THE EFFECT OF ADDITIVES ON THE MICROBIOLOGY OF GRASS SILAGE

by

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Dedicated to the memory of my father

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TABLE OF CONTENTS

A B S T R A C T

Formalin, formic acid, acetic acid and propionic acid were tested for use as silage additives on a laboratory scale in Section A. Laboratory silos of 40 g capacity were used, and the extent of fermentation was assessed by measuring pH and WSC changes. At low levels of application, the silage fermentation was unaffected by formalin addition but as the rate of application was increased, so the level of WSC increased until at an application rate of 7 g kg^{-1}, there was complete conservation of WSC in the silage. All levels of formic acid application tested resulted in silages in which the fermentation had been restricted, but acetic acid and propionic acid had very little effect on the course of the silage fermentation. When additives were used combined in pairs, the silage made with the addition of formalin/acetic acid mixture 9.6 g kg^{-1} was the only one in which the WSC content was higher than that of either of the silages made with the addition of one or other of the component parts of the mixture.

In pure culture studies, formalin was found to inhibit the growth of lactic acid bacteria at a much lower concentration than that found necessary to restrict fermentation in silage. Growth of Leuconostoc, Pediococcus and Streptococcus strains was inhibited by formic acid although acetic acid and propionic acid were not effective in inhibiting the growth of lactic acid bacteria at the maximum concentration tested.

The development of the microflora of a number of additive treated farm silages was investigated and reported in Section B. Formic

acid treatment of grass at the time of forage harvesting resulted in the material being loaded into the silo with a lower level of lactic acid bacteria than the corresponding control. Formic acid treated silages had lower counts of lactic acid bacteria than their respective controls though there was not such a marked difference in total count. Additive mixtures containing formalin did not show such a specific effect although formalin treatment itself depressed both total and lactic acid bacteria counts.

The susceptibility of silages to aerobic deterioration was studied in Section C. The available heat energy in k cal was calculated for a series of additive treated silages and it was found that the total energy used in raising the temperature of the silage was generally 10% of the available heat energy. Silages with high contents of residual sugar and high levels of lactic acid with very small quantities of other fermentation acids were found to deteriorate rapidly on exposure to aerobic conditions. The inclusion of acetic acid in additive treatments produced silages which were stable.

The changes in the microflora of a set of additive treated silages were examined over a 96 hour period of aerobic storage. The control silage was stable and yeasts were not isolated from it until the end of the storage time. Species of lactic acid assimilating yeasts, in particular Hansenula anomila were isolated from the deteriorating silages. Bacillus species were also isolated.

The concluding experiment reported in Section D was designed to provide a final assessment of formalin, formic acid, acetic acid and propionic acid as silage additives applied singly and in mixtures of two,

three and four at a constant application rate of 4.5 g kg^{-1}. All additives restricted silage fermentation. The additive which produced silage which fulfilled all the criteria set i.e. high WSC content, high pH and stability under aerobic storage conditions was a formic acid/acetic acid/propionic acid mixture.

G L O S S A R Y

BC	-	Buffering capacity
CP	-	Crude protein
DM_o	-	Oven dry matter
DM_t	-	Toluene dry matter
H_A	-	Available heat energy in 1 kg silage in k cal
H_T	-	Number of k cal required to raise the temperature of 1 kg silage by 1 C^o
MEA	-	Malt extract agar
S	-	Specific heat of silage
TAA	-	Tween acetate agar
TN	-	Total nitrogen
TSN	-	Total soluble nitrogen
VN	-	Volatile nitrogen
WSC	-	Water soluble carbohydrate
YEA	-	Yeast extract agar

INTRODUCTION AND LITERATURE REVIEW

THE SILAGE FERMENTATION

The main aim of the ensilage process is the preservation of high moisture crops, such as grass, which cannot be stored aerobically. The sequence of events which occurs during the preservation period has been well documented in reviews by Gibson and Stirling (1959), Watson and Nash (1960), Whittenbury, McDonald and Bryan-Jones (1967), Whittenbury (1968), Ruxton (1972), Woolford (1972) and McDonald and Whittenbury (1973).

For successful preservation by ensiling, the crop must be stored under anaerobic conditions and clostridial fermentation, leading to the production of carbon dioxide, ammonia and amines must be discouraged. The most common method of controlling clostridial activity is by encouraging a lactic acid fermentation of the sugars in the crop. The lactic acid and other fermentation acids produced reduce the pH of the crop to a level at which clostridial activity is restricted.

Lactic acid bacteria are the major agents of preservation within the silo but they are not usually part of the normal microflora of the plant (Stirling and Whittenbury, 1963). As the crop is harvested and in the process is chopped to release plant juices, the lactic acid bacteria proliferate and may reach high numbers as the crop is loaded into the silo (Henderson, McDonald and Woolford, 1972).

The majority of microorganisms found on herbage are strict aerobes with little fermentative ability. Gram negative facultative anaerobes are far less numerous than strict aerobes and of the facultative anaerobes, Escherichia is less frequently found than Klebsiella. Bacillus is found on herbage, but Langston, Bouma and Conner (1962) found it could not be detected after the first few hours of ensiling. Langston et al. (1962) have also recorded coryneforms (diphtheriods) on fresh herbage, and of their isolates, 58% were able to grow facultatively under anaerobic conditions.

When forage is packed into the silo, the aerobic microorganisms die rapidly as entrapped oxygen is consumed by the respiring herbage. Organisms capable of anaerobic growth start to ferment the plant sugars, organic acids are produced and the pH of the mass is lowered. Streptococcus and coliforms are the dominant organisms during the first 8-10 hours of ensiling, and as the pH falls, they are replaced by the more acid tolerant genera, Pediococcus and Leuconostoc. Finally Lactobacillus becomes the dominant genus isolated from silage.

In grass, the main sugar sources are glucose, fructose, sucrose and fructans all of which can be fermented by lactic acid bacteria with the production of lactic acid. In the case of the homofermentative lactic acid bacteria, the main product of carbohydrate fermentation is lactic acid.

glucose ⟶ 2 lactic acid

fructose ⟶ 2 lactic acid

pentose ⟶ lactic acid + acetic acid

The products of fermentation of sugars by heterofermentative lactic acid bacteria are more varied.

glucose ⟶ lactic acid + ethanol + CO_2

3 fructose ⟶ lactic acid + 2 mannitol + acetic acid + CO_2

2 fructose + glucose ⟶ lactic acid + 2 mannitol + acetic acid + CO_2

pentose ⟶ lactic acid + acetic acid

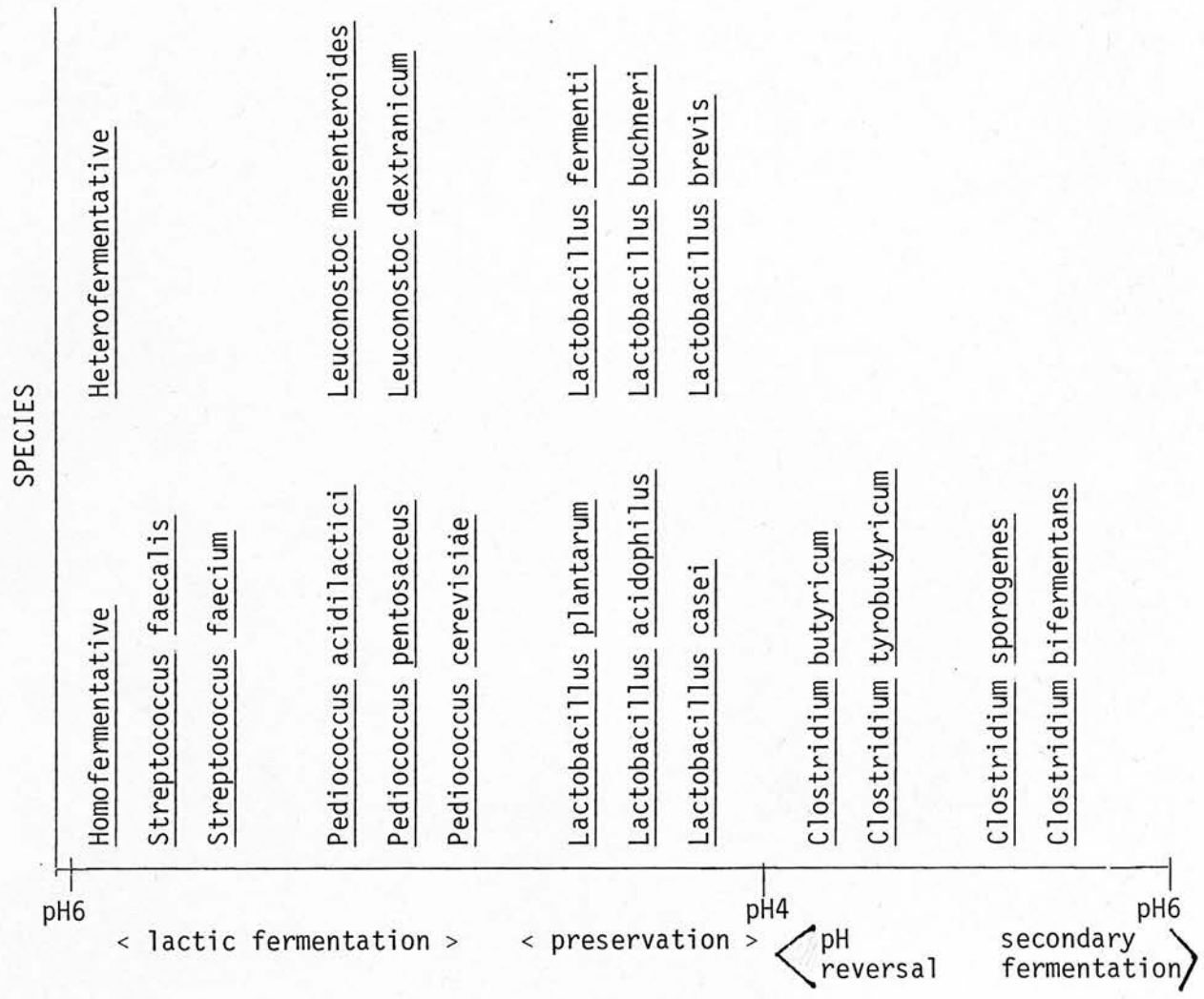
(Pathways from Whittenbury, 1968)

The activity of the heterofermentative lactic acid bacteria in silage is generally considered to be less desirable than that of the homofermentative lactic acid bacteria since the production of carbon dioxide represents a loss of dry matter.

