	17.74
GE content of silage DM (MJ kg^{-1} DM)	18.42

and Gordon[51] measured the in-silo losses during the ensilage of perennial ryegrass. Their results for DM, GE and crude protein in unwilted and wilted precision-chopped material are shown in Table 8.6. In this study 'invisible losses' included, in addition to fermentation loss, losses arising from initial plant respiration and from gaseous loss accompanying surface waste production. The results of this field-scale experiment confirm the conclusions from theoretical calculations that energy losses during ensilage are considerably less than DM losses.

As mentioned in Chapter 6, the amount of moisture present in the ensiled crop affects the total bacterial count and consequently the extent of fermentation. It seems reasonable to assume therefore that, unless the fermentation is predominantly homolactic, there will be an indirect inverse relationship between DM content of the ensiled crop and fermentation losses. While there appears to be no direct experimental evidence to confirm this, the results shown in Table 8.6 for fermentation plus oxidation (invisible) losses lend support to this view.

It seems clear from the foregoing that in well-preserved silages DM losses arising from fermentation should lie between about 2 and 4%, with energy losses being very much lower.

If clostridia or enterobacteria dominate the fermentation, losses of DM and energy will be much greater than if the lactic acid bacteria had been dominant. Such high losses arise from the extensive production of carbon dioxide and hydrogen from the fermentation of lactate or hexoses, and from the deamination and decarboxylation of amino acids. If yeasts are very active, as they may be in carbohydrate-rich crops such as maize and sugar beet, ethanol production can result in very high DM losses. However, the production of ethanol by these organisms results in the loss of very little energy (Table 8.4).

Table 8.6. In-silo nutrient losses during the ensilage of precision-chopped unwilted and wilted ryegrass silages stored in 100 tonne capacity bunker silos[51]

	Unwilted silages	Wilted silages
DM loss (%)		
Surface waste	1.0	1.2
Effluent	2.5	0
Invisible	9.9	4.8
Total	13 4	6.0
GE loss (%)		
Surface waste	0.9	1.3
Effluent	2.2	0
Invisible	6.7	3.1
Total	9.8	4.4
TN loss (%)		
Surface waste	0.9	1.2
Effluent	2.9	0
Invisible	12.3	10.5
Total	16.1	11.7

EFFLUENT LOSSES

The factors influencing the production of silage effluent, which have already been discussed in Chapter 6, include DM content of the ensiled crop, type of silo, degree of consolidation and pre-treatment of the crop. Of these, DM content is the most important factor. Table 8.7 shows the % DM loss in silage effluent calculated from the DM content of ensiled herbage using two different regression equations.[3,52] With very wet crops effluent DM losses can exceed 10%, whereas with crops ensiled with DM contents of 250 to 350 g kg^{-1} very little effluent will be produced, except in tall tower silos where pressures can be very high.

Since effluent contains highly digestible components such as soluble carbohydrates, organic acids, minerals and soluble nitrogenous compounds, it follows that production of large volumes of effluent will tend to increase the concentration of the nutritionally less desirable cell wall components in the silage.

The clear advantage in wilting wet crops prior to ensiling as a means of reducing effluent production is obvious, but unless it is carried out quickly in good weather conditions it can result in increasing nutrient losses instead of reducing them. An alternative method of restricting effluent flow is to add a suitable absorbent to the crop at the time of ensiling. The use of these materials has already been discussed in Chapters 6 and 7.

OXIDATION LOSSES

In Chapter 5, four aerobic phases occurring during the conservation of crops as silage were identified: the field phase, the initial aerobic phase in the silo, the air infiltration phase, and the aerobic deterioration phase after opening. Losses arising during the first of these aerobic phases have already been discussed in the first section of this chapter.

The second phase relates to the amount of oxygen trapped in the ensiled forage during the filling of the silo. In a moderately compacted silo containing, for

Table 8.7. Dry matter loss in effluent calculated from two different regression equations

DM content of ensiled herbage (g kg^{-1}) (D)	% DM loss (Y)	
	Miller and Clifton*	Bastiman and Altman#
125	10.9	11.0
150	9.5	6.5
175	8.2	3.5
200	6.9	2.0
225	5.5	1.1
250	4.2	0.6
275	2.8	0.4
300	1.5	0.2

* Miller and Clifton[52] $Y = 17.614 - 0.0538D$

\# Bastiman and Altman[3] $Y = 0.01 + 194.3e^{-0.023D}$

example, air and herbage in the proportion of 2:1, it can be calculated that the oxygen present, if used totally for respiration, would result in a glucose loss in the crop (DM content 200 g kg^{-1}) of only 0.27%.[53] It is clear that if silos are rapidly filled and effectively sealed, the losses arising from oxygen trapped within the herbage are of little significance. However, if sealing is delayed, the increased oxidation can have a dual effect. Firstly, DM loss arising from the direct oxidation of soluble carbohydrates will occur and, secondly, unless these substrates are present in the herbage in appreciable amounts, the resultant silages will be badly preserved.[54-57] As a consequence, fermentation losses will also increase. In addition to loss of WSC, delayed sealing also increases the loss of β-carotene.[58,59]

The importance of sealing the surface of the ensiled herbage in bunker silos with plastic sheeting has been adequately demonstrated in the studies of Oelberg *et al.*.[60] Using wilted lucerne, these workers obtained in-silo DM losses of 3.8% and 32.0% from covered and uncovered silos respectively. Addition of propionic acid to the lucerne prior to ensiling, at the rate of 0.2 g kg^{-1} (fresh basis), reduced the DM losses in the uncovered silo to 13.7%, probably by decreasing the activities of the aerobic microorganisms, but increased the losses in the covered silo to 15.9%.

Even in silos where the sealing of the surface is not delayed, gaseous exchange during the storage period can occur, caused by the production of carbon dioxide and its loss into the atmosphere.[50,61,62] The main factors influencing this gaseous exchange are the species of the crop and the degree of consolidation of the material in the silo. Honig[62] found that gaseous exchange was greater in ensiled maize than in ensiled grass, with lucerne being intermediate between the two. Chop length was also an important factor; reducing the chop length of maize from 7 to 4 mm had a positive effect in achieving slightly higher densities and lower loss rates.[50]

Prolonged oxidation of herbage leads to the formation of inedible waste material, resulting from the activities of aerobic bacteria, yeasts and moulds. As mentioned earlier, measurements of losses caused by the production of this waste are difficult and the weight of waste material produced at the end of the ensiling period bears little relationship to the weight of the ensiled crop which contributed to its formation. Using the technique of Wittwer *et al.*[27] (p. 239), McDonald and Whittenbury[46] reported that up to 73% of the original herbage DM was lost in the production of waste material. In another experiment using red clover, these workers[63] found that in eight silos DM losses during the formation of surface waste varied from 23.8% to 51.5%, with a mean loss of 38%.

Prevention of waste during ensiling depends essentially on silo design and management. If, at the end of the ensiling period, the silo is covered with plastic sheeting and weighted with suitable materials, surface losses can be virtually eliminated. This was well-illustrated in the study by Minson and Lancaster,[64] who covered the surface of bunker silos with plastic sheeting weighted down with 12.5 cm of soil. This treatment completely eliminated surface waste, except for a very small quantity at the edge of the plastic, and overall DM losses were reduced from 34% (uncovered silo) to 12%.

In baled herbage ensiled in large plastic bags or wrapped in plastic film, provided an adequate seal is obtained, DM losses will normally be low. In a study by Morrison *et al.*,[65] DM losses from grass ensiled in large black plastic bags, sealed at the neck, ranged from 3.4% to 8.9%. Some 30% of bales showed deep

mould patches greater than 50 mm in depth, most of which were located at the open end of the bag, indicating the difficulty in obtaining an effective seal. In a review of seven comparative sets of data involving wilted, big bale (bagged and wrapped) and unwilted, double-chopped conventional silages, Kennedy[66] found that mean in-store DM losses for bagged bales, wrapped bales and conventional silages were 10.9%, 9.9% and 27.2% respectively.

Finally, further losses of DM can occur on opening the silo during the feeding period (p. 158). Honig[67] has stated that losses of DM arising from secondary aerobic deterioration can be as high as 30%. There is very little information available about losses from this source measured *in situ*, and most studies have been carried out on samples of silage removed from the silo and examined in the laboratory. In studies with maize silages, Woolford and Cook[68] obtained DM losses of 7.4% after seven days and 12.7% after 13 days aerobic exposure. Henderson *et al.*[69] determined the DM losses in silages from 18 farms over a seven-day period of aerobic exposure. In six of the silages no DM loss occurred, while in the remaining 12 silages losses ranged from 0.8% to 20%, with a mean loss of 6.6%. Methods of preventing the aerobic deterioration of silages have already been discussed in Chapter 7.

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Chapter 9

Nutritive Value of Silages

The nutritive value of a particular silage is influenced by the nature of the crop at the time of cutting and the changes which have occurred as a result of the activities of plant and microbial enzymes during the harvesting and storage period. The main factors influencing the nutritional value of growing crops have already been discussed in Chapter 2; this final chapter is concerned mainly with the influence of the fermentation processes on the nutritional value of the product. In considering this topic silages can be classified into three main categories:

1. Naturally-fermented silages;
2. Additive-treated silages;
3. Aerobically-deteriorated silages.

NATURALLY-FERMENTED SILAGES

The chemical changes which occur during ensilage are the result of a series of biological activities and, although one particular group of microorganisms may eventually dominate the fermentation, other organisms also make a contribution, particularly in the early stages of ensilage. In spite of this variation it is possible to classify naturally-fermented silages into two main categories, generally described as 'well-preserved' and 'badly-preserved'. In the case of the former, lactic acid bacteria will have dominated the fermentation. In silages described as 'badly-preserved', enterobacteria and/or clostridia will have played a dominant role in their production. Well-preserved silages are produced from crops rich in WSC and containing a suitable population of lactic acid bacteria. The composition of these silages and their subsequent nutritional value are influenced by the DM content of the ensiled crop and it is convenient to consider unwilted and wilted silages separately.

Well-preserved unwilted silages

Composition

Typical compositions of silages of this type are shown in Table 9.1.[1,2] One of these silages was prepared from perennial ryegrass with a soluble carbohydrate content of 177 g kg^{-1} DM, and the other was made from maize with a soluble carbohydrate content of 145 g kg^{-1} DM. These silages are characterised by having low pH values, usually between pH 3.7 and 4.2, and containing high concentrations of lactic acid. In grass silages the lactic acid contents normally lie in the range 80 to 120 g kg^{-1} DM, although higher amounts may be present if silages are made from wet crops rich in soluble carbohydrates. In maize silages lactic acid contents are usually much lower than those of well-preserved grass silages[2] because of the higher DM content and lower buffering properties of the original

crop. For example, in the silages referred to in Table 9.1, the buffering capacities (mE kg^{-1} DM) of the original grass and maize crops were 350 and 170 respectively. As a result of the formation of fermentation acids, the buffering capacity of the ensiled crop may increase as much as three- or four-fold during the period of ensilage.

Very little sugar remains after fermentation, usually less than 20 g kg^{-1} DM, and this frequently comprises, in addition to glucose and fructose, pentoses released from enzymatic and acid hydrolysis of hemicelluloses.[1,3] Since the majority of lactic acid bacteria do not attack starch, most of this polysaccharide, if present, will be preserved. Although lactic acid is the main fermentation acid present in these silages, appreciable amounts of acetate also occur. Acetic acid is derived mainly from the action of heterofermentative lactic acid bacteria and enterobacteria on sugars, although some may also be formed from citrate, malate and amino acid degradation.

Under certain conditions, in which citrate appears to play a role,[4] some strains of lactic acid bacteria are able to metabolise lactate with the formation of acetate (see p. 98). An example of this type of fermentation is illustrated in the results of a study by Henderson.[5] The silage was prepared from a mixed ryegrass sward which was cut with a mower, lifted after a few hours with a precision-chop forage harvester and ensiled in a bunker silo. The standing crop had a DM content of 184 g kg^{-1}, a WSC content of 241 g kg^{-1} DM and a TN content of 17.6 g kg^{-1} DM. The composition of composite core samples taken after seven days and three months, and a composite sample of the silage offered to lambs after eight to nine

Table 9.1. Typical composition of lactate silages made from perennial ryegrass and maize[1,2]

	Perennial ryegrass silage	Maize silage
pH	3.90	3.91
DM (g kg^{-1})	190	285
Buffering capacity (mE kg^{-1} DM)	1120	—
TN (g kg^{-1} DM)	23	15
Protein-N (g kg^{-1} TN)	235	545
Ammonia-N (g kg^{-1} TN)	78	63
WSC (g kg^{-1} DM)	10	16
Glucose (g kg^{-1} DM)	2	—
Fructose (g kg^{-1} DM)	3	—
Fructans (g kg^{-1} DM)	1	—
Starch (g kg^{-1} DM)	—	206
Acetic acid (g kg^{-1} DM)	36	26
Propionic acid (g kg^{-1} DM)	2	0
Butyric acid (g kg^{-1} DM)	1	0
Lactic acid (g kg^{-1} DM)	102	53
Ethanol (g kg^{-1} DM)	12	<10
Mannitol (g kg^{-1} DM)	41	—
Crude fibre (g kg^{-1} DM)	298	—
Neutral detergent fibre (g kg^{-1} DM)	—	413
Acid detergent fibre (g kg^{-1} DM)	—	219
Ash (g kg^{-1} DM)	68	55

Table 9.2. Changes in the composition of a ryegrass silage over a period of time[5]

	7-day cores	3-month cores	8/9-months as fed
pH	4.07	4.12	4.11
DM (g kg^{-1})	190	207	206
TN (g kg^{-1} DM)	15.5	15.5	16.4
Ammonia-N (g kg^{-1} TN)	42	77	84
WSC (g kg^{-1} DM)	122	13	4
Acetic acid (g kg^{-1} DM)	16	43	67
Propionic acid (g kg^{-1} DM)	0	1	2
Butyric acid (g kg^{-1} DM)	0	0	0
Lactic acid ((g kg^{-1} DM)	98	80	48

months, is shown in Table 9.2. An analysis of samples from the face of the silo revealed that the silage from the lower layers contained the highest levels of acetic acid.

Small amounts of propionic and butyric acids are frequently found in well-preserved silages, the concentration of butyrate depending very much on the rate at which lactate is produced. Variable quantities of mannitol and ethanol, derived mainly from the activities of the heterofermentative lactic acid bacteria and yeasts, are also present in these silages. As a result of the losses of soluble nutrients in gaseous form, or in the effluent, the concentration of cell wall constituents (fibre) generally increases during ensiling.

The nitrogenous components of well-preserved silages are mainly in a non-protein soluble form in contrast to those present in fresh forage crops, where some 70% to 90% of the TN is present as protein. Table 9.3 summarises the major nitrogenous components of three different silages prepared from ryegrasses and red clover.[6-8] Residual protein-N levels are usually between 250 g kg^{-1} and 450 g kg^{-1} TN and most of the NPN is present in the form of amino acids. As mentioned in Chapter 3 (see p. 67 and Table 3.7), some amino acids are catabolised, especially arginine, which in some silages may totally disappear.[9] Appreciable losses of lysine, serine, threonine, and glutamic and aspartic acids also occur.[6-11] Some amino acids, especially alanine, may increase.[6,10-13]

Table 9.3. Nitrogenous components (g kg^{-1} TN) of herbages and naturally-fermented silages produced from them[6-8]

	Perennial ryegrass		Italian ryegrass		Red clover	
	Herbage	Silage	Herbage	Silage	Herbage	Silage
pH	5.65	3.79	6.00	4.50	—	4.23
Ammonia-N	11	136	2	121	10	144
Amide-N	10	16	3	trace	75	trace
Amino-N	43	285	17	369	43	250
Peptide-N	—	—	39	90	44	0
Protein-N	858	293	863	308	760	439
Nitrate-N	4	—	17	49	25	10

Chemical changes affect the colour of silage; fermentation acids convert chlorophyll into the brown, magnesium-free pigment phaeophytin.[14] In well-preserved silages losses of β-carotene are likely to be low,[15] although the relative proportions of different isomers of this provitamin may alter during ensiling.[16] Zubricky[17,18] found that in maize silage, in contrast to fresh maize, only stereoisomers of β-carotene of low biological activity remained. It has been reported[19] that when sheep were transferred from a hay diet to maize silage the thiamin concentration in ruminal fluid fell to about 25% of the control values on hay. This decrease was also reflected in lower levels in animal tissues.