Clostridia are almost the only microorganisms responsible for the spoilage of anaerobic silage, by fermenting lactic acid to butyric acid thus producing a pH reversal (Gibson, 1965). Lactate fermenters have been shown to multiply in the silo in the first 24 hours and to persist as spores in aged silage. The proteolytic clostridia, Cl. sporogenes and Cl. bifermentans commonly multiply just after the silo is filled, and in a silage with high levels of acid, they do not develop further. However, should the pH be markedly reversed, the proteolytic anaerobes will germinate and begin to ferment amino acids. Two factors are instrumental in the supression of clostridia in silage, the accumulation of lactic acid and a decrease in the water activity of the plant material. As the moisture content of the crop is decreased, the limiting pH value for clostridial growth is increased (Wieringa, 1958). In practice, ensiled crops containing 28% or more dry matter are preserved satisfactorily and very little clostridial activity occurs.

Representative species of the genera of bacteria commonly associated with the silage fermentation are summarised in Figure 1.

FIGURE 1. Representative species of genera commonly associated with the silage fermentation and their relationship to pH changes in silage.

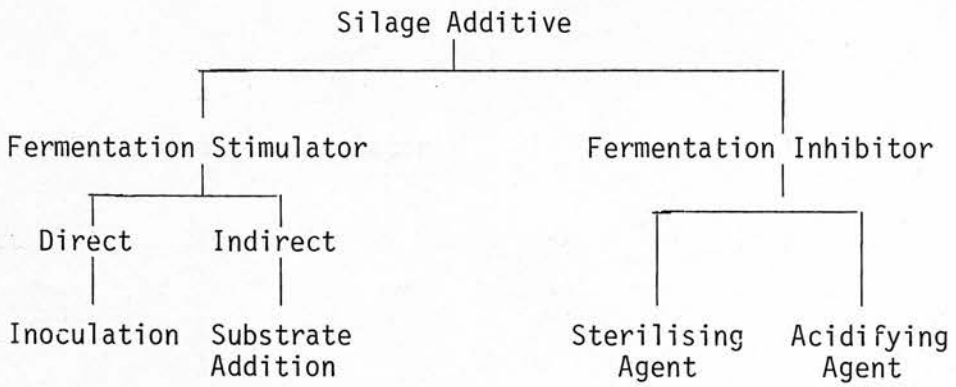


Data abstracted from:- Gibson, Stirling, Keddie and Rosenberger (1958); Keddie (1959); Langston and Bouma (1960); Langston, Bouma and Conner (1962); Gibson (1965); Whittenbury (1965); Whittenbury (1966).

SILAGE ADDITIVES

Originally, the purpose of a silage additive was to ensure satisfactory preservation of an ensiled crop by promoting a vigorous lactic acid fermentation. Now the emphasis is rather more on improving the quality of silage as a food product for animal consumption. Experimentally, many substances have been added to herbage at ensiling, but few of them have been used to give a marked improvement in the quality of the end product. The selection of additives for testing has been, in the past, generally unsatisfactory, the choice seeming to have been made in a completely random fashion with very little empirical research being done before starting substantial feeding trials with animals.

There are a number of ways of improving silage quality including the promotion of the lactic acid fermentation of the soluble carbohydrate component of the crop, elimination of undesirable proteolytic activity by clostridia and preservation of the carbohydrate content of the crop. Silage additives can be divided into two main categories, those which stimulate the silage fermentation and those which inhibit the fermentation.



A fermentation stimulator encourages the fermentation of soluble sugars to lactic acid in the crop ensiled. Direct stimulation is achieved by inoculation of the crop with cultures of lactic acid bacteria. Indirect stimulation of fermentation involves the provision of an additional carbohydrate source which can then be fermented by the indigenous population of lactic acid bacteria.

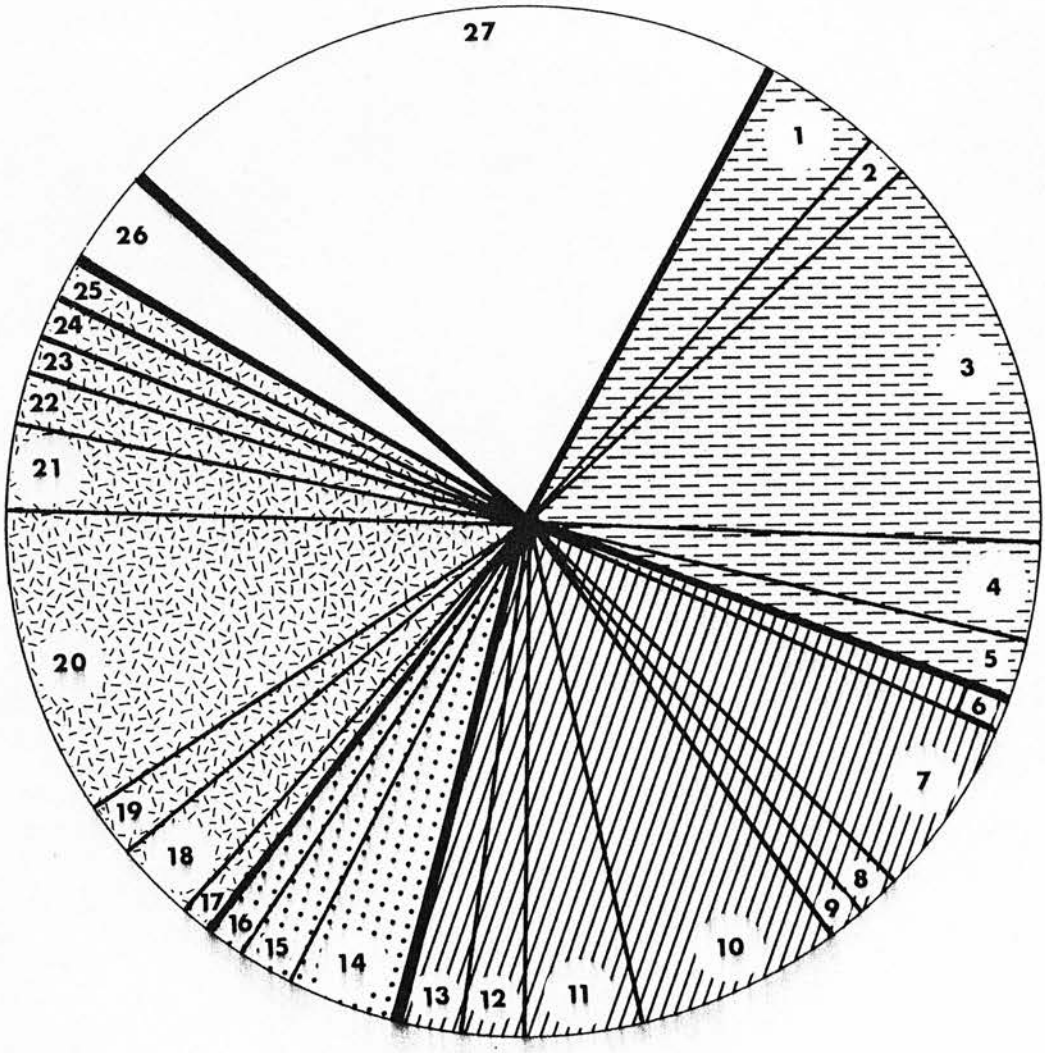
Additives which inhibit fermentation fall into two categories, those which prevent microbial activity within silage by direct sterilising action and those which acidify the crop to such an extent that microbial and enzyme activity is inhibited.

Nutritional supplements can also be added to silage. These additives are included to increase the feeding value of silage, but are not added specifically to influence the course of the silage fermentation. Urea is commonly added to increase the nitrogen content of silage and Barbier (1961) found that urea did not influence the contents of lactic, acetic or butyric acids in silage, but it was mainly converted to ammonium salts of acetic and lactic acids during fermentation. Diammonium phosphate is added to increase the nitrogen and phosphate content of silage to correct phosphorus deficiencies and according to Watson and Nash (1960) it does not appear to influence the fermentation.

A wide range of additives have been tested in silage making. Watson and Nash (1960) have summarised experimental work with additives up to 1960. Additives tested experimentally from 1950-1973 are listed in Appendix 1 and Figure 2 represents the frequency of publication of results using these additives. Additives which have been cited less than 5 times during the period 1950-1973 are included as "others".

1. Bacitracin
2. Formalin
3. Sodium metabisulphite
4. SO_2
5. Sovilon
6. AAZ
7. AIV
8. Amasil
9. Calcium formate
10. Formic acid
11. Kofa salt
12. Kofasil
13. Kylage
14. Urea
15. Limestone
16. Diammonium PO_4
17. Wheat bran
18. Sugar
19. Protosil
20. Molasses
21. Maize
22. Glucose
23. Citrus meal
24. Beet pulp
25. Barley meal
26. Lactic acid bacteria
27. Others

FIGURE 2 Pye diagram to show frequency of reported results of experiments using silage additives.



Direct stimulation of fermentation, inoculation

Sterilising agent

Acidifying agent

Nutritional supplement

Indirect stimulation of fermentation, substrate addition

1. Fermentation Stimulation

i. Direct stimulation, inoculation. Inoculation of crops for ensiling has been in use since the beginning of the 20th century when lacto-pulpe was in common use in France. There are several proprietary lactic acid cultures reported in the literature including Siloferm and Agrocym. According to Watson and Nash (1960) neither product had much effect on the course of fermentation and in silages made with Agrocym, abnormally high pH values were recorded.

Addition of cultures of lactic acid bacteria is useful where crops are unsuitable for ensiling. Wieringa and Hengeveld (1963) found that ensiling herbage of low water soluble carbohydrate content with the inclusion of 10 litres fluid culture of lactic acid bacteria per tonne was beneficial when the sugar content of the crop was between 6.3 and 11.0%. Above 11% WSC, inoculation was unnecessary and with a WSC level of 6.3% the silage was of poor quality, even when inoculated. Sedge is a difficult crop to ensile and Lesins and Schultz (1968) observed a marked decrease in pH compared with an uninoculated control when sedge was inoculated with lactic acid bacteria.

Inocula are commonly used in laboratory experiments where the method of harvesting may leave the crop deficient in lactic acid bacteria (Wieringa and Beck, 1964). Where forage harvested material is used, inoculation of the crop should not be necessary as it has been shown that the forage harvester is a major source of lactic acid bacteria (Stirling and Whittenbury, 1963; Henderson, McDonald and Woolford, 1972).

McDonald, Stirling, Henderson and Whittenbury (1964) found no advantage in inoculating material relatively rich in soluble carbohydrates

such as fairly mature ryegrass. With crops that are frequently low in soluble carbohydrates such as cocksfoot, or have high buffering properties as lucerne, inoculation may be beneficial in initiating the fermentation.

ii. Indirect stimulation, substrate addition. Carbohydrate-rich additives are included in silage to increase the available carbohydrate supply for fermentation by lactic acid bacteria. Among the additives used for this purpose are:- barley meal, beet pulp, citrus meal, glucose, maize, molasses, sugar and wheat bran. Molasses has been one of the more commonly used supplementary sources of fermentable carbohydrate in silage making. According to Whittenbury (1968) the fructose content of molasses may be a disadvantage in that it may encourage a heterofermentative type of fermentation. The addition of molasses may be unjustified also on an economic basis since it is no longer a cheap carbohydrate source.

Weise (1967) added 1% sugar to grass containing 15% DM and 10% WSC in DM. With the addition of sugar there was an initial increase in the numbers of lactic acid bacteria particularly those with a high capacity for producing lactic acid. In the absence of the additive, such lactic acid bacteria did not begin to develop until the second week of ensilage. With regard to yeast development in the silage, if air was allowed access during the ensiling period, the numbers of yeast cells were "one order of magnitude" higher where sugar had been added. The present high cost of sugar will probably prohibit its use as a silage additive.

Starch, in the form of cereal meal, is sometimes used as a carbohydrate source. Lactic acid bacteria do not normally ferment starch so it is necessary to include an amylase preparation in the form of an enzyme or an amylase rich material, such as malt, to hydrolyse starch to sugar (Rydin, 1961). Maize and barley meal can also be treated with boiling water to transform part of the starch into sugar (Salova, 1955).

2. Fermentation Inhibition

i. Sterilising agents. Among the substances which have been reported to have been tested experimentally for sterilising activity in the silo are antibiotics, formalin, sodium metabisulphite, SO_2 and Sovilon.

Many antibiotics have been proposed for use as silage additives and zinc bacitracin has been sold as Silotracin for this purpose. Bacitracin inhibits sporeformers as does nisin which is commonly used in the food manufacturing industry but neither antibiotic has been successfully used as a silage additive. Nisin had no effect on the course of fermentation in lucerne or red clover silage at 5-500 g / ton herbage (De Vuyst, Vanbelle, Arnould, Vervack and Moreels, 1965), though Whittenbury (1968) has proposed the inclusion of nisin-producing streptococci as an additive.

Aureomycin, streptomycin, oleandomycin, terramycin and penicillin have been suggested for use as silage additives. Fortunately none has improved silage quality (Wing and Wilcox, 1960) and indeed none should ever have been considered for use at all. Woolford (1974) screened the antibiotics tylosin and pimaricin in an in vitro trial and found

that tylosin had antibacterial properties at an application rate equivalent to 0.045 g kg^{-1} and pimaricin was antimycotic at a rate of 0.18 g kg^{-1}. A combination of pimaricin and tylosin was therefore suggested as a useful silage additive though no results of field tests were reported.

The indiscriminate use of any antibiotic as a silage additive should be discouraged since there is a distinct possibility of the production of a resistant population of lactic acid bacteria and that the drug resistance of this population might be transferred to another population by means of plasmids. However, when general protoplasmic inhibitors such as formaldehyde are used, this problem does not arise.

Formaldehyde is a well known sterilising agent and is commercially available as formalin, a 40% aqueous solution of formaldehyde or paraformaldehyde, a solid polymer that contains 91-99% formaldehyde. The use of formaldehyde as a silage additive is discussed below.

Sodium metabisulphite powder contains 65-67% by weight of SO_2, and is very soluble in water forming sodium bisulphite. In the presence of sufficient moisture, it is a strong reducing agent, capable of using large volumes of oxygen. Much work has been done on the value of metabisulphite as a silage additive. Several workers have reported that metabisulphite addition was ineffective compared with wilting and carbohydrate addition and its application was unjustifiable on a cost basis.

Protein deamination in silage is considerably reduced by metabisulphite addition. De Vuyst, Vervack and Arnould (1968) found that metabisulphite retarded amino acid breakdown, but had no effect on proteolysis as did Durand-Salomon and Zelter (1960). Macpherson, Wylam and Ramstad (1957) found that there was little fermentation in metabisulphite treated silages, but considerable proteolysis and carbohydrate hydrolysis occurred in the silage, the hydrolysed protein appearing as amino acids and peptides, while the carbohydrates accumulated as reducing sugars. Alderman, Cowan, Bratzler and Swift (1954) noted a considerable amount of hydrolysis of protein to amino acid in metabisulphite treated silage, but the ammonia nitrogen content of the silage was low.

Sulphur dioxide gas has been used to restrict fermentation, within the silo and Skaggs and Knodt (1952) found that SO_2-treated silage had low lactic acid content and lower ammonia nitrogen and volatile acid levels than the corresponding control. Kroulik, Burkley, Gordon, Wiseman and Melin (1955) found that SO_2 addition decreased the overall bacterial population in the silage, but did not greatly inhibit bacterial activity during the first few days after ensiling. However, the high acidity of the resulting silage and the difficulties involved in the use of SO_2, application from gas cylinders and evolution of SO_2 as the silo is opened, put the use of sulphur dioxide gas as a silage additive at a disadvantage, (Jensen, Mølle, Møller and Pedersen, 1962).

Sovilon, a polyhalogenated acetate of glycol is a bacteriostatic salt and has been used as a silage additive by Barnett (1955). Even the smallest concentrations of Sovilon used inhibited amino acid

breakdown. The concentration of volatile fatty acids and bases was reduced and lactic acid production was inhibited, indicating that fermentation had been restricted in the silo.

ii. Acidifying agents. One of the most widely used original methods to acidify silage was the AIV process, named after A.I. Virtanen. The AIV process involves the reduction of the pH of the crop to below pH 4.0. This inhibits undesirable clostridial fermentations since the activity of proteolytic enzymes is checked below pH 4.0. The Virtanen process includes the use of any acid solution which reduces the pH of the crop to below 4.0 though the most commonly used acid is a 2N mixture of hydrochloric acid and sulphuric acid. De Vuyst, Vervack and Arnould (1968) studied the mode of action of AIV acid and found that it reduced the pH to 3-3.5 and completely prevented fermentation. Amino acid breakdown was 25-33% of that in silage made without additives. The AIV process has never been widely used in the U.K. primarily because there are difficulties involved in the handling of mineral acids and corrosion factors. There is also a possibility of residual unneutralised acid in the silage, (Woodman, 1949).