There is very little information available on the effect of ensilage on the mineral content of silages. Stevenson and Unsworth[20] analysed 292 silages and reported wide variations for five major elements and four trace elements (Table 9.4). Similar wide variations in mineral contents of silages have been reported by other workers.[21,22] Stevenson and Unsworth[23] also studied the absorption of calcium, phosphorus, magnesium, zinc and copper in five silages, using re-entrant cannulated sheep. The gross changes in the flow and absorption of these minerals in the digestive tract were in general agreement with data for foods other than silage, although apparent magnesium availabilities and absorptions were higher and the corresponding calcium values lower than those reported for fresh herbage.

Ivan *et al.*[24] compared the flow and soluble proportions of zinc, manganese, copper and iron in the gastrointestinal tract of sheep fed maize or lucerne silages and found that the soluble proportions of manganese in rumen fluid, ileal digesta and faeces were much lower for lucerne silages than for maize silages. Suttle[25] found that copper in hay and dried grass was invariably more absorbable than copper in grass silage. Kalač[26] determined the contents of a number of trace elements in maize silage produced in Czechoslovakia and reported deficiencies in zinc, manganese and copper. De Boer *et al.*,[27] in studies with dairy cows, supplemented lucerne silage with phosphorus, copper, zinc and manganese and obtained no increases in milk yield, milk fat or milk lactose, but did obtain an increase in the content of protein in milk.

Some species of plants, such as cocksfoot, meadow fescue, lucerne and red clover, contain isoflavone and coumarine compounds which have oestrogenic effects. These phytoestrogens have a harmful effect on the fertility of cows and

Table 9.4. The mean contents of some nutritionally important mineral elements in 292 silages produced in Northern Ireland[20]

	Mean	s.e. (±)
Potassium (g kg^{-1} DM)	18.0	5.46
Calcium (g kg^{-1} DM)	6.2	1.50
Sodium (g kg^{-1} DM)	3.9	1.62
Phosphorus (g kg^{-1} DM)	3.0	0.57
Magnesium (g kg^{-1} DM)	1.4	0.50
Iron (mg kg^{-1} DM)	723	783
Manganese (mg kg^{-1} DM)	99.2	49.56
Zinc (mg kg^{-1} DM)	30.5	9.41
Copper (mg kg^{-1} DM)	8.3	2.47

particularly of sheep. During the making of hay and its storage the oestrogenic effects decrease, but in silage the effects are retained and may even increase.[28,29]

Digestibility

The stage of growth of the crop at the time of harvesting is the main factor influencing the digestibility of forages, although other factors, such as particle size and level of feeding, play a lesser but important role. The decline in digestibility with increasing maturity of crops, already referred to in Chapter 2, is mainly a result of the increasing content of structural carbohydrates, which are less digestible than the soluble components of plants. In particular, the proportion of lignin in the plant is significant, since lignin is resistant to hydrolysis by rumen microorganisms and the quantity of this component in the matrix determines the protective ability of the matrix against the biodegradation of cellulose.[30]

Several workers have examined the effect of stage of growth on the nutritive value of silages. Castle *et al.*[31] made well-preserved silages (pH 4.0 to 4.2) from perennial ryegrass grown in Scotland and harvested on 25 May, 13 June and 25 June and obtained organic matter digestibilities with cows of 0.776, 0.700 and 0.663 respectively. Using sheep, Bergheim[32] determined the digestibilities of silages made from timothy harvested at weekly intervals in Norway from 21 June to 23 July. Over two seasons he obtained mean organic matter digestibilities of 0.777, 0.753, 0.762, 0.707 and 0.617 for five consecutive cuts. Other studies[33-35] have shown similar effects of stage of growth on silage digestibility.

The digestibility pattern of cereal silages with varying stage of growth is quite different from that of the grasses, in that there is much less variation, as the declining quality of the leaf and stem is balanced by an increasing proportion of highly digestible ear and grain[36-38] (see p. 32). Typically, maize silage has an organic matter digestibility of about 0.72,[39] although the exact value varies with different cultivars, high grain genotypes having higher digestibilities than low grain ones.[40]

The effects of silage fermentation on digestibility are generally considered to be small. Ciotti *et al.*[41] compared Italian ryegrass and cocksfoot cut at three stages of growth and conserved by artificial barn drying and ensiling. They found that organic matter digestibilities were influenced only slightly by the type of conservation. Mean digestibility values of the dried materials and silages were 0.685 and 0.667 respectively.

In studies carried out at Edinburgh with sheep,[42] the mean DM digestibilities of 36 different silages and grasses from which they were made were 0.768 and 0.767 respectively.

In contrast, Rogers *et al.*[43] obtained significantly higher DM digestibilities with cows for fresh ryegrass/clover pasture (0.758) compared with silages made from similar material (0.688). In this study the modified acid detergent (MAD) fibre content of the silage was high (353 g kg^{-1} DM) compared with the grass (297 g kg^{-1} DM) and the DM values of both (137 and 157 g kg^{-1} respectively) were low, suggesting that effluent losses of soluble nutrients might have been extensive.

Aerts *et al.*[44] examined the effects of fine chopping (4 to 5 mm length) and coarse chopping (12 to 16 mm) on the digestibility of maize silages, using sheep and cattle. With sheep, fine chopping resulted in a decreased digestibility, especially of the crude fibre and the crude protein, presumably as a result of a

higher rate of passage through the digestive tract. With cows, fine chopping also decreased the crude fibre digestibility but this decrease was smaller than with sheep. In a study by Deswysen *et al.*,[45] the results of which are shown in Table 9.5, decreasing the particle length of Italian ryegrass from 53 mm to 18 mm prior to ensiling had no significant effect on DM digestibility.

Voluntary food intake

The voluntary DM intake of forage crops is a major factor influencing their value for animal production. Intake is influenced by the characteristics of the animal and also of the forage.[46,47] Although ruminants possess, in common with other animals, chemostatic mechanisms for regulating the intake of concentrate foods,[48,49] the intake of forages is limited by the rate of removal of forage particles from the reticulo-rumen.[50,51] This rate of removal is related to the chemical composition of the forage, to its particle size, to the digestion rate of its digestible constituents and to the reduction rate of its indigestible components.[47] The intake of silage DM by ruminants is thought to be controlled by the same basic mechanisms regulating fresh forage intake,[46,52] but several workers have noted that intakes of silage DM are generally lower than those of fresh or dried forage made from similar material.[43,53-60]

Dulphy[47] has quoted reductions in DM intakes of up to 50% in direct cut, flail-harvested grass silages compared with fresh herbages fed to sheep, but daily intakes are extremely variable and Demarquilly and Weiss[61] quoted daily intake values for sheep in the range 40 to 100 g DM (kg $W^{0.75}$)$^{-1}$ for fresh forages and 20 to 75 g DM (kg $W^{0.75}$)$^{-1}$ for silages. In 86 comparisons on sheep made by Demarquilly[62] the magnitude of the decrease on ensiling was from 1% to 64%, with an average DM intake reduction of 30%. Ensiling appears to have a much greater depressing effect on intake by sheep than by cattle[63-65] and the extent of the reduction is much greater with grass than with legume[66-70] or with maize silages.[47,55,71,72] In one study in which steers were fed maize silage or dried maize forage reconstituted with water, Phillip *et al.*[73] obtained no significant differences in DM intakes.

Table 9.5. The effect of silage chop length on the composition, intake and digestibility of Italian ryegrass silages[45]

	Silage		
	Short	Long	Long/short*
Mean particle length (mm)	18	53	18
DM (g kg^{-1})	189	195	189
pH	4.34	4.39	4.39
Ammonia-N (g kg^{-1} TN)	235	198	198
Acetic acid (g kg^{-1} DM)	59	24	24
Butyric acid (g kg^{-1} DM)	26	51	51
Lactic acid (g kg^{-1} DM)	93	122	122
DM intake g (kg $W^{0.75}$)$^{-1}$ day^{-1})	41.2	35.1	38
DM digestibility	0.67	0.68	0.68

* Long silage which was chopped prior to feeding.

Several attempts have been made to correlate various chemical parameters of silage with DM intake. Water content *per se* seems to have very little influence.[74,75] Using 25 different silages fed to dairy cows, Ogura[76] found that crude fibre was negatively correlated with DM intake. Henderson *et al.*[77] obtained a positive correlation between the residual WSC content of silages and DM intakes in lambs. The correlation was improved by including the protein-N content of the silages and the WSC content of the grasses, which both had positive effects on intake, and the ethanol content of the silages, which had a negative effect.

There is evidence to indicate that low intakes of silage are associated with high organic acid contents.[78,79] In studies using 70 silages fed to sheep, Wilkins *et al.*[80] found that intake of DM was positively correlated with the contents of silage DM, TN, and lactic acid as a percentage of total acids, and negatively correlated with the contents of acetic acid, and ammonia-N expressed as a percentage of TN. In a later study, Wilkins and co-workers[81] found that correlations with TN and ammonia-N were in line with the earlier results, but correlations with acetic acid and lactic acid were much lower than those found previously. Several workers have shown that intraruminal infusion of acetate depresses intake.[82-84] However, intraruminal studies by Mbanya *et al.*[85] using sodium acetate and sodium chloride indicated that the depressing effect of the former on silage DM intake may result from osmotic effects rather than the acetate *per se*.

Confirmation that osmolality may be an important factor in regulating silage DM intake was also obtained in studies by Phillip *et al.*[86] These workers infused into the rumens of lambs extracts from fresh and ensiled maize and measured the intake of the animals fed on an *ad libitum* diet of pelleted lucerne. Although the maize silage extract caused a reduction in DM intake, it was concluded that this effect was not caused by the products of fermentation in the extract but by an increase in ruminal osmolality. This conclusion was substantiated by the finding that food intake was depressed to a similar extent when rumen osmolality was manipulated with sodium chloride solution.

Some workers[87,88] have found that partial neutralisation of silage acids with sodium bicarbonate increases DM intake, while others have found that bicarbonate addition has no effect.[89,90] Snyder *et al.*[91] added 1.2% sodium bicarbonate to a maize silage:grain diet which was fed to dairy cows. Although the sodium bicarbonate had no significant effect on DM intake, milk fat content and milk production were both increased by the treatment.

There are conflicting reports about the influence of lactic acid *per se* on intake. McLeod *et al.*[87] found that lactic acid added to silage to raise the concentration of acid from 54 g kg^{-1} DM to 113 g kg^{-1} DM decreased the intake of DM of sheep by 22%. Thomas *et al.*[92] fed ryegrass silage to calves, with and without the addition of lactic acid and fishmeal (both added at the rate of 50 g kg^{-1} silage DM). The addition of lactic acid depressed DM intake in the absence of fishmeal, but not in its presence.Thomas *et al.*[74] showed that lactic acid itself, when added to hay, had no effect on DM intake with dairy heifers but, when it was mixed with acetic acid, intake was reduced. Morgan and L'Estrange[93] added lactic acid to grass meal to give three acid concentrations of 81, 108 and 135 g kg^{-1} DM. When these diets were fed to sheep the results (Table 9.6) indicated that lactic acid addition did not significantly affect feed intake, total fatty acid concentration of rumen fluid, blood acid-base balance, nor blood lactate concentration. In an

experiment by Morgan *et al.*[94] two well-preserved grass-clover silages of different DM (175 g kg^{-1} and 365 g kg^{-1}) and lactic acid (165 g kg^{-1} DM and 34 g kg^{-1} DM respectively) contents were fed to sheep. Daily intakes of DM were relatively high on both silages (117 g (kg $W^{0.75}$)$^{-1}$ and 106 g (kg $W^{0.75}$)$^{-1}$ respectively) and the values did not differ significantly. On balance, lactic acid *per se* does not appear to influence the voluntary intake of silage DM, although when expressed as a concentration of the total organic acids the content can give a relative indication of silage intake.[80,95]

In silages containing low concentrations of TN, such as those made from maize, the addition of a nitrogen supplement such as urea can increase the intake of DM.[96] The extent of improvement in intake appears to be related to the TN content of the silage, since Demarquilly *et al.*[97] observed a 48% increase in DM intake when the TN content was 8.6 g kg^{-1} DM, while there was only a 9% increase when the silage TN content was 13.9 g kg^{-1} DM. In silage diets containing high levels of crude protein (>210 g kg^{-1} DM) and a high proportion of NPN, excessive absorption of ammonia from the rumen can result in a significant reduction in DM intake.[98]

Several workers have shown conclusively that the intake of silage DM by ruminants can be increased by reducing the chop length of the silage.[45,47,57,63,99-110]

Table 9.6. The effect of dietary additions of lactic acid to grass meal upon feed intake, rumen metabolites and blood acid-base balance[96]

Parameter	Level of lactic acid (mmol kg^{-1} DM)†					
	0	900	1200	1500	s.e.	Significance level‡
pH of diet	5.54	3.65	3.49	3.38	—	—
Feed intake						
g DM (kg $W^{0.75}$) $^{-1}$ day^{-1})	63.0	58.6	57.2	56.7	2.3	NS
Rumen fluid						
pH	6.60	6.65	6.35	6.55	0.088	NS
Total VFA concentration (mmol $litre^{-1}$)	106.5	94.5	93.2	104.8	11.6	NS
Molar concentration of VFA (%)						
Acetic	66.1^{a}	58.7^{b}	56.8^{b}	55.5^{b}	1.47	*
Propionic	16.7^{a}	24.3^{b}	25.6^{cb}	30.3^{c}	1.47	**
Isobutyric	1.1	1.6	1.2	1.1	0.09	NS
Butyric	15.1	14.5	15.1	12.3	1.07	NS
Isovaleric	0.6^{a}	0.6^{a}	0.5^{a}	0.3^{b}	0.03	*
Valeric	0.4	0.6	0.7	0.5	0.09	NS
Blood						
pH	7.43	7.43	7.43	7.45	0.007	NS
Plasma CO_2 (mmol $litre^{-1}$)	24.7	25.2	25.0	25.9	0.36	NS
Base excess (mmol $litre^{-1}$)	−0.1	0	−0.5	1.0	0.37	NS
L (+) lactate (mg $litre^{-1}$)	80.7	92.8	83.7	85.8	4.3	NS

† These levels are equivalent to 0, 81, 108 and 135 g kg^{-1} DM respectively.
‡ Values with different superscripts differ significantly.
* $p<0.05$.
** $p<0.01$.

Dulphy[47] has reported that, in nine comparisons with sheep, the DM intake of direct-cut grass silages was increased by 56% when the crop was harvested with a precision-chop forage harvester compared with a flail machine. In the study by Deswysen *et al.*[45] reported earlier, the results of which are shown in Table 9.5, decreasing the particle length of ryegrass from 53 mm to 18 mm prior to ensiling significantly increased silage DM intake by about 17% in sheep. Chopping the long silage prior to feeding showed a slight, but insignificant, increase in intake. Thomas *et al.*[100] fed silage before (chop length 10 to 20 mm) and after mincing (chop length 2 to 3 mm) and obtained significant increases in DM intake as a result of the mincing treatment.