Mineral acids have largely been replaced by organic acids as silage additives and the organic acid widely used is formic acid. It is sold under a variety of names including Add-F, Amasil, Myosil, Norsil and Ensilan. In addition to its acidifying properties, formic acid has specific antibacterial effects particularly with respect to coliforms and clostridia. The use of formic acid as a silage additive is discussed below.

Salts of formic acid have also been used as silage additives, the most common of these being calcium formate which is mixed with

sodium nitrite in the commercially available additives, Kofasalt, Kofa or Kylage. Kofasil is a mixture of calcium formate, sodium nitrite, sucrose and trace elements. Kofa salt is a mixture of 20 parts calcium formate and 3 parts sodium nitrite. According to Pfeiffer (1953) during the first 8 days of ensiling, silage is protected from harmful bacteria by the nitrous oxide which develops from Kofa. Wieringa (1967) found that Kofa was effective as an additive if lactic acid fermentation was rapid since the added nitrite limited butyric acid fermentation for a short time only. Martin and Buysse (1954) found Kofa to be effective for improving silage quality where unchopped grass was used but where the grass was chopped, Kofa addition had no effect on silage quality.

FORMALIN, FORMIC ACID, ACETIC ACID AND PROPIONIC ACID AS SILAGE ADDITIVES

1. Formalin

Formaldehyde, HCHO, has been used as a vapour phase decontaminant for over 80 years to treat rooms where patients with contagious diseases have been housed. It is commercially available in aqueous solution (formalin) containing 40% formaldehyde or as paraformaldehyde, a solid polymer that contains 91-99% formaldehyde. Formaldehyde is manufactured in very large quantities and is used to treat textiles, leather and paper, and other uses include embalming, medicine and photography (Hoffman, 1971).

Watson and Nash (1960) have summarised the early work done from 1931 to 1934 with formaldehyde as a silage additive. Formaldehyde was used as a sterilising agent in the commercial 'Silohilfe' process in which lactic acid was included, in the patented 'Fingerling' process and in the 'Toro-Silon' process. It is interesting to note that lactic acid was included with formalin in the 'Silohilfe' process to ensure the presence of this acid. This demonstrates how little was understood of the ensiling process just 40 years ago.

There was little research work done with formaldehyde as a silage additive between 1934 and 1970, but the accumulation of experimental evidence that voluntary intake of silage is negatively correlated with the degree of fermentation during the ensiling process has stimulated research work in this area (Gordon, Derbyshire, Wiseman, Kane and Melin, 1961; Jackson and Forbes, 1970; Brown and Radcliffe, 1972). McLeod, Wilkins and Raymond (1970) came to the conclusion that acids

produced during normal silage fermentation could limit silage intake, and suggested that it would be advisable to investigate chemical treatments to conserve the nutrients in grass without the presence or formation of large quantities of acids.

Formaldehyde, at a suitable concentration of application preserves grass without producing a silage of low pH, and at levels of application above 3.6 g formaldehyde per kg fresh grass, (≡ 9.1 cm^3 formaldehyde solution, formalin per kg) the silages produced are non fermented silages (Wilkins, Cook and Wilson, 1972). Non fermented silages have been defined as silages in which the fermentation acids comprise less than 5% of the crop DM. Such silages are further characterised by high pH, in the region of 5.5, low lactic acid and volatile fatty acid content and high levels of water soluble carbohydrate.

The rate of formaldehyde application is critical. Wilkins, Wilson and Woolford (1973) have shown in laboratory studies that an application rate of 0.92 g formaldehyde per kg fresh grass (≡ 2.2 g kg^{-1} rate of formalin application) resulted in a badly preserved silage with a high ammonia nitrogen level and a high percentage (>7%) of butyric acid in the dry matter. As the application rate of formaldehyde was increased, the extent of fermentation in the silo became extremely small. Barry and Fennessy (1972) applied formaldehyde at 1.1, 2.2, 4.4 and 8.8 g kg^{-1} fresh grass. Application rates up to 4.4 g kg^{-1} resulted in a decreased level of acetic acid in the silage with no effect on pH or the concentration of propionic plus butyric acids. The fermentation-product carbon (measured as

VFA and 2-3 butanediol) was unaffected by this range of application of formaldehyde. The 8.8 g kg^{-1} rate of application reduced the concentrations of propionic and butyric acid to trace amounts, decreased the amount of fermentation product carbon and increased silage pH. At the low level of formaldehyde application 1.1 g kg^{-1} Barry and Fennessy (1972) also noted a higher level of propionic plus butyric acids in the silage.

Though formaldehyde treatment at a suitably high level produces high pH, low VFA silages which according to McLeod et al. (1970) will have higher intake levels than the corresponding fermented silages, it has several disadvantages in use. To be effective, high levels of application above approximately 4 g formaldehyde per kg fresh grass must be used. This application rate is equivalent to the addition of 2 gallons formalin per ton fresh grass. Formaldehyde vapour has a pungent odour and is highly irritating to the eyes and mucous membranes. Extreme exposure can cause death.

Formaldehyde treated silages are also inherently unstable on unloading from the silo and consequent exposure to air. Wilson, Wilkins and Cook (1971) found formaldehyde treated silage (application rate 3 g kg^{-1} fresh grass) showed more extensive moulding by the 6th day than the control silage which did not show mould growth until the 8th day after unloading. The temperature of the treated silage rose more rapidly than in the control. Barry and Fennessy (1972), in work with clover dominant herbage, recorded a secondary fermentation in a 30 cm deep layer round a stack of formaldehyde treated silage compared with little surface waste in the control stack. Brown and Valentine (1972) noted that formaldehyde treatment did not prevent

partial decomposition of herbage, and mould growth. Contamination with Fusarium was visually evident in 66% of the experimental silos which contained formaldehyde treated silage.

The mechanism of action of formaldehyde in the silage fermentation is twofold. First, it acts as a sterilising agent inactivating the lactic acid bacteria and the clostridia in the silo and secondly formaldehyde acts as a protein binding agent forming intermolecular cross-linkages of protein amino groups (Hoffman, 1971).

2. Formic Acid

Watson and Nash (1960) summarising work with organic acids as silage additives, concluded that the process using formic acid had, on the whole, not been successful. However, the experiments reviewed by Watson and Nash (1960) were carried out before the importance of laceration of grass for ensiling was fully exploited and long grass was commonly ensiled (Saue and Breirem, 1969). Schukking (1972) reported that approximately 5.2 g pure formic acid per kg fresh long grass was required to have a beneficial effect on the silage fermentation. Saue and Breirem (1969) found that laceration of grass had an effect on the fermentation equivalent to the addition of approximately 4.88 g formic acid to 1 kg long grass.

With the introduction of harvesting machinery, which, in the case of the precision chop forage harvester, can chop forage into 1 cm lengths, the efficiency of formic acid as a silage additive was increased. An applicator for formic acid was devised by Aas and Naerland (1966) to spray the additive on to the grass as it is being harvested. Using the forage harvester and spray applicator, it is possible to achieve uniform dispersal of additive in finely chopped or lacerated grass.

Using these developments, formic acid was then able to compete with AIV acid as a silage additive since it had been shown that used on lacerated grass, formic acid compared well with AIV acid in producing silages with low butyric acid and ammonia contents. On long grass, AIV acid had been shown to be superior to formic acid (Saue and Breirem 1969). This was reflected in decreased sales of AIV acid in Norway, where in 1967 the manufacture of AIV acid was discontinued, with a corresponding increase in the sale of formic acid. AIV acid treatment of silage was never popular in the U.K. (Woodman, 1949). However, with the marketing of formic acid under the brand name of "Add-F"[1] in 1968, the use of this additive has become increasingly popular in the U.K.

The recommended rate of formic acid application is 2.3 g pure formic acid per kg fresh grass ensiled. For wilted material, Schukking (1972) proposed that the level of formic acid added should be doubled i.e. approximately 5 g kg^{-1}. Henderson, McDonald and Woolford (1972) added formic acid at a rate of 3.3 g per kg to grass wilted to a dry matter of 36% and the silages so produced had increased levels of water soluble carbohydrate, WSC (15% approximately) compared with wilted controls (WSC - 4.5% DM approximately), decreased acetic acid content and lactic acid content, and slightly higher pH than the control.

Henderson (1974) reporting the use of formic acid on low dry matter, low water soluble carbohydrate crops, found that the additive affected the fermentation pattern in the silo, but did not affect oxidative losses. Applied at the recommended level, 2.3 g kg^{-1} fresh

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[1] Marketed by BP Chemicals International

grass, formic acid reduced respiration and losses of water soluble carbohydrate, inhibited gas production in the early stages and reduced temperature increase compared with the control. Formic acid application at this level retarded the rate of lactic acid production and the production of ammonia. Castle and Watson (1970) added formic acid to timothy/ryegrass at the rate of 2.3 g kg^{-1} and found that the maximum temperature reached in the silo was reduced by 10-15°C and that treated silages invariably had lower pH and higher lactic acid contents than the control. When Wilkins and Wilson (1968) added formic acid to Lolium perenne, containing 18% WSC, at the rate of 2.3 g kg^{-1}, the control and treated silages had the same pH (3.8) but the lactic acid content of the control was higher than that of the formic acid treated silage (15.4% and 9.1% respectively on a dry matter basis). Henderson and McDonald, (1971) ensiled a timothy/ meadow fescue/perennial ryegrass mixture with the addition of formic acid at 2.2 g kg^{-1} and found that at this level, lactic acid production was not prevented. Production of acetic acid was reduced and there was no butyric acid present in any of the silages.