Chopping increases silage intake in two ways: firstly, through improving the fermentation quality and, secondly, through increasing the rate of passage of food through the rumen. According to Deswysen,[111] in long silage the formation of a mat of interwoven particles in the rumen delays the separation and backflow of small particles into the reticulum and, as a result, rumination is delayed. Since the rate of particle reduction of long silage is slower than that of short material, the reticulo-rumen retention time of digesta is longer, so that the voluntary intake of DM is lower. Animal performance in growing cattle has been shown in some studies[112-114] to be higher on finely chopped silage than on coarsely chopped material. However, fine chopping, compared with coarse chopping, does not necessarily result in improvements in animal performance. In a study with dairy cows, Gordon[115,116] obtained a 7% improvement in milk production from flail-harvested grass silage compared with precision-chopped material.

Energy

Several workers have commented on the high GE values of well-preserved grass silages compared with fresh herbage.[42,117-125] Alderman *et al.*,[122] reporting on the results of digestibility trials with 45 grass silages, stated that the mean GE value of silage was about 10% higher than the usual value of 18.4 MJ kg^{-1} DM for herbage. In studies involving 18 experimental well-preserved grass silages, McDonald and Edwards[42] obtained GE values for grass and silages of 18.4 MJ kg^{-1} DM and 20.0 MJ kg^{-1} DM respectively, representing an increase during ensiling of 8.7%. Givens *et al.*[126] determined the GE values of 124 grass silages made in clamps on commercial farms during the years 1980 to 1986. The GE ranged from 17.0 to 22.1 MJ kg^{-1} DM, with a mean value of 19.2 and a standard deviation of 1.0.

In an examination of the results of 238 determinations of the GE contents of grass silages obtained from a number of sources, Edwards[127] obtained a mean value of 19.1 MJ kg^{-1} DM, with a standard deviation of 0.71 and a range of 17.0 to 21.1. Consideration of the metabolic pathways of fermentation suggests that the increases in GE during ensiling result from losses of DM without concomitant losses of energy (p. 241). There is also some evidence to indicate that in wet crops DM losses in the effluent are comparatively greater than losses of GE. For example, in one study using ryegrass with a DM content of 160 g kg^{-1}, Henderson and McDonald[128] obtained GE values (MJ kg^{-1} DM) of 18.1, 21.5 and 15.3 for grass, silage and effluent respectively.

Of the energy deductions (faecal, urinary and methane) made from GE in the calculation of ME, faecal energy is the greatest. The effects of ensilage on

digestibility have already been discussed in an earlier section of this Chapter.

Losses of energy in the urine are usually higher on silage diets than on fresh herbage diets;[42] this can be attributed mainly to the increased nitrogen excretion arising from inefficient utilisation of silage-N in the rumen. In trials carried out by Wainman and co-workers[129,130] using 32 grass silages, urinary energy (UE) losses, expressed as a proportion of GE, ranged from 0.025 to 0.073 and averaged 0.048. This last value is much higher than the mean figure of 0.026 found by Wainman *et al.*[130] for 16 maize silages, which are normally of lower TN content than grass silages.

There is no evidence to indicate that methane energy losses in ruminants are increased as a result of ensiling. Methane energy losses (CH_4E/GE) on fresh grass diets have been reported by Ekern *et al.*[131] to be about 0.075. Ekern and Sundstol[132] found methane energy losses at maintenance of 0.081 and 0.083 of GE for two hays and 0.075 and 0.075 for two silages all made from the same parent material. Smith *et al.*[133] quoted methane energy losses of 0.086 and 0.077 of GE for a silage fed at maintenance and twice maintenance respectively, while Wainman[129] found mean methane energy losses of 0.072 and 0.075 for 16 grass silages fed to sheep with oats and barley respectively. In a later study, Wainman *et al.*[130] obtained a mean methane energy loss value of 0.079 of GE for 16 grass silages fed with wheat, and a mean value of 0.077 when the same silages were fed

Table 9.7. Composition and nutritive value of ryegrass and three silages[60]

	Grass	Silages		
		Naturally-fermented		Formic acid-treated, wilted*
		Unwilted	Wilted	
DM (g kg^{-1})	175	186	316	336
pH	6.10	3.94	4.18	4.39
TN (g kg^{-1} DM)	22.7	23.0	22.8	24.2
Protein-N (g kg^{-1} TN)	648	235	289	314
Ammonia-N (g kg^{-1} TN)	40	78	79	66
WSC (g kg^{-1} DM)	140	10	47	151
Acetic acid (g kg^{-1} DM)	—	36	24	8
Propionic acid (g kg^{-1} DM)	—	1.7	0.3	0.8
Butyric acid (g kg^{-1} DM)	—	1.4	0.6	0.6
Lactic acid (g kg^{-1} DM)	—	102	59	43
Ethanol (g kg^{-1} DM)	—	12	6.4	6.1
DM digestibility	0.784	0.794	0.752	0.776
OM digestibility	0.797	0.809	0.768	0.788
N digestibility	0.752	0.782	0.723	0.784
UE/GE	0.046	0.063	0.059	0.055
ME† (MJ kg^{-1} DM)	11.6	13.6	11.4	12.0

* Treated with formic acid (85%) at a rate of 3.3 litres $tonne^{-1}$.
† Assuming calculated values for methane energy losses.[134]

with wheat offals. These methane energy values relating to grass silages are remarkably constant and are similar to the mean figure of 0.080 obtained by the same workers for 16 maize silages.

Because the magnitude of the increase in GE during fermentation is normally greater than the increased urinary energy loss, well-preserved silages can be expected to have higher ME values than the original parent materials. This is well-illustrated in the results of an experiment carried out by Donaldson and Edwards,[60] shown in Table 9.7, in which unwilted, wilted and formic acid-treated wilted silages were made from the same ryegrass herbage. The ME value of the unwilted silage was significantly higher than that of the fresh grass and those of the other two silages in which fermentation had been restricted. In this study methane losses were calculated from a regression equation based on the digestible energy (DE) value.[134]

The efficiency with which ME is utilised by ruminants for maintenance (k_m) and for fattening and growth (k_f) is very variable.[135] Ekern and Sundstol[132] found similar k_m and k_f values for hays and silages made from the same source material. Although no direct comparisons of the net energy (NE) values of grass silages and parent materials appear to have been made, it is possible to compare determined values of k_f with calculated values based on ME/GE ratios determined at maintenance (q_m values).

Table 9.8. The efficiency of utilisation of metabolisable energy for growth and fattening (k_f) of a range of grass silages[136-145]

No.	Silage treatment	k_f	
		Determined	Calculated*
1.	Untreated	0.49	0.43
2.	Untreated	0.54	0.51
3.	Untreated	0.52	0.61
4.	Untreated	0.58	0.51
5.	Untreated	0.68	0.54
6.	Untreated	0.34	0.56
7.	Formic acid	0.45	0.58
8.	Formic acid	0.44	0.55
9.	Formic acid + formalin	0.40	0.57
10.	Wilted	0.42	0.42
11.	Wilted	0.23	0.61
12.	Wilted + formic acid	0.21	0.44
13.	Wilted + formic acid	0.51	0.56
14.	Wilted + formic acid	0.41	0.45
15.	Wilted + formic acid	0.61	0.61
16.	Wilted + formic acid	0.46	0.47
17.	Wilted + formic acid	0.43	0.52
18.	Wilted + formic acid + formalin	0.39	0.45
19.	Wilted + formic acid + formalin	0.43	0.50
20.	Wilted + formic acid + formalin + sucrose	0.54	0.53
	Mean	0.45	0.51

* $k_f = 1.32\ q_m - 0.318$ (reference 145).

Table 9.8 shows the results of 20 experiments[136-145] in which the k_f values of silages were determined with ruminants, together with values for grasses calculated using q_m. The mean determined k_f value for the 20 silages is 0.45, which is about 13% lower than the mean calculated value for grasses of 0.52. The data are, however, too limited to draw any definite conclusions about the effect of various treatments on the k_f values of silages, although it is interesting to note that the mean determined and calculated values for the six untreated silages are 0.47 and 0.43 respectively. It would appear also, from the data in Table 9.8, that there are instances when the k_f values of silages are considerably lower than those predicted from the equation; clearly more detailed research is needed to identify the conditions under which this occurs.

It is well established that legumes have higher k_f values than grasses of equal or of even higher digestibility.[146] This property of legumes also appears to apply to legume silages.[147,148] In a comparison of red clover silage with grass silage of similar metabolisability (ME/GE), Smith *et al.*[148] obtained k_f values for sheep of 0.54 and 0.49 respectively. Joyce and Brunswick[149] found no significant differences in the utilisation of ME for maintenance and growth when lucerne in the fresh form was compared with similar material conserved as silage or hay.

Van Es,[150] from a study of 280 balance trials, concluded that k_m and k_l (efficiency of utilisation of ME for lactation) did not differ for hays and silages of similar ME values. However, Thomas and Thomas[151] have stated that determined k_m values for silages are consistently lower than those predicted from the q_m of the diet using the Agricultural Research Council (ARC) equation;[139] on average the difference is about 5.5% of the calculated value.

There is some evidence to indicate that cattle and sheep may differ in their ability to digest and utilise the energy of certain silages. In a study by Smith *et al.*[152] maize silage was fed to steers and sheep at two levels of intake, maintenance and *ad libitum*. From the results of this experiment it was concluded that cattle digested maize silage more efficiently than did sheep, and this resulted in higher efficiencies in the utilisation of GE.

Nitrogen

Following ingestion of food by the ruminant, nitrogenous compounds undergo hydrolysis and deamination in the rumen under the influence of microbial enzymes to yield ammonia-N, which itself is one of the major nitrogenous substrates for the growth of rumen microorganisms.[153] The efficiency with which food-N is converted into microbial protein depends upon the relative rates of ammonia release and ammonia assimilation. If the former is faster than the latter, then the excess ammonia will be absorbed into the blood, carried to the liver and converted into urea. Although some of this urea is recycled to the rumen, generally most of it is excreted in the urine. The two main factors in this process are the rumen degradability of the dietary-N compounds and the level of energy available for microbial growth.[153]

Because a high proportion of the nitrogenous components of naturally fermented silages are in a non-protein, soluble form, as mentioned earlier, they are extensively and rapidly degraded in the rumen (Table 9.9),[154-165] resulting in extreme ammonia patterns, manifested by higher peak values immediately after feeding and lower minimum values before feeding than in those found in sheep

given grass diets.[42,59] In one study,[42] carried out with sheep, peak ruminal ammonia concentrations for six naturally-fermented silages ranged from 195 to 450 mg litre^{-1} and there was a highly significant correlation between peak concentration of ruminal ammonia and the NPN and ammonia-N contents of the silages. Other studies have shown similar high peak rumen ammonia-N levels in sheep on silage diets.[166]

There is some controversy about maximum ruminal ammonia levels for optimum microbial growth: Ciszuk and Eriksson[167] have suggested that levels over 150 mg ammonia-N litre^{-1} are associated with net losses from the rumen, while other workers[168] have reported various optimal ruminal ammonia-N levels ranging from 50 to 235 mg litre^{-1}.

Table 9.9. The degradability of dietary protein and the efficiency of microbial protein synthesis in sheep given hays and silages[154-165]

Diet	Degradability of dietary protein	Efficiency of microbial protein synthesis (g N kg^{-1} OMADR)*
Hays		
Ryegrass	0.51	33.4
Ryegrass	0.93	29.8
Subterranean clover†	0.57	35.7
Subterranean clover	0.64	32.3
Subterranean clover	0.54	28.5
Lucerne	0.67	29.5
Mean	0.64	31.5
Grass silages		
Untreated	0.86	33.4
Untreated	0.78	23.0
Wilted	—	24.3
Wilted	0.83	21.6
Wilted	0.86	27.2
Formic acid	0.54	10.3
Formic acid	0.52	16.4
Formic acid	0.73	20.0
Formic acid	0.81	24.0
Formic acid	0.72	23.0
Formic acid	0.63	21.0
Formic acid + wilted	0.82	27.2
Formaldehyde	0.36	13.1
Formaldehyde + wilted	0.33	27.2
Formaldehyde + formic acid + wilted	—	25.2
Formaldehyde + formic and sulphuric acids + wilted	0.80	26.5
Mean	0.69	22.7

* OMADR = Organic matter apparently digested in the rumen.
† *Trifolium subterraneum*.

The optimal utilisation of ammonia by rumen microorganisms depends upon the synchronisation of both nitrogen and a suitable energy source. The energy sources available in silage are not ideal, as most of the soluble carbohydrates present in the original forage have already been fermented to reduced products, which themselves may be end-products of rumen fermentation. An exception is lactate which, in the L(+) isomeric form, appears to be rapidly fermented in the rumen.[169] Unfortunately, in most silages the D(−) isomer predominates[170] and this form of lactic acid is metabolised by the animal more slowly than the L(+) form, which may lead to acidosis.[171-173] However, there is evidence that the lactate concentration in the rumen is maintained at a low level due to its rapid metabolism by the rumen microorganisms, and therefore little lactate is absorbed by the animal (see p. 87). Protozoa play a central role in ruminal lactate metabolism;[174] the major end-product of this fermentation appears to be acetate.[175]

As a consequence of the rapid production of ammonia in the rumen combined with a deficiency of a suitable energy source, ruminal microbial protein synthesis in animals given silage diets is lower than that in animals given fresh forage or hay diets. The mean value for the efficiency of microbial synthesis expressed as g N per kg organic matter apparently digested in the rumen (OMADR) for the six hays shown in Table 9.9 is 31.5, which is similar to the mean value for all diets (32) quoted by the ARC.[176] Corresponding values for silages are very variable, as is shown in Table 9.9, but they are generally considerably lower than those shown for hays.

Because of a deficiency of suitable energy sources and the highly degradable nature of the nitrogenous compounds in naturally-fermented silages, it is reasonable to assume that the addition of carbohydrate-rich or protein-rich foods to silage diets may improve the overall utilisation of nitrogen. Supplementation of grass silage with barley has, however, proved largely ineffective in stimulating the efficiency of microbial synthesis,[161,165,177] whereas consistent responses have been obtained by supplementing silage given to cattle with protein concentrates such as soya bean meal.[165,178-180]

Table 9.10. The effects of supplements of barley or soya bean meal on dry matter intake, digestion of organic matter and nitrogen retention in sheep given silage *ad libitum*[181]

	Silage alone	Silage + barley	Silage + soya bean meal	
			Low	High
Concentrate DM intake (g day^{-1})	—	252	57	231
Silage DM intake g (kg $W^{0.75}$)$^{-1}$ day^{-1}	38.0^{a}	29.8^{a}	37.9^{a}	35.3^{a}
OM digestibility	0.659^{a}	0.715^{b}	0.665^{a}	0.719^{b}
TN intake (g day^{-1})	12.2^{a}	14.3^{ab}	16.9^{b}	30.0^{c}
Abomasal TN flow (g day^{-1})	11.0^{a}	13.0^{ab}	12.9^{ab}	18.4^{b}
N retention (g day^{-1})	−0.7^{a}	1.2^{ab}	2.9^{bc}	5.2^{c}

Values with different superscripts differ significantly ($p<0.05$).