Wilson and Wilkins (1973) ensiled unwilted cocksfoot, perennial ryegrass and lucerne to which formic acid had been applied at 2.3 g kg^{-1}. Compared with unwilted controls, formic acid treatment resulted in reduced acetic acid content and reduced ammonia content in all silages examined. The pH of the treated silages was lower than that of the control. All silages had very little residual WSC in the region of 1-2% of the dry matter.

At higher levels of application than the recommended level of 2.3 g kg^{-1}, formic acid addition results in increasing inhibition of

lactic acid bacteria and corresponding conservation of the water soluble carbohydrate fraction (Henderson, 1974). Henderson and McDonald (1971) added formic acid to a meadow fescue/timothy/perennial ryegrass mixture at the rate of 3.4 g kg^{-1}. The silages thus produced had a water soluble carbohydrate content which was 65.3% of the watersoluble carbohydrate present in the original grass compared with a water soluble carbohydrate retention of 30.7% in the control. The formic acid treated silage contained no lactic acid while the control had a lactic acid content of approximately 8% on a dry matter basis.

3. Acetic Acid

The use of acetic acid as a silage additive is somewhat limited since large quantities of free acetic acid in silage may bring about depressions in silage consumption. In an investigation into the effects of intraruminal infusion of volatile fatty acids on voluntary food intake of sheep, Hutchinson and Wilkins (1971) examined the possibility that the acetate ion exerts a direct effect on silage intake. The pattern of eating over the day was affected by the acetate content in the silage, in a feeding trial with sheep, but the intake over 24 hours was unaffected. Results indicated that although acetate *per se* did not limit silage intake, large quantities of free acids might have been involved in depressed intakes.

De Vuyst, Arnould, Moreels and Romedenne (1973) used acetic acid as a silage additive and applied it at a level of 3 g kg^{-1} ryegrass. The acetic acid treated silage had an increased ammonia content (14.88% TN) compared with the control (11.84% TN) and a decreased lactic acid content.

De Vuyst et al. (1973) recommended the use of acetic acid particularly on the basis of cost. McDonald and Henderson (1974) added acetic acid to Lolium multiflorum at a rate of 3 g kg^{-1} and obtained silage with similar pH, water soluble carbohydrate and total nitrogen levels to the control silage indicating that acetic acid had had no effect on fermentation. The acetic acid treated silages had increased ammonia contents compared with the control.

4. Propionic Acid

The antimicrobial properties of propionic acid have long been recognised and used in food preservation, particularly in the bakery industry, where the inclusion of a maximum concentration of 0.3% propionic acid, calculated on the weight of flour, is permitted by the Preservatives in Food Regulations (1962) in the U.K. (Huitson, 1968). Propionic acid is also used as a preservative in the storage of cereal grains for animal feed and is marketed under the trademark Propcorn.[1]

McDonald and Henderson (1974) added propionic acid to L. multiflorum at a rate of 3.7 g kg^{-1}. The silage thus produced was similar in chemical composition to the control silage with a slight retention of water soluble carbohydrate compared with the control. The ammonia nitrogen content of the treated silage was greater (9.8% TN) than that of the control (7.9% TN).

De Vuyst, Arnould, Vanbelle and Moreels (1972) studied propionic acid as a silage additive and concluded that it had potential as an additive because it lowered the pH of the crop at ensiling. They did not, however, obtain any fungicidal activity in the silos or with hay of high moisture content. At an application rate of 1.5 g kg^{-1}, the

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[1] Propcorn manufactured by BP Chemicals International

treated silage had a higher pH (4.21) than the control (4.19), the lactic acid level was lower, (2.81% fresh weight) than that of the control (3.15% fresh weight) but the ammonia nitrogen content was greater in the propionic acid treatment (13.73% TN) than in the control (11.77% TN). Daniel, Honig, Weise and Zimmer (1970) found that propionic acid addition significantly reduced the lactic acid content of silage, and deamination was also significantly reduced.

THE STABILITY OF SILAGE UNDER AEROBIC STORAGE CONDITIONS

The main condition which has to be met in the preservation of wet crops by ensiling, is the rapid achievement and maintenance of anaerobic conditions throughout the period of ensiling. However, when the silo is opened and the contents removed for feeding, the anaerobic environment of the silage is changed to a more aerobic environment. Factors (apart from anaerobiosis) which operate within the silo to prevent spoilage of the crop may not be effective in preventing spoilage in the aerobic conditions which prevail on unloading. Such factors are low pH, produced naturally by lactic acid fermentation or artificially by inclusion of an acid additive, high dry matter, which prevents clostridial activity, and suppression of microbial activity by addition of a sterilising agent such as formaldehyde.

According to Gross and Beck (1970) temperature increases were observed in silage after its removal from the silo particularly with wilted silages and carbohydrate-rich silages. This is the so-called 'after-fermentation' or 'after-heating' defined by Daniel, Honig, Weise and Zimmer (1970) as the metabolism of lactic acid and acetic acid by yeasts. In silages which deteriorate on unloading, there is an appreciable temperature rise within the mass which can reach as much as 50°C in some cases (Gross and Beck, 1970). This is coupled with a decrease in lactic acid content of the silage (Zimmer, 1963) and increases in CO_2 production, pH value and yeast numbers with a concurrent degradation of protein. The nutrient losses can be as high as 20-30% and the silage pH may reach 6 or 7 or even higher (Gross and Beck, 1970). Such a feedstuff could cause dietary problems and limited intakes.

The initial cause of aerobic deterioration of silage is the metabolism of lactic acid and residual sugar in silage by yeasts (Daniel et al., 1970). The role of yeasts in ensilage is, at present, unknown. However, yeasts are commonly found in silage, and Henderson, McDonald and Woolford (1972) showed that formic acid-treated silages had higher yeast counts than the corresponding control. In a study of the ecological aspects of yeast actions in the ensiling process, Endo (1955) found that at the end of the ensiling period, the oxidative activity of yeasts isolated from silage was considerable whereas their fermentative ability was much weaker. According to Beck and Gross (1964) yeasts can reach populations of up to 10^{12} per gram silage within 3 days of aerobic storage. This led to the postulation of a fixed population density of yeasts in silage, above which the silage was unstable. Experimentally it was shown from yeast counts in both stable and unstable silages that silages which contained over 10^5 yeast propagules per gram had a high probability of deteriorating under aerobic conditions, while silages with yeast populations below this level were not at such a risk.

In investigations into the keeping quality of silage, Beck and Gross (1964) found that silage stability under aerobic conditions was not dependent upon the Fleig quality of the silage, but upon the content and growth of yeasts of two specific types, lactic acid and acetic acid assimilating yeasts such as Candida krusei, Pichia fermentans and Hansenula anomila and yeasts which can assimilate lactic acid to a very small extent belonging to the genus Torulopsis.

All silages with high contents of residual sugar or lactic acid are very susceptible to yeast attack on unloading. Gross and Beck (1970) studied the effects of the organic acids acetic, propionic and butyric acids on yeast respiration and found that 0.6 - 0.85% acetic acid, 0.15 - 0.2% propionic acid or 0.15 - 0.25% butyric acid was required for complete prevention of lactic acid respiration. These results reinforced experimental observations in which butyric acid or acetic acid rich silages showed no signs of aerobic deterioration. Ohyama and Masaki (1971) examined the changes in chemical composition and temperature of wilted ryegrass silages. Two silages remained stable over storage periods of 5 days and 10 days respectively while two silages deteriorated after 1 and 2 days aerobic storage periods respectively. The acetic acid contents of the stable silages were higher than those of the deteriorated silages, 0.45% and 0.63% compared with 0.30 and 0.13% respectively (% acetic acid based on silage fresh weight).

A qualitatively good silage is considered to have either a high lactic acid content with low levels of volatile acids or a high water soluble carbohydrate content resulting from a restriction in fermentation. Such silages are therefore open to yeast attack when exposed to aerobic conditions (Gross and Beck, 1970). Accordingly, attempts have been made by various workers to improve the keeping qualities of such silage.

Schukking (1972) added formic acid, acetic acid, propionic acid, lactic acid and sodium bisulphite directly to unstable wilted silage at a level of 10 g per kg of silage. The control silage showed a temperature increase of 20C^{o} by the 3rd day of the deterioration period.

Formic acid, acetic acid and sodium bisulphite-treated silages showed similar temperature profiles reaching a maximum temperature of 40-43°C by the 11th day of the aerobic storage period. Propionic acid treatment rendered the silage stable over 15 days and butyric acid addition resulted in an intermediate temperature profile between the propionic acid treatment and the formic acid, acetic acid, sodium bisulphite treatments.