This is illustrated in the study by Morgan *et al.*,[181] in which dietary supplements of barley and soya bean meal to a basal diet of grass silage were fed to sheep. Some of the results of this experiment are shown in Table 9.10. In this experiment, four diets were fed: (1) silage *ad libitum*, (2) silage + barley, (3) and (4) silage + soya bean meal. The supplements were isonitrogenous in (2) and (3) and isoenergetic in (2) and (4). Intake of silage DM was lower with the barley supplement than with the other diets, and nitrogen retention was improved by the addition of the soya bean meal but not by the barley.

Although starch, in the form of barley, appears to be an unsuitable energy source for stimulation of microbial protein synthesis in ruminants on silage diets, several workers have shown that a more readily fermentable carbohydrate source, such as sucrose, can be beneficial. Huhtanen and Ala-Seppälä[182] obtained a 60% increase in the production of microbial-N in the rumen of cattle on a silage/barley diet, by the intraruminal infusion of sucrose (Table 9.11). In this study, however, the additional supply of sugar had a negative effect on fibre digestion.

Other workers have also demonstrated improvements in ruminal microbial-N synthesis by the addition of soluble carbohydrates. Chamberlain *et al.*[183] compared three different carbohydrate sources, glucose, sucrose and starch, added to a basal diet of grass silage fed to goats (Table 9.12). Starch gave a small reduction in ruminal ammonia-N concentration and an increase in protozoal numbers. In contrast, glucose and sucrose produced much larger reductions in ammonia-N concentrations, with no change in protozoal numbers. Marked increases in protozoal numbers in response to barley supplementation had been observed previously by these workers, who had also demonstrated that defaunation led to a reduction in ammonia concentration. Infusions into the rumen of the protein casein, along with sucrose have recently been shown by Rooke and Armstrong[184] to increase the microbial-N entering the small intestine of cattle on grass silage diets, when compared with infusions of sucrose only.

The beneficial effects of adding a protein supplement to silage diets on nitrogen utilisation and animal performance can be explained in a number of ways. Firstly, it has been suggested[185,186] that the efficiency of microbial protein synthesis is considerably increased, or indeed optimised, when the rumen microorganisms have access to both amino acid-N (as protein) and ammonia-N. Since the proportion of nitrogen present as protein in silage diets is normally low, it is

Table 9.11. The effect of intraruminal infusion of sucrose on nitrogen metabolism of cattle given a basal diet of 12 kg silage + 1 kg rolled barley[182]

	Sucrose level (g day^{-1})		
	0	450	900
Rumen ammonia (mmol litre^{-1})	8.30	5.05	4.26
N at proximal duodenum (g 24 hour^{-1})			
Non-ammonia-N	74.3	102.3	113.5
Microbial-N	42.0	58.7	67.9
Microbial-N (g N kg^{-1} OMADR)*	24.4	31.6	31.6
Digestion of neutral detergent fibre	0.668	0.617	0.576

* OMADR = Organic matter apparently digested in the rumen.

Table 9.12. Effects of supplementing a grass silage diet with starch, glucose and sucrose on ruminal pH, ammonia concentrations and protozoa counts in goats[183]

Diet*	pH	Ammonia-N (mg litre^{-1})	Protozoa counts ($\times 10^5$ ml^{-1})
Silage	6.35	231	7.7
Silage+maize starch	6.33	205	15.1
Silage+sucrose	6.17	160	6.3
Silage+glucose	5.79	155	6.1

* Basal silage diet of 1350 g DM day^{-1}. Carbohydrates added at the rate of 1.1 mol of glucose or an equivalent amount of maize starch or sucrose.

possible that absorption of intact amino acids by rumen microorganisms is a limiting factor in their growth. Secondly, for efficient utilisation of acetate and butyrate, which are produced in the rumen and which may also be present in silage, the body tissues need an adequate supply of gluconeogenic substrates to supply the necessary amounts of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and glycerol for fat synthesis.[146] Gluconeogenic amino acids, surplus to requirements for tissue protein synthesis, may play an important role in this respect in sparing glucose.

Finally, that part of the dietary protein which is undegraded in the rumen may provide the animal with essential amino acids necessary for tissue synthesis. Amino acids which appear to be of particular importance are methionine,[161,163,187] threonine and lysine.[188,189] Attention has concentrated on methionine, which is frequently the first limiting amino acid for protein synthesis in ruminants. It has been shown that the proportion of this amino acid in the duodenal digesta protein of animals given grass silage diets is lower than that of animals on hay diets.[158,188-190] Several experiments with sheep have been carried out to determine the changes in nitrogen retention and blood plasma methionine concentration in response to intraperitoneal or intravenous supplements of methionine.[191-194] Most of these experiments have supported the view that methionine is limiting for protein synthesis, but increases in nitrogen retention have not always been obtained. In studies with cows on silage diets, Rogers *et al.*[195] obtained a significant increase in the yield and content of protein in milk in response to intraabomasal infusion of methionine. However, other workers[191,196-198] have obtained no marked effects of methionine infusions or dietary supplements on the yield and protein content of the milk in the lactating dairy cow.

Maize silages are of lower TN contents than grass silages (see Table 9.1), although exceptions to this generalisation are grass silages produced from tropical pasture species, which can have TN contents of less than 10 g kg^{-1} DM.[199] The studies of Thomas and Wilkinson[200] on the utilisation of maize silage by growing cattle clearly demonstrated the necessity of supplying the diet with extra nitrogen to achieve adequate rates of liveweight gain. Similar beneficial effects have been observed with dairy cows.[201]

Several workers have examined the effects of adding urea to maize before ensiling and the results of such studies have already been described in an earlier chapter (p. 218). An alternative approach is to add urea or ammonia to maize

Table 9.13. The effect of pre-wilting grass on the composition and nutritional value of silages[212,213]

	Treatment							
	1		2		3		4	
	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage
DM (g kg^{-1})	198	190	295	273	345	323	462	432
pH	—	3.95	—	4.14	—	4.28	—	4.48
Organic matter (g kg^{-1} DM)	916	903	912	896	910	898	911	899
Crude fibre (g kg^{-1} DM)	266	309	282	305	282	308	275	305
WSC (g kg^{-1} DM)	117	11	110	34	96	33	108	55
TN (g kg^{-1} DM)	19.1	22.6	18.6	22.0	19.2	22.0	18.8	21.2
Protein-N (g kg^{-1} TN)	823	470	766	457	763	445	798	482
Ammonia-N (g kg^{-1} TN)	—	109	—	106	—	92	—	73
Acetic acid (g kg^{-1} DM)	—	32	—	14	—	7	—	9
Butyric acid (g kg^{-1} DM)	—	28	—	30	—	27	—	11
Lactic acid (g kg^{-1} DM)	—	71	—	55	—	45	—	32
Digestibility of organic matter*	0.739	0.759	0.720	0.744	0.737	0.725	0.733	0.725
Digestibility of TN*	0.640	0.675	0.613	0.648	0.648	0.651	0.633	0.667
GE (MJ kg^{-1} DM)	18.03	18.66	18.03	18.49	17.91	18.66	17.99	18.16
ME (MJ kg^{-1} DM)†	10.38	10.96	9.96	10.71	10.08	10.59	10.13	10.21
Intake of DM (g $(kgW^{0.75})^{-1}$ day^{-1})‡	—	78.4	—	89.9	—	95.5	—	92.8

* Using sheep.

† Determined at maintenance level of feeding and using a calculated methane energy value.[134]

‡ Using cattle.

silage before feeding.[202-206] Teller *et al.*[207] concluded that urea can contribute as much as 30% of the total dietary nitrogen without causing a reduction of the nitrogen utilisation for milk production in cows with moderate milk yields.

Well-preserved wilted silages

Composition

The term 'wilted silage' describes silage made from herbage usually cut with a mower and left for varying periods of time in the field prior to lifting and ensiling, regardless of weather conditions. Under poor weather conditions, DM contents may increase very little, if at all,[208] and if the wilting period is extended over several days, soluble carbohydrates and protein-N contents may be reduced and deamination of amino acids may increase.[209-211]

The main effects of wilting under good weather conditions on the crop and on the composition of the silage have already been discussed in Chapters 3 and 6. In general, fermentation is restricted as DM content increases, and this is reflected in higher pH and soluble carbohydrate values, and in lower levels of fermentation acids. This is well-illustrated by the results of experiments carried out by Jackson and Forbes,[212,213] in which timothy-meadow fescue-white clover was ensiled after wilting under good weather conditions to four different DM levels. The herbages and silages were used in digestibility trials with sheep and the silages were fed to cattle in order to measure the voluntary intake of DM. The results of this study are shown in Table 9.13 and it can be seen that wilting had little effect on the composition of the herbage although, compared with unwilted material, there was a tendency for the WSC and protein-N contents to decrease. The protein-N contents of the silages varied only slightly with DM content. This is in contrast to the results obtained in a study by Henderson *et al.*,[214] who found that protein-N contents of silages increased progressively with increasing DM content (Table 9.14).

Papadopoulos and McKersie[215] wilted two grass and three legume species over a 24-hour period. They found that proteolysis was more extensive in wilted silages made from first cut herbages than in wilted silages made from second cut material where wilting was more rapid. In silages made from first cut herbage, soluble NPN levels increased as DM contents increased, whereas in silages from second cut forages, the reverse trend was observed, with soluble NPN levels being highest in the direct-cut and lowest in the wilted samples. Not shown in Table 9.13 are changes in the contents of β-carotene, but it is known that losses of this provitamin from forage crops can occur during wilting.[16,216]

Table 9.14. Influence of wilting on the protein-N and ammonia-N components of ryegrass silages[214]

	DM content of herbage (g kg^{-1})					
	150	200	250	300	350	400
Protein-N (g kg^{-1} TN)	302	319	350	384	370	447
Ammonia-N (g kg^{-1} TN)	101	102	85	93	95	85

Digestibility

The results of the animal trials shown in Table 9.13 suggest that digestibility is not influenced by wilting to any marked extent, although some workers have noted reductions in digestibility as a result of wilting,[217-220] while others have obtained increases.[221] In a comparison of the data from 20 sources, Marsh[222] obtained for wilted and unwilted silages mean DM digestibility values of 0.685 and 0.700 respectively, and from nine sources corresponding nitrogen digestibilities of 0.629 and 0.658. Using steers, Petit *et al.*[223] compared digestibilities of lucerne, timothy and a grass-red clover mixture preserved either as hays or wilted silages (DM content 345 to 419 g kg^{-1}) and found that DM and energy digestibilities were similar for all treatments.

The effect of wilting on digestibility is likely to be influenced very much by weather conditions. If wilting is prolonged and carried out under poor weather conditions, losses of highly digestible nutrients through oxidation and leaching can be relatively high and this could have an effect in reducing DM digestibility.

Voluntary food intake

Several workers have reported increases in voluntary DM intake with increasing DM content,[53,60,224-234] although in some studies increases in DM intake as a result of wilting have not always been obtained.[235-237] Marsh[222] found that in ten comparisons with sheep, wilting increased DM intake by 44%. Savoie *et al.*,[238] however, noted an increase of only 20% with wilted grass silages compared with direct-cut silages. Demarquilly[62] obtained an increase in DM intake with wilting of only 12.6% in 14 comparisons, but the particle length of the silages was only 4 to 5 cm. It seems likely that the effect of wilting on DM intake is less with short-chop silage than with long material;[47] also, the response in intake to silage DM content is likely to be small when low DM silages are well-preserved.[151]

Wilkins *et al.*[80] and Demarquilly[62] found that correlations between DM intake and DM content were not as high as those between DM intake and content of volatile fatty acids. Gross[239] has stated that silage intake normally increases at a decreasing rate with increasing DM content, and reaches its peak value at about 400 g DM kg^{-1} for grass silages with crude fibre contents less than 260 g kg^{-1} DM. At higher crude fibre levels, the intake increases only up to 300 to 350 g DM kg^{-1}.

Energy

Because wilting a crop prior to ensiling restricts fermentation, both GE and ME values of wilted silages are likely to be less than those of silages made from unwilted material. In general, the data shown in Tables 9.7 and 9.13 support this view. In a comparison of 25 grasses, 18 unwilted, well-preserved silages and seven wilted silages, McDonald and Edwards[42] obtained GE values (MJ kg^{-1} DM) of 18.3, 20.0 and 19.1 respectively. Other workers have also demonstrated that wilted silages have lower GE and ME values than direct-cut silages made from similar material.[240,241]

Using sheep, Anderson[140] compared directly the energy values of direct-cut grass silage and silage made from grass wilted for a prolonged period (120 hours) under poor weather conditions. The results of this study are summarised in Table 9.15. The urine (UE) and methane (CH_4E) energy losses were similar in both

silages, but the ME value of the wilted silage was significantly less than that of the direct-cut material. Although the efficiency of utilisation of the ME for growth and fattening (k_f) of the wilted silage was slightly lower than that of the unwilted silage, the differences were not statistically significant. Of the nine wilted silages on which k_f values were determined, shown in Table 9.8, only two samples were markedly different from the ARC predicted values.

Unsworth *et al.*[242] determined the efficiency of utilisation of ME for lactation (k_l) of unwilted and wilted formic acid-treated silages in two experiments. The k_l values in the first experiment, which were not significantly different, were 0.50 and 0.53 for the unwilted and wilted silages respectively. In the second experiment the two k_l values were identical (0.58). All these values were about 10% lower than the values predicted from the ARC equation.[145]

Nitrogen

It might be reasonable to assume that silages made from high DM herbages, and containing relatively high protein-N and WSC levels, would provide a better substrate for microbial protein synthesis. However, there is little evidence to indicate that the nitrogenous components in wilted silages are utilised any more efficiently by ruminants than those in silages made from direct-cut material. The mean efficiency of microbial protein synthesis (g N kg^{-1} OMADR) value calculated for the three wilted silages shown in Table 9.9 is 24.4, which is considerably below the mean value of 32 for foods quoted by the ARC,[176] although slightly higher than the mean value of 22.7 calculated for the 16 silages shown in Table 9.9.

Grenet *et al.*[237] conserved 10 fresh crops (lucerne, ryegrasses and cocksfoot) as direct-cut silages (182 g DM kg^{-1}), wilted silages (381 g DM kg^{-1}) or direct-cut silages treated with formic acid (190 g DM kg^{-1}) and, when these were fed to sheep, found that nitrogen retention was lowest and ruminal ammonia-N highest in the wilted silages. However, in this study the mean voluntary DM intake of the wilted silage was only 49.7 g (kg $W^{0.75})^{-1}$ compared with 57.5 for the direct-cut silage and 61.8 for the formic acid-treated material.

Table 9.15. Energy values of direct-cut and wilted silages made from perennial ryegrass*[140]

	Direct-cut silage	Wilted silage
pH	3.76	4.14
DM (g kg^{-1})	170	220
D value†	0.710	0.624
GE (MJ kg^{-1} DM)	19.4	18.4
DE/GE	0.767	0.682
UE/GE	0.043	0.038
CH_4E/GE	0.065	0.066
ME/GE (q_m)	0.658	0.577
ME (MJ kg^{-1} DM)	12.57	10.29
k_f	0.44	0.40

* Both direct-cut and wilted herbages were precision-chopped and treated with formic acid (85%) at the rate of 2.5 litres $tonne^{-1}$ before ensiling.

† Digestible organic matter expressed as a proportion of DM.

In a comparison of the digestion of unwilted and wilted silages with the original fresh crop, Thomson and Beever[243] found that the flow of amino acid-N at the duodenum was 15% lower on the unwilted silage and 25% lower on the wilted silage compared with the fresh crop. Merchen and Satter[244] examined the nitrogenous changes in three lucerne silages made from fresh herbage of DM content 290 g kg^{-1}, and herbages wilted to two different DM levels (400 and 660 g kg^{-1}). These silages were fed along with maize grain to cannulated cows. They found that the amounts of amino acids apparently absorbed from the small intestine were higher in the two wilted silages compared with the unwilted silage, but the differences were not statistically significant.

Co-ordinated experiments

In terms of overall animal performance on wilted silages, some studies report increases in liveweight gains and milk production[53,221,231] as a result of wilting. Some workers have obtained no benefits in animal performance arising from wilting, in spite of improvements in voluntary DM intake,[235,236,245,246] while others have reported a reduction in output.[211,247,248]

The co-ordinated experiment, involving eight European countries, referred to in Chapter 8,[249,250] was designed to clarify these problems associated with wilted silages. In this study, silages were made from grass ensiled directly or after wilting to a target DM of 300 to 400 g kg^{-1}. A precision-chop harvester was used in all experiments. The unwilted silages were made with the addition of either formic acid or formic acid plus formalin, whereas in most experiments the wilted silages were made without additive. The average composition and nutritive value of the

Table 9.16. Average composition and nutritive value of unwilted and wilted silages obtained in 24 European experiments[249,250]

	Unwilted silages	Wilted silages
Composition of silages		
DM (g kg^{-1})	215	348
pH	4.0	4.4
Lactic acid (g kg^{-1} total acid)	760	740
Ammonia-N (g kg^{-1} TN)	72	96
Animal performance		
Dairy cows		
Silage DM digestibility	0.746	0.723
Relative DM intake	100	104
Milk yield (kg day^{-1})	23.4	22.8
Milk composition (g kg^{-1})		
Fat	41.3	41.9
Protein	30.9	31.2
Lactose	47.6	47.3
Liveweight gain (g day^{-1})	111	103
Growing cattle		
Silage DM digestibility	0.698	0.678
Relative DM intake	100	109
Liveweight gain (g day^{-1})	865	828

24 silages fed to dairy cows (eight experiments) and growing cattle (16 experiments) are shown in Table 9.16.

Dry matter digestibility was slightly higher for unwilted than for wilted silages. The mean DM intake of wilted silage was marginally higher than that of unwilted material, but this increased intake was not reflected in improved animal performance. Significant reductions in performance on wilted silages were associated with high levels of soil contamination or a prolonged field period under adverse weather conditions. The main conclusion from this extensive study was that the two ensiling systems differed little in feeding value provided the silages were well-preserved and were made without soil contamination or an extended field wilting period. The main benefit of pre-wilting crops prior to ensiling would appear to arise from reduced effluent production.

Badly-preserved silages

Composition

The term badly-preserved silages refers to silages in which either enterobacteria or clostridia or both have usually dominated the fermentation. It does not include those silages which have deteriorated as a result of oxidation; these aerobically-deteriorated silages will be discussed in a later section.

Badly-preserved silages are produced from crops which are either ensiled too

Table 9.17. The composition of three badly-preserved silages and the parent materials from which they were made[8,255,256]

	Ryegrass		Cocksfoot		Lucerne	
	Original herbage	Silage	Original herbage	Silage	Original herbage	Silage
pH	6.3	5.4	—	5.4	6.3	7.0
DM (g kg^{-1})	160	174	175	162	140	131
TN (g kg^{-1} DM)	37	39	32	37	44	46
Protein-N (g kg^{-1} TN)	—	566	803	302	731	260
Ammonia-N (g kg^{-1} TN)	—	205	6	323	2	292
WSC (g kg^{-1} DM)	108	4	62	4	54	nil
Acetic acid (g kg^{-1} DM)	—	116	—	37	—	114
Propionic acid (g kg^{-1} DM)	—	14	—	15	—	8
Butyric acid (g kg^{-1} DM)	—	23	—	36	—	8
Lactic acid (g kg^{-1} DM)	—	trace	—	1	—	13
Crude fibre (g kg^{-1} DM)	220	284	254	316	—	—
Ash (g kg^{-1} DM)	99	150	97	108	—	—
Buffering capacity (mE kg^{-1} DM)	320	1300	—	—	570	2530

wet or which contain low levels of fermentable carbohydrates. They may also be produced if the ensiled forage is deficient in lactic acid bacteria. Silages rich in acetate and low in lactate are often produced in laboratory silos[251] unless precautions have been taken to inoculate the herbage prior to ensiling. Such silages, which are generally rich in acetic acid content, may arise from contamination of the herbage with enterobacteria. High acetate silages have also been produced in studies with tropical grass species[252-254] and, under certain conditions as mentioned earlier (p. 251), by some strains of lactic acid bacteria using lactate as an energy source. In the case of the latter, as acetate is a weaker acid than lactate, there is usually a pH shift, which may allow the further development of undesirable microorganisms.

It is often difficult, from a consideration of the chemical composition of the silages, to distinguish which groups of microorganisms have been dominant, as the end-products of the fermentations are often very similar. If a silage contains a high proportion of butyric acid relative to the other fermentation acids, it is highly likely that saccharolytic clostridia have been active. However, the presence of butyric acid is not conclusively an indication of the activity of these bacteria, as butyric acid is also a by-product of the deamination of lysine by proteolytic clostridia and enterobacteria. Similarly, acetic acid, which is often characteristically associated with enterobacteria, can also be produced from the fermentation of glucose and pentoses by lactic acid bacteria. It is also a by-product from the deamination of amino acids by both clostridia and enterobacteria.

In general, badly-preserved silages are characterised by having high pH values, usually within the range 5.0 to 7.0. The main fermentation acid present is either acetate or butyrate. Lactate and WSC are present in relatively low concentrations or frequently absent. The ammonia-N levels are usually high, often above 200 g kg^{-1} TN. This ammonia, which is derived from the catabolism of amino acids, is accompanied by other degradation products such as amines and various keto- and fatty-acids (see p. 115).

Table 9.17 shows the composition of three badly-preserved silages together with the parent materials from which they were made.[8,255,256] The ryegrass silage was made from herbage in which sealing of the silo had been delayed for 72 hours and, because of high losses of sugars through oxidation, insufficient substrate was available for the lactic acid bacteria to produce enough lactic acid to lower the pH to below the critical level to prevent development of enterobacteria and clostridia. Because of the low DM content of the original crop (160 g kg^{-1}) this critical pH level would have been very low. In this example, the main fermentation acid produced was acetate.

In the case of the second example shown in Table 9.17, both the WSC and DM contents were initially low and this combination resulted in a silage containing similar amounts of acetate and butyrate, and very high concentrations of ammonia-N. With the third example, very little butyrate was produced and the main fermentation acid was acetate. In the studies with ryegrass and lucerne, buffering capacity determinations were carried out and in both species there was a fourfold increase during ensilage.

In the case of the lucerne, analysis of amino acids, after hydrolysis, was carried out and the data obtained for the badly-preserved silage can be compared with those for a well-preserved, additive-treated silage made from similar forage (Table 9.18). With the exception of hydroxyproline, alanine, valine, isoleucine

and leucine, extensive breakdown of the amino acids occurred in the badly-preserved silage. The high ammonia content in the lucerne silage suggests that a large proportion of the amino acids was deaminated, but the results of amine analysis (Table 9.19) indicate that several amino acids were also decarboxylated, particularly glutamic acid, which after decarboxylation produces γ-aminobutyric acid.

Digestibility

There is very little information available on the digestibility of badly-preserved silages. Flynn[257] obtained DM digestibilities of 0.74 for a well-preserved silage and 0.71 for a badly-preserved one. In the three silage examples shown in Table 9.17 only one, the cocksfoot silage, was fed to animals. The digestibilities of the organic matter by sheep were 0.76 for the original grass and 0.74 for the silage.

It is conceivable that when losses from the silo are high, as they usually are in the production of badly-fermented silages, concentrations of crude fibre, including lignin, can increase and this may result in a significant reduction in digestibility in silages made from relatively mature herbage. In young herbages the fibre is often as highly digestible as the other nutrients; for example, in the case of the cocksfoot referred to in Table 9.17 the digestibility of the crude fibre was 0.87 compared with a value of 0.84 for the organic matter. Corresponding values for the badly-preserved silage were 0.91 and 0.85.

Table 9.18. Changes in total amino acids of hydrolysates of lucerne during ensilage[8]

Amino acid	Original lucerne (g amino acid (16 g N)$^{-1}$)	Untreated silage (% recovery)	Additive-treated silage* (% recovery)
Hydroxyproline	0.8	145	178
Aspartic acid	17.0	16	87
Threonine	3.7	32	95
Serine	4.1	29	89
Glutamic acid	9.4	28	102
Proline	3.9	47	96
Glycine	4.3	59	94
Alanine	5.4	121	91
Valine	5.4	97	91
Cystine	1.2	31	64
Methionine	1.5	56	80
Isoleucine	3.9	89	89
Leucine	7.6	86	91
Tyrosine	3.4	27	75
Phenylalanine	4.6	76	98
Histidine	2.2	29	40
Lysine	6.2	26	50
Tryptophan	1.8	66	80
Arginine	4.9	19	86

*Treated with 3.2 g of formic acid (85%) + 7.4 g of formalin (40%) kg^{-1}.

Voluntary food intake

It is generally accepted that intakes of DM by ruminants fed badly-preserved silages are less than those by animals fed well-preserved silages. Reference has already been made to the negative correlation between DM intake and the acetic acid and ammonia contents of silage (p. 256). The exact cause of low intakes of DM is unclear although amines have been implicated. Histamine, if absorbed, can be physiologically harmful.[258] Normally, this amine appears to be broken down in the rumen[259,260] but, under certain conditions, it can be absorbed and lead to a depression in food intake[261,262] (see p. 117).

Gamma-aminobutyric acid is often a major component of badly-fermented silages. Buchanan-Smith[263] demonstrated that infusion of this amine into the jugular vein of lambs fed on lucerne pellets caused a depression in food intake. When a silage extract containing 40 g of γ-aminobutyric acid was infused into the rumens of lambs fed on high DM lucerne-grass silage, intake was depressed for up to four hours following feeding.[264] In a similar experiment in which a mixture of α-aminobutyric acid and four other amines (excluding γ-aminobutyric acid) was infused into the rumen, intake was depressed for up to eight hours following feeding. It was concluded from this study that soluble constituents in silage, including amines, can inhibit intake but no single constituent is primarily responsible.[264]

Flynn[257] compared the nutritive value of well-preserved silage (pH 4.2) with badly-preserved silage (pH 4.8) and obtained DM intakes (% body weight) of 1.9 and 1.4 respectively. Corresponding liveweight gains (g day^{-1}) were 895 and 472.

Energy

From a consideration of the fermentation pathways shown in Table 8.4 (p. 242), DM losses during the fermentation of sugars and lactic acid by enterobacteria and clostridia are very high. These losses are only partially balanced by energy losses and, as a consequence, the GE values of badly-fermented silages are likely to be slightly higher than those of the original herbage. Although faecal energy losses are unlikely to be markedly different from those obtained by feeding parent

Table 9.19. Changes in amines (g (16 g N)$^{-1}$) of lucerne during ensilage.*[8]

	Original lucerne	Untreated silage	Additive-treated silage
Glucosamine	0.04	0.25	0.19
Ethanolamine	0.10	trace	0.05
Tyramine	nil	1.74	nil
Histamine	nil	1.03	0.18
Cadaverine	nil	2.33	nil
Putrescine	nil	2.65	nil
α-aminobutyric acid	nil	1.00	nil
γ-aminobutyric acid	0.71	6.48	0.39
δ-aminovaleric acid	nil	2.85	nil

* For details of amino acid changes see Table 9.18.

material, unless this is very mature, urine energy losses can be expected to be high from badly-fermented silage because of poor utilisation of silage nitrogen.

There appears to be no information available about the NE values of badly-fermented silages, although it may be assumed that silages containing high levels of acetate are likely to be utilised less efficiently than those containing high lactate or soluble carbohydrates. Acetate is known to be used less efficiently as an energy source for tissue growth than propionate or butyrate,[265] especially if there is a deficiency of glucose or glucose precursors.[266] Both acetate and butyrate are themselves end-products of rumen fermentation and consequently are unlikely to feature as suitable energy sources for rumen microorganisms.

A further consequence of feeding silages low in gluconeogenic substances is the possibility of increasing the risk of hyperketonaemia. In a study with dairy cows fed on a range of silages, some well-preserved and others containing varying levels of butyric acid, Andersson and Lundström[267] found that ketone bodies in milk were significantly positively correlated with butyric acid intake.

Nitrogen

A large part of the nitrogenous components of badly-preserved silages is in a highly degradable form and this, coupled with a deficiency of suitable energy substrates, is likely to result in poor utilisation of silage nitrogen by the rumen microorganisms. Catton and co-workers[268] compared the *in sacco* rumen degradability of a well-preserved silage (pH 3.9) containing a low level of

Table 9.20. The composition and nutritive value of ryegrass silages made with varying levels of formic acid.[162]

	Formic acid (85%) added (litres tonne^{-1})			
	0	2.3	4.6	5.9
DM (g kg^{-1})	200	196	194	193
pH	3.72	3.76	4.32	4.54
TN (g kg^{-1} DM)	21.3	21.4	23.4	23.6
Protein-N (g kg^{-1} TN)	440	500	500	600
Ammonia-N (g kg^{-1} TN)	90	60	50	60
WSC (g kg^{-1} DM)	14	36	36	21
Acetic acid (g kg^{-1} DM)	29	36	19	24
Butyric acid (g kg^{-1} DM)	3	2	2	3
Lactic acid (g kg^{-1} DM)	143	120	73	76
OM digestibility	0.78	0.79	0.78	0.75
N digestibility	0.68	0.68	0.72	0.68
Microbial-N (g N kg^{-1} OMADR)*	23	20	24	23
Amino acids (g amino acid kg^{-1} determined amino acids)				
Glutamic acid	101	120	136	131
Aspartic acid	76	101	101	101
Arginine	42	50	57	62
Alanine	136	101	92	90

* OMADR = Organic matter apparently digested in the rumen.

ammonia-N (8 g kg^{-1} TN) with a badly-preserved silage (pH 4.9) rich in ammonia-N (370 g kg^{-1} TN) and high in butyric acid content (42 g kg^{-1} DM). Although the protein degradabilities of the two silages were similar (0.88 and 0.86), the pattern of release in the well-preserved and the badly-preserved silages was markedly different, with 67% and 90% respectively of the degradable nitrogen disappearing from the bags during the first four hours of incubation in the rumen. It was concluded that a rapid release of nitrogen with no concurrent carbohydrate release was likely to lead to high absorption of ammonia from the rumen and consequent wastage of nitrogen.

Inefficient utilisation of ammonia-N in the rumen may lead to increases in blood urea levels, and it has been suggested[269] that this may be associated with poor conception rates and reduced reproductive efficiency. Ammonia itself may have a detrimental effect on a number of liver functions.

The potential toxic nature of the amines in badly-fermented silages, together with the possible contamination of milk and cheese with clostridial spores,[270] make it imperative that the production of badly-fermented silage is avoided at all costs.

ADDITIVE-TREATED SILAGES

The influence of various additives on the composition of silage has been discussed in Chapter 7; in this chapter the effect of only a few selected additives on the nutritional value of silages will be considered.

Formic acid-treated silages

Composition

The main effects of formic acid on the composition of silage have already been described. In general, increasing levels of formic acid application result in decreasing concentrations of fermentation acids, especially lactic acid, and increasing contents of soluble carbohydrates in silages. The preserving effect of formic acid is enhanced with increasing DM levels (see p. 203). In studies in which the control (untreated) silage is well-preserved, beneficial effects of formic acid on preservation are usually small.