Gross and Beck (1970) added propionic acid to maize silage at rates of 1 g kg^{-1}, 2 g kg^{-1}, 4 g kg^{-1} and 8 g kg^{-1}. At the 4 g kg^{-1} and the 8 g kg^{-1} rates of application, there was a significant reduction in the temperature increase observed in the untreated and 1 g kg^{-1} treated silages. At the 2 g kg^{-1} rate of propionic acid application the temperature increase was somewhat retarded since it was not until the 4th day of the experiment that a temperature increase from ambient was observed, but by the 10th day, the temperature of the unloaded silage had reached 50°C. Aerobic degradation losses were very heavy within a very short time. These were reduced by the addition of 1-2 g kg^{-1} propionic acid and were almost completely prevented by rates of propionic acid addition of 4 g kg^{-1} and above. Increase in temperature was accompanied by decrease of lactic and acetic acids and a rise in pH.

Ohyama and Masaki (1971) added sodium propionate at a rate of 10 g kg^{-1} and 20 g kg^{-1} to unloaded wilted ryegrass silage. At the lower level of application, the rise in temperature of the silage was retarded for 2 days and 1 day compared with the sharp temperature increase observed in the control silage immediately after time zero. The addition of 20 g sodium propionate per kg unloaded silage

maintained the silage temperature slightly above ambient for 12 days in one experiment, but in a second experiment with the same rate of application the deterioration process was delayed for 2 days, but the temperature of this silage reached 50°C approximately after 5 days. The addition of nitrofrazone (sic) reinforced the effect of sodium propionate, but nitrofrazone (sic) itself did not have the effect of retarding rise in temperature. Schukking (1972) compared the temperature changes of unloaded silage treated with 10 g kg^{-1} propionic acid with 10 g kg^{-1} sodium propionate solution addition and 10 g kg^{-1} sodium propionate (powder) addition, and found the acid addition to be more effective than the addition of sodium propionate in either powder or solution form in maintaining the stability of the unloaded silage. Cook (1973) improved the stability of maize silage by the addition of 5 g kg^{-1} propionic acid, the propionic acid resulting in direct inhibition of mould and yeast growth.

OBJECTS OF STUDY

Formalin, formic acid, acetic acid and propionic acid were chosen for a systematic study of their effects on the silage fermentation. The reason for the selection of these particular additives was three-fold. They are readily available and their application as silage additives can be justified on an economic basis. It was already known that formalin was effective in restricting silage fermentation, formic acid was used as a silage additive and propionic acid was used as a hay preservative. With the exception of formalin, the acids selected are products of rumen fermentation and so the feeding of silage treated with these acids would not be expected to affect the rumen microflora adversely.

The experimental work has been divided into four sections. Section A deals with the effect of the additives on the silage fermentation using 40 g laboratory silos. Two screening trials are described in which formalin, formic acid, acetic acid and propionic acid were used as additives applied singly, in pairs and in mixtures of three and four. Formalin, formic acid, acetic acid and propionic acid were assessed for their antimicrobial activity against pure cultures of microorganisms isolated from silage.

Additive-treated silages made on a farm scale are examined in Section B and the changes in the microflora of a series of these silages were investigated from the standing crop in the field, through the harvesting procedure to the finished product.

During the course of work with additive treated silages, it became apparent that another factor would have to be considered in the choice of an efficient silage additive. Several observations had been made that silages which had been apparently stable in the silo were deteriorating rapidly when unloaded and exposed to aerobic conditions. Silages with low levels of fermentation acids were particularly susceptible to this aerobic deterioration and as such silages were being produced as a result of additive treatment preliminary investigations were undertaken into this process and reported in Section C. In the final assessment of formalin, formic acid, acetic acid and propionic acid as silage additives reported in Section D, the stability of the additive treated silage on exposure to aerobic conditions was one of the most important parameters considered.

METHODS

EXPERIMENTAL SILOS

Since the prime aim of ensiling is to exclude oxygen from the crop, and to maintain anaerobic conditions throughout the ensiling period, the ideal silo should be an airtight container. Where necessary, provision should be made for effluent release and collection. Wilson and Wilkins (1972) have evaluated laboratory ensiling techniques using PVC silos of 1 tonne capacity, test tube silos of 100 g capacity and polythene bag silos of 6 kg capacity. Test tube silos and the polythene bag silos gave silages with compositions which correlated closely with those of the larger silos.

1. 40 g silos

Glass jars, capacity 70 ml were packed with 40 g chopped grass (fresh weight) and sealed with neoprene rubber stoppers which were fitted with U tube fermentation seals filled with water, (Leigh Williams and Sons, Tattenhall, Nr. Chester). There was no provision for effluent drainage. The silos were incubated at laboratory temperature in the dark.

2. 3 kg silos

Plastic bags of a type used in the meat packing industry were filled with 3 kg grass and sealed with Strip-Seal (Stevens Plastics, Corsham, Wilts). The bags were placed in a 500 kg PVC silo which was evacuated and filled with nitrogen before sealing with Strip-Seal. Air was thus excluded from round the bags. Control silages were

also made which were stored in the open. In practice it was found that oxygen did not permeate the bags as no moulding was seen on the surface of the silages stored in the open. There was no provision for effluent drainage.

3. 10 kg silos

Silos with capacity for 10 kg silage were manufactured from PVC reinforced with Terylene, Plastolene (Gordon Low Prefabrications Ltd., Cowes, Isle of Wight). The silos were 0.36 m in diameter and 0.36 m in depth and sealed with Strip-Seal. No provision was made for effluent drainage.

4. 500 kg silos

Silos with capacity for 500 kg silage were made from Plastolene as described above. They were 1.2 m in diameter and 1.35 m in depth, and were fitted with an effluent pipe 2.54 cm diameter and 0.9 m long with 0.3 m of pipe projecting from the silo. The effluent pipes were plugged with neoprene rubber stoppers and effluent was collected and weighed at regular intervals over the ensiling period. The silos each rested on a timber fork lift pallet 1.3 m x 1.3 m.

5. 25 Mg silos

Silos with capacity for 25 Mg silage were of the commercial vacuum type and were sited at Woodhouselea Farm, Midlothian.

6. 100 Mg silos

Concrete bunker silos with capacity for 100 Mg silage were situated at Easter Howgate Farm, Midlothian.

CHEMICAL ANALYSIS OF GRASS AND SILAGE

1. Oven Dry Matter DM_o

Duplicate 10 g samples of grass or silage were dried in porcelain crucibles at 80°C to constant weight.

2. Toluene Dry Matter DM_t

The method of Dewar and McDonald (1961) was used in which volatile losses were considered.

3. pH

The pH was determined on an aqueous macerate of grass or silage (30 g in 250 ml distilled water) using PYE Model 291 pH Meter.

4. Buffering capacity BC

The buffering capacity was determined by the electrometric titration method of Playne and McDonald (1966). The buffering capacity of a sample was calculated as the number of milliequivalents of alkali required to raise the pH of an aqueous extract from pH4-6 per 100 g dry matter.

5. Water soluble carbohydrates WSC

The initial hydrolysis of the sugars present in a grass or silage sample followed the procedure outlined by McDonald and Henderson (1964). Hydrolysed reducing sugars were then determined by the colorimetric method of Nelson (1944) incubating 1 ml hydrolysate and 1 ml Somogyi reagent in a boiling bath for 20 min. After cooling, 1 ml arsenomolybdate reagent was added and made up to 25 ml with distilled water. The absorbtion of the solution was measured at

540 nm in an EEL Spectra flowthrough colorimeter (Evans Electroselenium Ltd. Halstead, Essex, England). The result was converted to hexose concentration with reference to a standard graph and expressed as a percentage of the dry matter.

6. Analysis of the nitrogenous fractions, ethanol and lactic acid contents of grass and silage were carried out by methods routinely used in the analytical laboratories of the Edinburgh School of Agriculture, (Henderson, 1974).

i. Nitrogen. Total nitrogen (TN) was determined in fresh grass and silage samples by the Kjeldahl method. Total soluble nitrogen (TSN) which gives a measure of the degree of proteolysis was determined by extracting a sample twice with boiling water, making to volume, and determining TN of the filtrate by micro-Kjeldahl. Volatile nitrogen (VN) which is an index of deamination in silage is determined in the TSN filtrate by steam distillation using excess 0.05M sodium borate (pH 9.2) to liberate ammonia. The crude protein (CP) content of samples was obtained by multiplying TN by 6.25.

ii. Ethanol was determined in the filtrate of macerate of fresh silage by steam distillation and digestion with sulphuric acid/potassium dichromate. Potassium iodide was added and the iodine liberated was titrated with sodium thiosulphate using starch as indicator.

iii. Lactic acid was determined in a 0.6N sulphuric acid extract of silage by the silicic acid chromatographic method of Lessard and McDonald (1966).

MICROBIOLOGICAL TECHNIQUES

1. Microbiological examination of grass and silage

Grass and silage samples were collected in sterile plastic bags or in sterile wide neck jars with screw caps. Samples were processed in x g amounts in 10 x ml sterile water using either an "Osterizer Blender" or the Colworth Stomacher 400 (A.J. Seward, UAC House, Blackfriars Road, London SE19 9UG). Decimal dilutions of the macerate were made in sterile water to the 10^{-8} dilution and suitable dilutions were plated out for total count, number of lactic acid bacteria, yeasts and moulds in Yeast Extract Agar (YEA), Tween Acetate Agar (TAA) and Malt Extract Agar (MEA) respectively.