In a study by Chamberlain *et al.*,[162] silages were made from perennial ryegrass with the addition of 0, 2.3, 4.6 and 5.9 litres of formic acid (85%) per tonne of fresh herbage. Some of their results are shown in Table 9.20. It can be seen that the acid addition had very little effect on acetate and butyrate production, but suppressed the production of lactate and ammonia-N and inhibited the breakdown of protein. Although a detailed study of the amino acids in the silages was carried out, only the concentrations of a selected few are shown in Table 9.20. The proportions of glutamic acid and arginine in the total amino acids increased progressively with the level of formic acid applied, whilst that of alanine progressively decreased. All three treated silages were substantially higher in aspartic acid than the untreated silage.

In studies in which control silages have been badly fermented, comparisons between treated and untreated silages are more striking. This is well illustrated in the experiments of Lancaster *et al.*[271] in which six lucerne silages made with formic acid (3.63 to 4.87 litres $tonne^{-1}$) were compared in feeding trials with

untreated silages (Table 9.21). In this example, the addition of formic acid effectively inhibited the development of enterobacteria and clostridia.

Digestibility

Waldo[272] has carried out a comprehensive survey of the literature concerning the effects of formic acid on the digestibility of silages. For direct-cut silages, formic acid treatment increased the digestibility of the organic matter by 7% and for wilted silages by 2%. Several workers, however, have been unable to show such differences. In a comparison of 19 silages preserved with and without formic acid (5 litres tonne^{-1}) Grenet,[273] using sheep, obtained digestibility values for organic matter of 0.702 and 0.706 and for TN of 0.629 and 0.655 respectively. McDonald and Henderson,[274] working with very low DM grass (120 g kg^{-1}), obtained lower organic matter digestibility values for formic acid-treated silages than for untreated ones and this they attributed to the higher loss of digestible nutrients in the effluents from the treated silages.

In the study of Chamberlain *et al.*[162] referred to earlier (Table 9.20), the digestibilities of the four silages were not significantly different. Hingston and Christensen[275] compared the digestibilities, using Hereford steers, of four cereals harvested at the mid-dough stage and ensiled with and without formic acid (10 kg tonne^{-1}). In all cases the digestibilities of DM, GE and TN were similar for the treated and untreated silages, which were all well-preserved.

There is no controversy about formic acid treatment improving organic matter digestibility when the control silage is badly preserved, and this is illustrated in the results shown in Table 9.21. Parker and Crawshaw[276] analysed the data from 22 comparisons of untreated and formic acid-treated silages carried out on ADAS Experimental Husbandry Farms and concluded that when the untreated silages

Table 9.21. The composition and nutritive value of lucerne silages made with and without formic acid (3.63 to 4.87 litres tonne^{-1})[271]

	Treated	Untreated	s.e. difference†
pH	4.36	5.22	0.7***
DM (g kg^{-1})	241	230	5.4
Organic matter (g kg^{-1} DM)	857	826	8.4*
TN (g kg^{-1} DM)	30.3	31.9	0.9
Ammonia-N (g kg^{-1} TN)	94	242	24.4**
Acetic acid (g kg^{-1} DM)	26	41	7.8
Propionic acid (g kg^{-1} DM)	1	5	1.1*
Butyric acid (g kg^{-1} DM)	4	30	5.7**
Lactic acid (g kg^{-1} DM)	30	14	6.9
MAD fibre (g kg^{-1} DM)	359	408	10.0
Organic matter digestibility	0.657	0.588	0.018*
DM intake (g (kg W$^{0.75}$)$^{-1}$ day^{-1})	62.6	45.6	4.06**
LWG (g day^{-1}) (sheep)‡	19	−32	17 *
LWG (g day^{-1}) (cattle)‡	442	60	71 **

† * $p<0.05$, ** $p<0.01$, *** $p<0.001$.
‡ Liveweight gains (LWG) relate to four experiments with sheep and five experiments with cattle.

were well-preserved, organic matter digestibility and animal performance associated with both types of silage were similar, but when the fermentation quality of the control silage was unsatisfactory, formic acid treatment resulted in significantly higher organic matter digestibility values and liveweight gains.

Voluntary food intake

The beneficial effects of formic acid in improving the voluntary intake of silage DM are well known.[57,235,277-283] The extent of the improvement in intake depends very much on the preservation quality of the untreated control silage. For example, the DM intake of the formic acid-treated silage referred to in Table 9.21 was 37% higher than that of the poorly-preserved control silage. This large difference was also reflected in the liveweight gains of both sheep and cattle.

In a general review of the literature, Waldo[272] reported that with young cattle fed no supplemental concentrates, formic acid treatment increased the digestible energy intake by 20% for direct-cut silages and 6% for wilted silages. With lactating cattle fed supplemental concentrates, formic acid treatment increased the DM intake of direct-cut silages by 12% and wilted silages by 9%. In the study by Grenet[273] referred to earlier, a 15% increase in DM intake with sheep was obtained as a result of the formic acid treatment.

In direct comparisons between formic acid-treated unwilted silages and untreated wilted silages, the latter tend to result in slightly higher DM intakes. This is illustrated in the European co-ordinated experiment reported on p. 270. From the data shown in Table 9.16, DM intakes of the wilted silages were 4% higher in dairy cows and 9% higher in growing cattle than DM intakes in animals on the formic acid-treated unwilted silages. These differences, however, were not reflected in terms of animal performance.

Table 9.22. The composition and nutritive value of ryegrass silage made with and without a high level of formic acid[285]

	Untreated	Formic acid-treated*
DM	204	212
pH	4.0	3.8
Protein-N (g kg^{-1} TN)	397	445
Ammonia-N (g kg^{-1} TN)	86	56
WSC (g kg^{-1} DM)	9	117
Acetic acid (g kg^{-1} DM)	57	25
Butyric acid (g kg^{-1} DM)	0	1
Lactic acid (g kg^{-1} DM)	65	46
Ethanol (g kg^{-1} DM)	26	14
DM digestibility	0.665	0.684
ME (MJ kg^{-1} DM)	10.9	11.3
Silage DM intake (g day^{-1})	626	916
Total DM intake (g day^{-1})†	681	971
Liveweight gain (g day^{-1})†	64	161
Feed conversion efficiency (g LWG kg DM $intake^{-1}$)	92	165

* Formic acid (85%) applied at a rate of 5 litres $tonne^{-1}$ grass.
† Silage offered to lambs and supplemented with 60 g brewers' dark grains per day.

Some workers have reported improved DM intakes by feeding precision-chopped, formic acid-treated silages compared with long-chopped, formic acid-treated silages,[65] while others have reported no benefit.[284]

In the past, the recommended commercial minimum level of application of formic acid (85%) to grass crops in the UK has been about 2.3 litres tonne^{-1}. Recently there has been considerable interest in the application of the acid at higher levels than this. In one study by Henderson *et al.*,[285] ryegrass was ensiled direct-cut, untreated or treated with 5 litres formic acid (85%) tonne^{-1}. Some of the results of this study are shown in Table 9.22. In this experiment, the application of formic acid was sufficient to lower the pH of the grass immediately to 4.0 and this had the effect of producing a silage in which a large proportion of the WSC had been preserved. The beneficial effects of this are seen in the improved intakes and performances of the lambs given the treated silage supplemented with brewers' grains.

Energy

Because formic acid restricts fermentation in the silo, it may be expected that the GE values of treated silages will be lower than those of untreated ones. In general this appears to be the case. From a survey of the literature[211,249,286-290] involving 23 different formic acid-treated grass silages, a mean GE value of 19.0 MJ kg^{-1} DM was obtained. This compares with mean values quoted earlier in this chapter (p. 258) of 18.4 MJ kg^{-1} DM for fresh grass and 20.0 MJ kg^{-1} DM for untreated silages. In a direct comparison of four samples of fresh grass and silages made with formic acid, Anderson[288] obtained mean GE values of 18.5 and 19.2 MJ kg^{-1} DM respectively.

It has been shown that when silage fermentation is restricted, losses of energy in the urine are lowered.[42] In the experiments referred to earlier by Grenet,[273] nitrogen excretion values (expressed as % of nitrogen intake) of 47.0 and 53.7 were obtained for formic acid-treated and untreated silages respectively.

In the European co-ordinated experiment described on p. 270 the ME values of the original grasses, unwilted formic acid-treated silages and the wilted silages were not statistically significantly different. There is very little information in the literature on the utilisation of the ME of formic acid-treated silages. In Table 9.8, the k_f values of only two unwilted silages made from herbage treated with formic acid are listed and these are about 20% less than the predicted values. However, in the case of the six silages which were wilted in addition to being treated with formic acid, four of the k_f values are very similar to the predicted values. In a comparison of grass cut at three stages of growth and fed to sheep either fresh, ensiled with formic acid or barn dried, Sundstøl and Ekern[291] found the ME concentrations of the silage DM and the k_m values of the silages were higher than those for the corresponding cuts of grass and hay. Van der Honing *et al.*[292] compared the energy values of hay and formic acid-treated silage and found no difference in the efficiencies with which dairy cows converted ME into NE.

Nitrogen

The addition of formic acid to direct-cut herbage can have a beneficial effect in protecting forage protein, although at the recommended commercial minimum level of application of the acid to grass (2.3 litres of 85% acid tonne^{-1}),

proteolysis is still fairly extensive.[42,209] Several workers have shown that formic acid treatment decreases the rumen degradability of silage protein.[293,294] In a review of the literature, Thomas and Chamberlain[191] concluded that silages made without additive had a mean rumen protein degradability coefficient of 0.82 and silages made with formic acid about 0.63. These values agree generally with those shown in Table 9.9. Chamberlain *et al.*[162] showed that the effect of formic acid on protein digestion in sheep was similar, irrespective of the rate of application, over a range 2.3 to 5.9 kg tonne^{-1} fresh grass (Table 9.20).

The efficiencies of microbial protein synthesis in the rumens of animals fed formic acid-treated, unwilted silages are very variable. In the data presented in Table 9.9, the values range from 10.3 to 24.0 g N kg^{-1} OMADR, which are all lower than the values obtained for animals on hay diets.

Formic acid treatment appears to have a beneficial effect on the subsequent metabolism of silage-N by ruminant animals, compared with untreated silages, as the results shown in Table 9.23 indicate.

Formaldehyde-treated silages

Composition

The effects of formaldehyde as an additive on the composition of silages have already been discussed in Chapter 7. It is generally agreed that formaldehyde should be used in low concentrations and in combination with an acid, usually formic or sulphuric. The acid functions as the main fermentation inhibitor, while formaldehyde protects the protein from excessive degradation.

In general, when used with an acid, increasing levels of formaldehyde produce silages with decreasing levels of fermentation acids and ammonia-N and increasing concentrations of soluble carbohydrates and protein-N. These trends are clearly evident in Table 7.15 (p. 208) and also in Table 9.24, which summarises the results of a study carried out by Kaiser *et al.*[295] using calves.

Digestibility

Because of the reactions of formaldehyde with proteins described on p. 208, the level of this additive which should be applied to harvested crops is critical, as excessive formaldehyde can seriously lower the digestibility of the proteins and often other constituents. Even at moderate levels of application, digestibility can

Table 9.23. The effect of formic acid treatment* of silages on the intake and retention of nitrogen in sheep[273]

	Fresh forages	Untreated silages	Formic acid-treated silages*
Number of silages	17	18	18
TN (g kg^{-1} DM)	23.0	22.6	22.3
Intake of TN (g day^{-1})	32.4	25.0	26.5
Retained N (g day^{-1})	5.1	3.0	4.2
Retained N (g kg^{-1} TN)	157	120	158

* Formic acid (85%) applied at a rate of either 3.5 litres or 5 litres tonne^{-1} grass.

be reduced.[43,214,295-299] For example, in the study by Kaiser *et al.*[295] referred to earlier (Table 9.24) formaldehyde treatment, even at the lowest level (47 g kg^{-1} crude protein), significantly reduced the digestibility of organic matter, cellulose and energy as well as of TN. These workers obtained similar effects of formaldehyde on digestibility in a different experiment using red clover silages.[297]

When lower levels of formaldehyde are used, digestibility of nitrogen may be reduced but not that of other components of the organic matter. For example, in the experiment carried out by Henderson *et al.*,[214] the results of which are shown in Table 9.25, a formic acid/formalin additive applied at a rate equivalent to 26 g formaldehyde kg^{-1} crude protein on the harvested grass significantly reduced the digestibility of the nitrogen but not of the DM, organic matter and energy compared with the untreated silage. At a higher level of formaldehyde application (56 g formaldehyde kg^{-1} crude protein) applied with sulphuric acid instead of formic acid, the digestibilities of DM, organic matter, energy and TN were all significantly depressed by the treatment. It appears that the effects of formaldehyde on digestibility are directly related to the level of application.

Voluntary food intake

The adverse effects of high levels of formaldehyde on DM intake have already been referred to in Chapter 7. When used at relatively low levels (15 to 56 g formaldehyde kg^{-1} crude protein), and combined with an acid, the additive does not reduce DM intake and may even result in small increases.[214,273,295,297,300,301]

Table 9.24. Composition and nutritive value of ryegrass silages preserved with the addition of formic acid (2 litres $tonne^{-1}$) and different levels of formaldehyde[295]

	Formaldehyde applied at ensiling (g kg^{-1} crude protein)		
	0	47	100
DM (g kg^{-1})	218	230	226
pH	4.0	4.4	5.0
TN (g kg^{-1} DM)	24.3	23.7	23.7
Protein-N (g kg^{-1} TN)	439	659	773
Ammonia-N (g kg^{-1} TN)	75	31	12
Acetic acid (g kg^{-1} DM)	20	11	4.1
Butyric acid (g kg^{-1} DM)	0.9	2.3	1.0
Lactic acid (g kg^{-1} DM)	112	38	6.9
GE (MJ kg^{-1} DM)	18.3	18.6	18.0
OM digestibility	0.76	0.73	0.71
Cellulose digestibility	0.83	0.82	0.77
Energy digestibility	0.71	0.69	0.65
N digestibility	0.61	0.51	0.48
N intake (g day^{-1})	76.4	74.3	63.0
N in faeces (% of N intake)	38.8	48.7	51.8
N in urine (% of N intake)	39.1	22.9	18.6
N retention (g day^{-1})	20.5	27.0	28.2
Ruminal ammonia-N (mg $litre^{-1}$)	85	38	16
DM intake (g (kg W)$^{-1}$ day^{-1})	25.7	25.7	21.4

Table 9.25. Composition and nutritive value of silages treated with different additives[214]

	Untreated	Formic acid (3.4 litres tonne^{-1})	Sulphuric acid +formalin (4.6 litres tonne^{-1})*	Formic acid +formalin (4.8 litres tonne^{-1})†
DM (g kg^{-1})	181	184	176	189
pH	3.8	3.7	4.0	3.6
TN (g kg^{-1} DM)	26.5	23.0	25.1	24.6
Protein-N (g kg^{-1} TN)	400	490	509	630
Ammonia-N (g kg^{-1} TN)	65	49	44	31
Acetic acid (g kg^{-1} DM)	34	15	25	12
Butyric acid (g kg^{-1} DM)	0.02	0.03	0.21	0.16
Lactic acid (g kg^{-1} DM)	98	44	64	36
Ethanol (g kg^{-1} DM)	6.7	9.2	18	12
GE (MJ kg^{-1} DM)	19.6	18.6	18.0	18.2
OM digestibility	0.76	0.77	0.74	0.77
Energy digestibility	0.75	0.74	0.70	0.76
ME (MJ kg^{-1} DM)	12.1	11.3	10.3	11.1
N digestibility	0.73	0.69	0.64	0.67
N retention (g N day^{-1})	7.21	5.38	6.17	8.47
DM intake (g (kg W)$^{-1}$ day^{-1})	28.2	29.8	27.7	30.7
Liveweight gain (g day^{-1})	200	231	236	267

* Equivalent to 56 g formaldehyde kg^{-1} crude protein.
† Equivalent to 26 g formaldehyde kg^{-1} crude protein.

In a study with growing cattle, reported by Haigh *et al.*,[300] these increases in intake resulted in significant improvements in daily liveweight gain. Other workers have reported similar improvements in liveweight gain when feeding formaldehyde/acid-treated silages.[214,272,300]

Energy

As mentioned in an earlier section in this chapter, any additive treatment which restricts microbial activity during ensilage should result in silages with GE values lower than those of untreated ones. In the experiment referred to in Table 9.25, the GE value of the well-preserved, untreated silage was 19.6 MJ kg^{-1} DM, and that of the formalin/formic acid-treated silage was 18.2 MJ kg^{-1} DM. In comparisons with silages treated with formic acid only, GE values of formaldehyde-treated silages are unlikely to differ markedly.

The ME values of formaldehyde-treated silages are likely to vary depending upon the relative balance between the increased losses of energy in the faeces and the reduced losses of energy in the urine. There appears to be no evidence to indicate that methane losses vary greatly. In the study reported in Table 9.25, the ME values of the three treated silages were all lower than the value of the untreated silage.

Very few NE determinations of formaldehyde-treated silages have been carried out. In Table 9.8 four such silages are listed. With the exception of the silage fed

with sucrose, the k_f values determined experimentally are all lower than those predicted from the ARC equation. In a direct comparison of a formaldehyde-treated silage with an untreated silage made from similar ryegrass, McLellan and McGinn[141] obtained k_f values of 0.40 and 0.34 respectively.

Nitrogen

The effect of formaldehyde in reducing proteolysis and rumen degradability has been stressed in Chapter 7. In a study with sheep, Beever *et al.*[159] fed ryegrass silage, pre-treated with formaldehyde, at a rate equivalent to 60 g kg^{-1} crude protein, and noted an increase of 33% in total amino acid flow to the small intestine when compared with untreated silage, and a marked change in the proportions of microbial and feed protein entering the duodenum. Ruminal protein degradation was reduced from 0.85 to 0.22. This in turn led to a reduction in the supply of available nitrogen for microbial growth and, as a consequence, the extent of microbial protein synthesis declined from 26.7 to 9.0 g N kg^{-1} OMADR. Changing the composition of duodenal protein caused the overall availability of amino acids within the small intestine to be reduced from 75% in the control to 67% in the formaldehyde treatment, with the net result that amino acid uptake on the treated diet was increased by only 13%.

Siddons *et al.*[160] used a lower level of formaldehyde (35 g kg^{-1} crude protein) combined with formic acid and confirmed that amino acid supply to the small

Table 9.26. Composition and nutritive value of wilted ryegrass silages treated with either formic acid or a formic acid-formalin mixture[299]

	Formic acid treated (3.14 g kg^{-1})	Formic acid + formalin treated (2.86g acid + 1.44g formalin kg^{-1})
pH	4.09	4.08
DM (g kg^{-1})	272	280
TN (g kg^{-1} DM)	21.6	21.8
Protein-N (g kg^{-1} TN)	383	508
Ammonia-N (g kg^{-1} TN)	70	50
WSC (g kg^{-1} DM)	57	99
Formic acid (g kg^{-1} DM)	12	12
Acetic acid (g kg^{-1} DM)	92	74
Propionic acid (g kg^{-1} DM)	0.3	0.2
Butyric acid (g kg^{-1} DM)	0.2	0.7
Lactic acid (g kg^{-1} DM)	92	74
Ethanol (g kg^{-1} DM)	21.0	11.0
GE (MJ kg^{-1} DM)	18.7	18.6
ME (MJ kg^{-1} DM)*	10.35	11.14
Digestibility of organic matter*	0.725	0.725
Digestibility of N*	0.702	0.683
N retention (g day^{-1})*	5.66	7.09
Intake of ME (MJ per head)†	9260	10018
Liveweight gain (g day^{-1})†	831	977

* Using sheep.
† Using cattle.

intestine was increased. Although the concentration of ammonia in the rumen declined from 241 mg litre^{-1} to 107 mg litre^{-1}, this still appeared to be sufficient to sustain an active microbial population in the rumen, as no apparent reduction in microbial protein synthesis could be detected. Ruminal degradation of silage protein declined from 0.86 in the control to 0.43 in the additive treatment; this extra feed protein accounted for 85% of the increased flow of amino acids to the duodenum. With this lower level of formaldehyde application, in contrast to the higher level used by Beever *et al.*,[159] the availability of the amino acids was not affected by formaldehyde treatment and the net effect of using the additive was to increase the proportion of the dietary-N absorbed in the form of amino acids from 35% in the control to 68% in the treated material.

In a study by Rooke *et al.*,[164] using wilted perennial ryegrass a very low level of formaldehyde was used (15 g kg^{-1} crude protein) combined with formic and sulphuric acids as an additive. The degradability of the dietary protein in the treated silage (0.8) and the efficiency of microbial protein synthesis (26.5 g N kg^{-1} OMADR) were unaffected by the additive treatment.

Hinks *et al.*[299] compared ryegrass silages treated with either formic acid (3.14 g kg^{-1}) or a formic acid/formalin (2.86 g kg^{-1}/1.44 g kg^{-1}) mixture in digestibility studies with sheep and feeding trials with growing cattle. The formaldehyde addition in this experiment was similar to that used by Rooke *et al.*[164] (15.3 g kg^{-1} crude protein). Differences in digestibility between the two silages were small and non-significant, but the ME value of the silage treated with the additive mixture was significantly higher than that of the silage treated with acid alone (Table 9.26). When the silages were fed to cattle, supplemented with 1.5 kg day^{-1} of barley, the intakes of ME and daily liveweight gains were significantly higher on the acid/formalin silage than on the silage treated with acid alone.

Ammonia-treated silages

Composition

Pre-treatment of maize with ammonia reduces carbon dioxide production during the early stages of ensiling and reduces DM and energy losses during ensiling by 5 to 10%.[302] Recovery of ammonia varies[302,303] but 70 to 80% recovery can be obtained, with a concomitant increase in crude protein in the silage.[304]

The concentration of fermentation acids depends upon the rate of application of ammonia. When Heinrichs and Conrad[304] treated whole-crop maize plants at 350 g DM kg^{-1} with 3.2 or 4.5 kg anhydrous ammonia tonne^{-1} at ensiling, the final pH values increased with treatment but the treated silages contained less acetic and propionic acids and more butyric acid than the untreated silage. The lactic acid content was higher in the low ammonia-treated silage (83 g kg^{-1} DM) but lower in the higher ammonia-treated silage (35 g kg^{-1} DM) than in the untreated silage (60 g kg^{-1} DM). In a study by Deschard *et al.*,[305] in which they ensiled immature wheat at a DM content of 410 g kg^{-1} with ammonia applied at 35 kg tonne^{-1} DM, lactic acid and ethanol production were greatly reduced and the silages had a pungent smell, with pH values of 8.4 to 8.9 compared with 4.0 for the untreated silage.

Treatment with ammonia reduces hemicellulose concentrations[305,306] and this may affect the digestibility of the forage.

Digestibility

Ammonia treatment of maize silage has been shown by Goering and Waldo[302] to have no effect on DM or energy digestibilities. Cell wall digestibility increased with treatment (3.5 kg ammonia tonne^{-1} fresh matter) from 0.539 to 0.582 but the difference was not significant. However, when Deschard *et al.*[305] applied a higher rate of ammonia (35 kg tonne^{-1} DM) to wheat the apparent digestibilities of organic matter were 0.621 and 0.711 and those of the acid detergent fibre (ADF) fractions were 0.457 and 0.688 for the untreated and treated silages respectively.

Voluntary food intake

In the study by Heinrichs and Conrad[304] described earlier, in which untreated and ammonia-treated maize silages were offered to dairy cattle, there were no significant differences in intake among the silages, although the patterns of feed intake were altered. The weights of both the initial and spontaneous meals were increased significantly for the high ammonia-treated silages when compared with the untreated and low ammonia-treated silages. Voluntary intake of wheat silage[305] by sheep was increased with ammonia treatment despite the high ammonia-N content (668 g kg^{-1} TN) of the silage, but it was consumed in smaller portions and over an extended period.

Energy

Energy losses during ensiling are reduced with ammonia treatment,[302] and energy recovery is further improved as ammonia has mould inhibiting properties. Soper and Owen[307] have noted improved stability of maize silages with ammonia addition at ensiling. A similar improvement in the stability of whole-crop barley silage treated with 15 or 30 g anhydrous ammonia kg^{-1} DM was observed by Witt and Mølle.[308]

When Goering and Waldo[302] offered ammonia-treated maize silage rather than maize silage plus urea to yearling Holstein heifers, 12% less DE was required for 1 kg gain in liveweight.

Nitrogen

Many workers have noted the inhibition of protein breakdown during ensilage with the addition of ammonia.[303,309,310,311] When Johnson *et al.*[311] treated chopped whole maize at a DM content of 330 g kg^{-1} with 0, 0.25, 0.52 or 1.08% added nitrogen as ammonia to the dry matter, the water insoluble nitrogen in the silages increased with added ammonia, from 7.6 to 11.4 g kg^{-1} DM for the untreated and ammonia-treated (1.08%) silages respectively. In addition, large increases in alanine were noted in the water phase of the silage treated with the highest level of ammonia. Results have shown that anhydrous ammonia, when used to treat maize silage, can be an economical substitute for proteins in rations for dairy cows.[304]

Inoculated silages

Well-preserved silages have been described as those in which lactic acid bacteria have dominated the fermentation (p. 250). When viable homofermentative lactic

acid bacteria are applied to a crop in numbers greater than those of the epiphytic microflora, lactic acid is the main product of the fermentation. Excessive ingestion of lactic acid-rich foods may cause lactic acidosis, probably due to the D(−) isomer of lactic acid which, unlike the L(+) isomer, is very slowly metabolised by gluconeogenesis.[312] Vanbelle *et al.*[313] have therefore suggested that it would be beneficial if added lactic acid bacteria not only inhibited other organisms but also moved the fermentation in the direction of the L(+) isomer of lactic acid.

Composition

Silages treated with an inoculum of lactic acid bacteria will have a faster rate of fall of pH and will usually contain less ammonia-N and acetic acid and more lactic acid and residual sugars than untreated silages. In silages in which there is an adequate supply of substrate for the lactic acid bacteria, other changes in composition will be similar to those found in well-preserved silages (p. 250). In silages made from low sugar crops, the conversion of lactic acid to acetic acid which occurs with time will be less but may not be prevented by inoculating the

Table 9.27. Composition and nutritive value of silages treated with 85% formic acid (3 litres tonne^{-1}), *L. plantarum* (10^5 cfu g^{-1}) or *L. plantarum*/*P. pentosaceus* (10^6 cfu g^{-1})[316]

Treatment*	U	F	I_5	I_6	s.e.
DM (g kg^{-1} DM)	168	182	163	181	
pH	4.55	4.44	4.40	4.09	
TN (g kg^{-1} DM)	33.1	32.5	33.0	31.9	
Protein-N (g kg^{-1} TN)	386	455	358	407	
Ammonia-N (g kg^{-1} TN)	130	109	131	88	
WSC (g kg^{-1} DM)	0	11	0	20	
Acetic acid (g kg^{-1} DM)	46	35	45	30	
Propionic acid (g kg^{-1} DM)	8.7	5.4	7.4	6.7	
Butyric acid (g kg^{-1} DM)	5.2	2.1	5.7	5.3	
Lactic acid (g kg^{-1} DM)	59	51	71	84	
Ethanol (g kg^{-1} DM)	13	33	9.3	6.8	
GE (MJ kg^{-1} DM)	19.4	19.7	19.5	19.9	
DOM (g kg^{-1} DM)	710^{a}	726^{b}	723^{b}	737^{c}	4.9
Silage DM intake (g day^{-1})	681^{a}	692^{ab}	753^{bc}	792^{c}	34.3
Total DM intake (g day^{-1})	857^{a}	863^{a}	929^{ab}	968^{b}	41.8
Liveweight gain (g day^{-1})	71^{a}	94^{a}	124^{b}	129^{b}	14.8

*U = Untreated.
F = 85% formic acid.
I_5 = *L. plantarum*.
I_6 = *L. plantarum*/*P. pentosaceus*.
Means with same superscripts within rows are not significantly different ($p<0.05$).

crop with homofermentative lactic acid bacteria.[314] Despite the more efficient conversion of sugars to lactic acid in the inoculated silages, DM losses (g kg^{-1} DM ensiled) are frequently similar to those from untreated silages.[315]

The composition of silages which were treated with 85% formic acid (3 litres $tonne^{-1}$), with an inoculum of *L. plantarum* (10^5 cfu g^{-1}) or with an inoculum of *L. plantarum* and *P. pentosaceus* (10^6 cfu g^{-1}) is shown in Table 9.27.[316] The grass, direct-cut precision-chopped perennial ryegrass, was cut pre-ear emergence with a DM content of 146 g kg^{-1} and a WSC content of 151 g kg^{-1} DM. The higher rate of application of lactic acid bacteria produced the silage with the lowest pH value, the lowest ammonia-N and acetic acid contents, and the highest residual sugar and lactic acid contents. The lower rate of application of lactic acid bacteria had little effect on the composition, with the exception of the lactic acid content.

Digestibility

In the majority of trials reported in the literature, the silages treated with an inoculum of lactic acid bacteria appeared to be more digestible than the untreated silages.[316-322] In others, however, the additive had no effect or a negative effect on digestibility.[290,315,323] In the feeding trial carried out by Henderson *et al.*[316] (Table 9.27), both inoculants had a significant effect on DM digestibility but the effect was greater with the higher rate of application of lactic acid bacteria.

Voluntary food intake

In the early 1980s several experiments were reported in which the application of an inoculant had no effect on the composition of the silage nor on voluntary food intake.[324-326] In these trials the species, numbers and viability of the added microorganisms may have been unreliable. In later studies with improved commercial inoculants, a number of workers have reported increased silage DM intakes with no obvious improvements in fermentation.[316,319,320,323] In the trial referred to in Table 9.27,[316] in which the silage was treated with 10^5 lactic acid bacteria g^{-1}, and in the trial carried out by Steen *et al.*,[323] the treated silages appeared to be similar in composition to the poorly-fermented untreated silages. In the former trial, lambs ate significantly more of the treated silage and gained significantly more weight, as did steers in the latter trial. In both trials the pH declined more rapidly initially with the application of the inoculant. This is known to have inhibited the activity of the enterobacteria[316] and may have reduced the production of endotoxins, as suggested by Lindgren *et al.*,[327] thus influencing the intake of the silage.

Similar improvements in silage DM intake were reported by Gordon[319,321] when silages treated with an inoculum of lactic acid bacteria (10^6 *L. plantarum* g^{-1}) were evaluated with lactating cattle. In both trials the untreated silages were well-preserved, but in one[319] the inoculant increased silage DM intake over the final four weeks on treatment by 12% and milk yield by 10% and in the other[321] by 5% and 5% over the untreated silage. The results of one of these trials are summarised in Table 9.28.[319] The grass ensiled was the first regrowth of a perennial ryegrass sward with a DM content of 153 g kg^{-1} and a WSC content of 24.1 g kg^{-1} fresh weight; the silages were offered to British Friesian cows of mixed parity (16 per treatment).