TABLE 1

Dilutions used in counting microorganisms in grass and silage.

		Dilutions	
		Grass	Silage
Total Count	YEA	10^{-3} - 10^{-6}	10^{-5} - 10^{-8}
Lactic acid bacteria	TAA	10^{-1} - 10^{-4}	10^{-5} - 10^{-8}
Yeasts	MEA	10^{-1} - 10^{-5}	10^{-2} - 10^{-6}
Moulds	MEA	10^{-1} - 10^{-5}	10^{-2} - 10^{-6}

Dilutions for the total count and the lactic acid bacteria count were plated in duplicate by the pour plate method, and 0.1 ml of dilution for yeast and mould count were spread on prepared MEA plates. Wherever possible, the counts were expressed per gram dry matter of the sample.

2. Maintenance of microorganisms

Lactic acid bacteria were maintained in 7 ml vials of Cooked Meat Medium and routinely subcultured every 6 months. Yeasts and moulds were maintained on Wort Agar slopes in 25 ml Universal Containers and subcultured every 6 months. Bacillus strains were maintained on Nutrient Agar slopes in 25 ml capacity Universal Containers and were discarded after identification and testing.

3. Staining

i. Gram stain. Jensen's modification of Gram's stain was used with dilute carbol fuchsin as counterstain, (Cruikshank, 1968).

ii. Spore stain. Malachite green heated over a beaker of boiling water for 1 minute was used to stain spores with 0.05% basic fuchsin as counterstain, (Cruikshank, 1968).

4. Catalase

A few drops of hydrogen peroxide (10 vol) were placed on the surface of young colonies on an agar plate. The immediate appearance of bubbles of gas indicated the presence of the enzyme catalase.

5. Voges-Proskauer Test

Barritt's modification was used adding 0.5 ml 6% alcoholic solution of α-naphthol and 0.5 ml 16% KOH solution to 1 ml of culture. The appearance of a red colour up to within 2 hours was deemed a positive reaction, (Holding and Collee, 1971).

6. Decomposition of casein

Casein decomposition was demonstrated by clearing of the medium containing skim milk round the inoculated streak.

7. Gelatin liquefaction

The organism was streaked on to a plate of Nutrient Agar containing 0.4% gelatin. Gelatinase activity was demonstrated by zones of clearing round the streak when the plate was flooded with a solution of 15% $HgCl_2$ and 20% conc HCl in water, (Holding and Collee, 1971).

8. Amylase activity

The test strains were spot inoculated on to Nutrient Agar containing 2% starch. After incubation, the plate was flooded with Lugol's iodine solution (diluted 1 in 10) and a clear zone of variable diameter around the colony indicated the starch had been hydrolysed.

9. Lecithinase production

Lecithinase producing colonies grown on Nutrient Agar containing egg yolk showed wide zones of opalescence round the colonies.

10. Characterisation of the lactic acid bacteria

Lactic acid bacteria are defined as Gram-positive, non-sporulating cocci or rods, catalase negative (some strains may possess a 'pseudocatalase' detectable on low sugar containing media) usually non motile, obligate fermenters, producing mainly lactic acid and sometimes also volatile acids and CO_2.

The lactic acid bacteria were characterised according to the scheme of Sharpe and Fryer (1966). They were subdivided into genera as follows.

Streptococcus	Homofermentative cocci in pairs or chains
Leuconostoc	Heterofermentative cocci in pairs or chains
Pediococcus	Homofermentative cocci dividing in 2 planes to give tetrads
Lactobacillus	Homofermentative or heterofermentative rods

11. Identification of Bacillus isolates

Members of the Bacillus genus were recognised as rod-shaped organisms which are spore bearing, usually Gram-positive, catalase positive and capable of sporulating aerobically. The Bacillus strains isolated were placed in Group I, II or III according to the key of Wolf and Barker (1968) and species names were allocated according to a modified key of Knight and Proom (1950) using Voges-Proskauer reaction, nitrate reduction, glucose and arabinose fermentation, amylase, lecithinase and gelatinase production and the ability to hydrolyse casein as criteria for identification.

12. Identification of yeasts

Yeasts isolated from silages were identified as far as possible using the morphological and biochemical keys of Beech, Davenport, Goswell and Burnett (1968). The morphological key divides yeasts into pigmented and non-pigmented groups. The non pigmented yeasts are further separated on the basis of fermentative ability and the ability to assimilate nitrate. The biochemical key is based on sugar fermentation patterns of glucose, galactose, maltose, sucrose, raffinose and lactose, nitrate assimilation and sugar assimilation patterns.

Fermentation tests were carried out with filter sterilised solutions of sugars in a basal medium without a carbon source. A positive reaction was the production of acid and gas. Nitrate assimilation was checked by inoculating a tube of nitrate assimilation medium with 0.2 ml of a washed suspension of yeast cells, incubating for 7 days and inoculating a second tube with 0.1 ml from the first. Turbidity was assessed after a further 7 days incubation. Sugar assimilation was recorded by an auxanographic method (Smith 1969). The basal, carbohydrate-free solid medium was melted and cooled to 40°C. It was poured in 10 ml quantities into a Petri dish containing 2 ml of a washed yeast cell suspension in distilled water. The medium was well mixed in order to seed the yeast throughout and allowed to set. Very small quantities of the sugars to be tested were placed on the surface of the medium. The sugars diffused into the medium and, if utilised, stimulated into growth the cells lying within the diffusion zone.

The yeast isolates were coded according to the biochemical key of Beech et al. (1968) and species names allocated wherever possible. Yeasts isolated from deteriorating silages were also examined for growth temperature range by inoculating the yeast on to Wort Agar and incubating at 0°C, 10°C, 20°C, 30°C and 37°C and recording the presence or absence of growth. They were also tested for their ability to utilise lactic acid and acetic acid as sole source of carbon by the auxanographic method used in the sugar assimilation tests. Lactic and acetic acid (0.01 ml of each) were spotted on to the basal medium seeded with the test yeast and after incubation at 30°C the result was recorded.

13. Media

a. Yeast Extract Agar. Yeast extract (Oxoid L21) 5 g; peptone (Oxoid L37) 5 g; 'Lab Lemco' (Oxoid L30) 5 g; glucose 5 g; $MgSO_4$ 0.5 g; agar (Oxoid L13) 12 g; distilled water 1000 ml; pH 6.8; autoclave 121°C for 15 min.

b. Yeast Extract Broth. As Yeast Extract Agar omitting agar.

c. Tween Acetate Agar. Yeast extract (Oxoid L21) 5 g; peptone (Oxoid L37) 5 g; 'Lab Lemco' (Oxoid L30) 5 g; fructose 10 g; Tween 80 0.5 ml; agar (Oxoid L13) 15 g; distilled water 1000 ml; pH 5.4; autoclave 121°C for 15 min. Before final dispensing, 10 ml filter sterilised 2M acetic acid sodium acetate buffer (pH 5.4) added per 100 ml medium.

d. Tween Acetate Broth. As Tween Acetate Agar omitting agar.

e. Malt Extract Agar. Oxoid CM59; acidified with 5 ml filter sterilised 10% lactic acid per 100 ml medium.

f. Malt Extract Broth. Oxoid CM57.

g. Wort Agar. Oxoid CM247.

h. Nutrient Agar. Oxoid CM3.

i. Cooked meat medium (Cruikshank, 1968). Fresh bullock's heart 500 g; distilled water 1000 ml; 1 N NaOH 1.5 ml; the heart was minced and simmered in alkali solution for 20 min; it was then placed

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Oxoid Ltd., Southwark Bridge Road, London, SE1

in 1 g approximate amounts in 7 ml capacity glass screw top vials and covered with 4 ml nutrient broth (Oxoid CM1); autoclave 121°C for 15 min.

j. Media for yeast identification (Beech, Davenport, Goswell and Burnett, 1968)

(i) Dalmau plates (for pseudomycelium formation). Corn meal Agar (Difco 0386) prepared and dried in Petri dishes for 2 days before streaking with up to 5 cultures per plate. A sterile coverslip was placed over part of each streak.

(ii) Fermentation Medium. Yeast extract (Difco 0127) 4.5 g; peptone (Difco 0118) 7.5 g; distilled water 1000 ml; pH 6.4; bromothymol blue sufficient to give an intense blue colour; dispensed in 4 ml quantities in 150 x 15 mm test tubes each with an inverted 5 x 10 mm Durham tube; autoclave 121°C for 15 min. When cool, added to different tubes 2 ml of filter sterilised 6% solutions of the sugars glucose, galactose, sucrose, maltose, lactose, 8% melibiose and 12% raffinose.

(iii) MYPG broth. Malt extract (Oxoid L39) 3 g; yeast extract (Oxoid L21) 3 g; peptone (Oxoid L37) 5 g; glucose 10 g; distilled water 1000 ml; autoclave 121°C for 15 min.

(iv) Nitrate Assimilation medium. Difco carbon base 117 g; KNO_3 7.8 g; distilled water 1000 ml; sterilised by filtration. 0.5 ml of solution added aseptically to 4.5 ml sterile distilled water.