In trials in which inoculated silages were offered to beef cattle, neither Appleton and Done[328] nor Henderson *et al.*[329] reported significant differences in intakes over well-preserved untreated silages, but there were differences in liveweight gain which could not be related directly to differences in silage composition.

In trials in which lucerne,[330-332] sorghum[330,333] and maize[330,334] were treated with a range of additives containing lactic acid bacteria, no improvements were detected in animal performance over the untreated silages but in one trial there was an improvement in the quality of low moisture lucerne silage.[332]

Differences in the efficacy of inoculants of lactic acid bacteria are to be expected since they, unlike chemical additives such as formic acid, do not have identical properties; even different strains of *L. plantarum* vary in their fermentation characteristics.

Energy

In general, the GE values of inoculated silages are similar to those of untreated silages but, when the digestibilities are greater, ME values tend to be higher. In

Table 9.28. Silage composition, feed intake and animal performance during final four weeks on treatment[319]

Treatment	Untreated	Formic acid-treated*	Inoculant-treated	s.e.
DM (g kg^{-1})	184^{a}	186^{a}	185^{a}	1.89
pH	3.73^{a}	3.79^{a}	3.74^{a}	0.02
TN (g kg^{-1} DM)	24.2^{a}	25.3^{b}	25.1^{b}	0.06
Ammonia-N (g kg^{-1} TN)	64^{a}	50^{b}	51^{b}	1.80
WSC (g kg^{-1} DM)	8.9^{a}	13.0^{b}	12.9^{b}	0.62
Acetic acid (g kg^{-1} DM)	22.1^{a}	20.0^{a}	13.7^{b}	1.14
Propionic acid (g kg^{-1} DM)	0.76^{a}	0.46^{b}	0.57^{ab}	0.08
Butyric acid (g kg^{-1} DM)	0.81^{a}	0.23^{a}	0.74^{a}	0.18
Lactic acid (g kg^{-1} DM)	114	93	109	5.50
Silage DM intake (kg day^{-1})	9.89^{a}	10.06^{a}	11.09^{b}	0.13
Concentrate DM intake (kg day^{-1})	4.3	4.3	4.3	
Milk yield (kg day^{-1})	22.1^{a}	21.9^{a}	24.2^{b}	0.52
Milk composition (g kg^{-1})				
Fat	37.0^{a}	40.2^{b}	36.8^{a}	0.66
Protein	27.8^{a}	27.7^{a}	28.0^{a}	0.21
Lactose	46.0^{a}	45.9^{a}	46.4^{a}	0.20
Milk constituent output (kg)				
Fat	838^{a}	908^{b}	905^{b}	22.4
Protein	618^{a}	605^{a}	693^{b}	14.1
Lactose	1030^{a}	1002^{a}	1108^{b}	24.5

* Formic acid (85%) applied at the rate of 2.27 litres tonne^{-1}.
Means with the same superscripts within rows are not significantly different ($p<0.05$).

most trials improved animal performance is related to increased ME intake. In the trial referred to earlier[319] (Table 9.28), the cows on the inoculated silage consumed 15.6 MJ day^{-1} above that of animals on the control silage. The resulting response in milk energy output of 6.1 MJ day^{-1} represented a milk energy response of 0.39 MJ milk energy output per additional MJ of ME intake.

Nitrogen

There is little evidence to show that the nitrogenous components in inoculated silages are utilised any more efficiently than those in the untreated silage, but Rooke *et al.*[290] observed a marked difference in both nitrogen digestibility and nitrogen retention in sheep between treated and untreated silages. Decreases in urinary-N excretion were largely responsible for the improvement in nitrogen retention with the treated silage, and it is probable that this resulted from improved microbial-N synthesis within the rumen and/or reduced silage-N degradability within the rumen. However, Gordon[319] detected no improvement in nitrogen retention in the cows when the silages were supplemented with concentrates.

Enzyme-treated silages

The addition of cell wall degrading enzymes to release additional substrate may

Table 9.29. Composition and nutritive value of perennial ryegrass silages treated with or without a hemicellulase/cellulase mixture[339]

	Untreated	Enzyme-treated	s.e.d.
DM (g kg^{-1})	180	202	
pH	4.20	3.72	
Organic matter (g kg^{-1} DM)	902	902	
TN (g kg^{-1} DM)	25.8	27.1	
Protein-N (g kg^{-1} TN)	492	538	
Ammonia-N (g kg^{-1} TN)	87	61	
WSC (g kg^{-1} DM)	3	31	
Acetic acid (g kg^{-1} DM)	62	32	
Propionic acid (g kg^{-1} DM)	4	2	
Butyric acid (g kg^{-1} DM)	1	0	
Lactic acid (g kg^{-1} DM)	70	110	
Ethanol (g kg^{-1} DM)	6	4	
Acid detergent fibre (g kg^{-1} DM)	286	246	
Neutral detergent fibre (g kg^{-1} DM)	471	425	
Hemicellulose (g kg^{-1} DM)	185	179	
Buffering capacity (mE kg^{-1} DM)	1327	1179	
GE (MJ kg^{-1} DM)	19.3	20.3	
Silage DM intake (g day^{-1})†	785	770	36.0
Liveweight gain (g day^{-1})†	72.0	81.8	14.38
DOM (*in vivo*) (g kg^{-1} DM)†	691	656	8.6
DE (MJ kg^{-1} DM)†	14.5	14.9	0.17
ME (MJ kg^{-1} DM)†	11.9	12.4	0.18

† Using sheep.

be beneficial to crops in which the lack of substrate rather than the numbers of viable lactic acid bacteria may be the limiting factor in the production of a well-preserved silage. The ability of cellulases and hemicellulases to produce additional sugars and the effects of pH, temperature and crop DM on the hydrolysis of the structural carbohydrates have been discussed in Chapter 7 (p. 195).

Composition

In general, silages treated with cell wall degrading enzymes have lower pH values and contain more lactic acid and less acetic acid and ammonia than the corresponding untreated silages.[335-338] In some trials, but not all, enzyme treatment produces silages which contain more residual sugars than the untreated silages. Neutral detergent fibre and acid detergent fibre contents are lower in enzyme-treated silages,[339,340] the extent of the decrease being related to the rate of application of the enzymes, the stage of maturity of the crops and their DM contents. With increasing maturity and DM content the effects of the enzymes diminish.

The composition of a typical low DM, enzyme-treated silage is shown in Table 9.29.[339] Perennial ryegrass with a WSC content of 158 g kg^{-1} DM was ensiled direct-cut (DM 154 g kg^{-1}) with and without a hemicellulase/cellulase mixture derived from *Trichoderma viride (reesei)* applied at the rate of 0.3 litre $tonne^{-1}$.

Digestibility

Claims have been made that cell wall degrading enzymes will upgrade grasses during ensilage by increasing their digestibility. However, it appears that cellulase enzyme systems will first attack the most easily digested fraction of the fibre in the plant and that the residual fibre will be less digestible; this in turn may result in the treated silage having a lower organic matter digestibility than that of the untreated silage. This is illustrated in Table 9.29,[339] which gives the results of a digestibility trial in which the untreated and enzyme-treated silages were each offered to lambs, four per silage; the *in vivo* organic matter digestibility of the treated silage was significantly lower than that of the untreated silage. Increased effluent flow has been observed in some enzyme-treated silages[341,342] and this may also have contributed to the reduction in the digestibility of the silage organic matter in this trial.

Olsen and Voelker[343] and Owen[344] reported that added cellulase failed to increase the digestibility of lucerne silages by ruminants, and when Jacobs and McAllan[341] treated perennial ryegrass with a mixture of hemicellulases, cellulases and glucose oxidase the untreated and treated silages were well digested and no differences were observed in whole tract organic matter digestibilities. However, McHan[345] improved the *in vitro* digestibility of Coastal bermuda grass silage with the application of 30 g NaOH kg^{-1} plus 5 g cellulase kg^{-1}, and Baintner *et al.*[346] increased the digestibility *in vivo* of maize and maize/sorghum silages with the application of a cellulase and urea. Urea was used to slow down the drop in pH value during fermentation and to increase the efficiency of the cellulase by raising the final pH of the silages.

Huhtanen *et al.*[335] and van Vuuren *et al.*[340] studied the effect on degradability *in sacco*[347] of enzyme treatment. The very rapidly degradable fraction of organic

matter increased with treatment[335,340] and with the rate of application of the cellulase[335] but the undegradable organic matter fraction was not affected by treatment.[335,340] Van Vuuren *et al.*[340] found no change in the rate of degradation of the potentially degradable fraction but in the trials carried out by Huhtanen *et al.*[335] the rates of degradation with treatment were variable.

Voluntary food intake

The increase in the instantly degradable organic matter fraction with enzyme treatment may speed up the rate of passage through the rumen and Mo[348] has suggested that this may lead to an increase in intake. Where there has been an improvement in fermentation quality with enzyme treatment, an increase in silage DM intake has not always been evident, as illustrated in Table 9.29.[339] However, in another trial in which the ammonia-N content was reduced from 240 g kg^{-1} TN to 163 g kg^{-1} TN with treatment with a different enzyme mixture[349] there was a significant increase in silage DM intake from 6.75 kg day^{-1} to 8.19 kg day^{-1} when the silages were offered to dairy cattle. In trials in which the untreated silages were well-preserved,[342,350,351] no significant increases in silage DM intakes were obtained with enzyme treatment. Kennedy[342] found no significant increase in liveweight gain with enzyme treatment when treated silages were offered to beef cattle. However, when Chamberlain *et al.*[350] and Chamberlain and Robertson[351] offered enzyme-treated silages to dairy cows they produced higher milk yields than those offered untreated silages. In one trial[351] both the milk yields and the milk protein yields were significantly higher with enzyme treatment.

When Jaster and Moore[352] treated lucerne (DM 550 g kg^{-1}) with an enzyme preparation having cellulolytic and amylolytic activity, the high DM silage (haylage) pH values were 4.4 for the untreated and 4.2 for the treated material; there were no differences in DM intake, DM digestibility, milk production or milk composition when the haylages were offered to dairy cows.

When enzymes were applied to timothy grass[352] (DM approximately 260 g kg^{-1}) or to ryegrass/white clover[354] (DM approximately 240 g kg^{-1}) ensiled in big bales, the treated silages had lower modified acid detergent fibre contents, pH values and ammonia-N contents and higher residual sugar and lactic acid contents than the untreated silages. The higher silage intakes and, in one trial,[354] the significantly higher daily liveweight gains reflected the better fermentations in the treated silages.

Energy

Any prediction of energy value based on the fibre content of a silage which has been treated with a cell wall degrading enzyme may be misleading. There will be less fibre in a treated silage than in an untreated silage but the fraction which remains may be less digestible.[339,340] The GE value of a treated silage will depend upon the extent of the fermentation in the silo, the loss of DM in the effluent and the GE value of the effluent DM. In the experiment described earlier (Table 9.29) the higher GE value of the treated silage was partially offset by the lower digestibility of this silage. If the increase in rumen solubility of enzyme-treated silages results in an increase in the synthesis of microbial protein, this should lead to a reduction in the loss of energy in the urine and, partly as a result of this, to improved ME:DE ratios.[355]

Nitrogen

In the trials described by Huhtanen *et al.*[335] and van Vuuren *et al.*,[340] enzyme treatment increased the instantly degradable crude protein fraction, the greatest increase occurring in the least mature silages. Van Vuuren *et al.*[340] used the *in sacco* technique to demonstrate that the insoluble degradable fraction of crude protein decreased with increasing maturity whereas the undegradable fraction increased. Effective crude protein degradabilities estimated at a rate of passage of 0.03 hour^{-1} ranged from 0.80 to 0.90.

Jacobs and McAllan[356] compared the duodenal nitrogen constituent flows and weight gains in growing steers offered formic acid-treated silage (3.0 litres tonne^{-1}) or enzyme-treated silage (0.3 litres cellulase/hemicellulase/glucose oxidase tonne^{-1}) with or without supplements of rapeseed meal or fish meal. Efficiencies of microbial protein synthesis were not significantly different among the formic acid-treated silage diets nor between these diets and the unsupplemented enzyme-treated silage diet, but they were significantly higher for the enzyme-treated silage supplemented with rapeseed meal. Microbial nitrogen flows at the duodenum were 59, 75, and 81 g day^{-1} with rapeseed meal supplements of 0%, 6% and 12% of the DM respectively. The higher protein flows at the duodenum were reflected in higher liveweight gains. Huhtanen *et al.*[335] have also demonstrated an interaction between enzyme treatment of silage and protein supplementation.

Inoculated/enzyme-treated silages

As the epiphytic lactic acid bacteria are a mixture of homofermentative and heterofermentative strains, Setälä[357] has recommended that enzymes should be used as silage additives together with an inoculum of homofermentative lactic acid bacteria to encourage an efficient fermentation of the sugars released by the enzymes. Many commercial products do contain both[358] but the cost of enzymes limits their concentration in these products and, therefore, the degradation of the structural carbohydrates during ensilage. In laboratory studies the combination of lactic acid bacteria with enzymes has been shown to improve the fermentation,[336,357] but there is little information available on the nutritive value of silages treated with effective levels of both lactic acid bacteria and cell wall degrading enzymes.

AEROBICALLY DETERIORATED SILAGES

Many silages when exposed to air immediately start to deteriorate. If the period of aerobic exposure is long, extensive changes in the composition of the silages can occur and this is likely to influence adversely their nutritional value. The changes are brought about firstly by bacteria and yeasts and subsequently by moulds. Initially the soluble components of silages, WSC, organic acids and soluble nitrogenous compounds, act as substrates for the development of these microorganisms, and the losses of these nutrients result in corresponding increases in crude fibre and ash contents.[359,360] Increases in pH and ammonia-N also frequently occur, although changes in the latter are inconsistent because of variable losses through volatilisation. In the terminal stages of deterioration, structural carbohydrates can also be decomposed.[359]

Information on the nutritive value of deteriorated silages is sparse.[361-363] Two experiments using maize silage were carried out by Deoka *et al.*.[361] In the first of these, whole crop maize silages, undeteriorated and aerobically deteriorated for three days, were fed to sheep. There were no significant differences between the two silages in DM intakes, digestibilities and concentrations of ammonia-N and volatile fatty acids in the rumen. In the second experiment, in which maize silage was aerobically deteriorated for five days, the aerobic treatment resulted in a significant decrease in DM intake.

In a study with grass silages, Fujita and co-workers[362] compared the nutritional values of silages aerobically deteriorated for three to seven days with those of undeteriorated silages. Significant decreases in the digestibilities of organic constituents were observed in the deteriorated silages. Cows fed the latter had greater nitrogen losses in their faeces and urine, and significantly lower nitrogen retention values. Oxygen consumption, carbon dioxide production and heat production per unit of metabolic body weight were increased in animals fed the deteriorated silages, suggesting that there was a decrease in the availability of NE.

High losses of β-carotene can occur from silages exposed to air.[16] In studies with wilted lucerne, Másková and Havelik[364] demonstrated that during the aerobic deterioration of wilted lucerne silages over a 10-day period losses of β-carotene could amount to 73%.

In addition to a reduction in nutritional value, aerobic deterioration of silages can result in serious health risks through the development of mycotoxin-producing moulds, such as *Aspergillus* spp. and *Fusarium* spp., and undesirable pathogens such as *Listeria monocytogenes*.[365]

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