Difco Laboratories, PO Box 14B, East Molesey, Surrey

(v) Sugar Assimilation medium (Smith, 1969). $(NH_4)_2SO_4$ 5 g: KH_2PO_4 1 g: $MgSO_4 7H_2O$ 0.5 g: agar (Oxoid 'Ionagar No.2' L12) 20 g: distilled water 1000 ml; autoclave 121°C for 15 min. Dispensed in 10 ml quantities at 40°C into Petri dishes containing 2 ml of thick suspension of yeast cells in sterile water. When the agar had set, very small quantities of the sugars glucose, galactose, sucrose, maltose, lactose, melibiose and raffinose were placed on the surface, three or four sugars in the one dish. Lactic acid and acetic acid assimilation were checked on this medium by dropping 0.01 ml of each acid on to the agar surface.

(vi) Wort Agar. Oxoid CM247.

k. Media for Bacillus identification (Wolfe and Barker, 1968)

(i) Fermentation medium. $(NH_4)_2HPO_4$ 1 g; KCl 0.2 g; $MgSO_4$ 0.2 g; yeast extract (Oxoid L21) 0.2 g; agar (Oxoid L13) 15 g; distilled water 1000 ml; bromocresol purple 0.04% solution 20 ml; autoclave 121°C for 15 min. Glucose and arabinose added as filter sterilised solutions to give 0.5% overall concentration.

(ii) Casein Hydrolysis medium. Nutrient Agar (Oxoid CM3) + 10% skim milk.

(iii) Gelatin hydrolysis medium. Nutrient Agar (Oxoid CM3) + gelatin 0.4% (Oxoid L8).

(iv) Lecithinase medium. Nutrient Agar (Oxoid CM3) + 5% egg yolk. Egg yolk was obtained by swabbing the outside of a hen's egg with

alcohol, aseptically breaking the shell and separating the yolk into a sterile screw-top jar. The yolk was then mixed with an equal volume of physiological saline (sterile).

(v) Starch hydrolysis medium. Nutrient Agar (Oxoid CM3) + 0.2% soluble starch.

(vi) Voges-Proskauer medium. Peptone (Oxoid L37) 7 g; glucose 5 g; NaCl 5 g; distilled water 1000 ml; autoclave 121°C for 15 min.

1. Oxidative fermentative medium (Hugh and Leifson, 1953). Peptone (Oxoid L37) 2 g; NaCl 5 g; K_2HPO_4 0.3 g; agar (Oxoid L13) 30 g; 1% aqueous solution bromothymol blue 3.0 ml; distilled water 1000 ml; pH 7.1; autoclave 121°C for 20 min. Add 10 ml 10% aqueous solution of glucose (filter sterilised) to 100 ml sterile melted medium; dispensed in 5 ml amounts in sterile 150 x 50 mm tubes.

SECTION A

LABORATORY STUDIES

LABORATORY STUDIES

ADDITIVE SCREENING TRIALS IN LABORATORY SILOS

The effect of additives on the conservation of grass as silage was examined in two laboratory screening trials. The extent of fermentation within the silo was monitored using the parameters pH, dry matter and water soluble carbohydrate content.

Experimental

1. Screening Trials. Two screening trials were carried out, Trial 1 and Trial 2. In Trial 1, formalin, formic acid, acetic acid and propionic acid were tested as silage additives, singly and in pairs. In Trial 2, each of the four additives was tested singly and in combinations of three.

2. Silos. Experimental silos of 40 g capacity were used with three replicates per treatment in Trial 1 and two replicates per treatment in Trial 2.

3. Grass. In Trial 1, the grass used was cut by scythe in the morning of 19.6.72, from Tower Field, Langhill Farm. The seeding mixture of this field was Leda Daehnfeldt Italian Ryegrass, 5lb: Barrestra Perennial Ryegrass, 8lb: S23 Perennial Ryegrass, 6lb: N.Z. White Clover, 2lb, 23lb/acre, undersown 1971. The grass was chopped in a hand chopper before ensiling. The grass for Trial 2 was obtained from the Seafield and Ploverhall Field, Langhill Farm in the morning of 18.4.73. The grass was harvested with hand shears, wilted

in a forced draught oven to a dry matter of approximately 30% and chopped in the hand chopper before ensiling. The field was seeded with Leda Daehnfeldt Italian Ryegrass, 30lb/acre.

4. Analysis of grass and silage. Oven dry matter (DM_o), water soluble carbohydrate content (WSC) and pH analyses were done. No microbiological analysis was carried out. In Trial 1, one set of chemical determinations was done per replicate, giving three sets of readings per treatment and similarly in Trial 2, two sets of readings per treatment were obtained.

5. Additives. Formalin (40% solution of formaldehyde), formic acid, acetic acid and propionic acid were screened for use as silage additives. Table 2 shows the chemical formula, molecular weight, acidimetric assay and specific gravity for each of the test additives.

Table 2

The chemical compositions of the additives examined

Additive	Formula	Molecular Weight	Assay (acidimetric)	Specific Gravity
Formalin *	HCOH		37-40%	1.08
Formic acid	HCOOH	46.03	98.0%	1.22
Acetic acid	CH_3COOH	60.05	99.5%	1.05
Propionic acid	CH_3CH_2COOH	74.08	99.0%	0.99

* Formalin - composition, 37-40% solution of formaldehyde containing 11-14% methanol as polymerisation inhibitor.

The test additives were all laboratory grade reagents supplied by BDH Chemicals, Poole, England.

6. Rate of application of additives. The rate of application of additive was based on the recommended rate of application of Add-F* which is 0.5 gallon Add-F per ton herbage ensiled.

0.5 gallon per ton ≡ 2.25 cm^3 kg^{-1}
But specific gravity Add-F = 1.2
∴ 2.25 cm^3 Add-F ≡ 2.7 g Add-F
But Add-F contains 85% formic acid
∴ 2.7 g Add-F ≡ 2.3 g formic acid

In Trial 1, the additives were applied at two levels, on a volume basis. Each additive was applied at a rate of 1.1 cm^3 and 4.5 cm^3 per kg grass ensiled. The corresponding rates of additive application in g additive per kg grass ensiled are, formalin, 1.2: formic acid, 1.3: acetic acid, 1.2: propionic acid 1.1 for an application rate of 1.1 cm^3 kg^{-1}, and formalin, 4.9: formic acid, 5.5: acetic acid, 4.7: propionic acid, 4.5 for an additive application rate of 4.5 cm^3 kg^{-1}.

In Trial 2, additive application was based on a maximum rate of application of 4.6 g additive per kg grass ensiled (equivalent to 1 gallon additive per ton herbage). The rate of application was adjusted to accommodate the dry matter content of 30% of the grass used in Trial 2. Additives were applied at three rates 1.8, 3.5 and 6.9 g kg^{-1}.

The additives used in Trial 1 had the compositions shown in Table 3.

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*Add-F - a proprietory silage additive manufactured by BP Chemicals International Ltd., containing 85% formic acid

Table 3

Composition of additives used in Trial 1

Additive	Rate of application		
	g kg^{-1} fresh weight		g kg^{-1} DM*
	Components	Total	
1. Formalin	-	1.2	7.9
2. Formalin	-	4.9	32.2
3. Formic acid	-	1.3	8.6
4. Formic acid	-	5.5	36.2
5. Acetic acid	-	1.2	7.9
6. Acetic acid	-	4.7	30.9
7. Propionic acid	-	1.1	7.2
8. Propionic acid	-	4.5	29.6
9. Formalin:Formic	1.2:1.3	2.5	16.5
10. Formalin:Formic	4.9:5.5	10.4	68.4
11. Formalin:Acetic	1.2:1.2	2.4	15.8
12. Formalin:Acetic	4.9:4.7	9.6	63.2
13. Formalin:Propionic	1.2:1.1	2.3	15.1
14. Formalin:Propionic	4.9:4.5	9.4	61.8
15. Formic:Acetic	1.3:1.2	2.5	16.4
16. Formic:Acetic	5.5:4.7	10.2	67.1
17. Formic:Propionic	1.3:1.1	2.4	15.8
18. Formic:Propionic	5.5:4.5	10.0	65.8
19. Acetic:Propionic	1.2:1.1	2.3	15.1
20. Acetic:Propionic	4.7:4.5	9.2	60.5

* DM grass = 15.2%

Additives used in Trial 2 had compositions shown in Table 4.

Table 4

Composition of additives used in Trial 2

Additive	Rate of application		
	g kg^{-1} fresh weight		g kg^{-1} DM*
	Components	Total	
1. Formalin	-	1.8	5.7
2. Formalin	-	3.5	11.1
3. Formalin	-	6.9	21.8
4. Formic acid	-	1.8	5.7
5. Formic acid	-	3.5	11.1
6. Formic acid	-	6.9	21.8
7. Acetic acid	-	1.8	5.7
8. Acetic acid	-	3.5	11.1
9. Acetic acid	-	6.9	21.8
10. Propionic acid	-	1.8	5.7
11. Propionic acid	-	3.5	11.1
12. Propionic acid	-	6.9	21.8
13. Formalin:Formic:Acetic	1.8:1.8:3.5	7.1	22.5
14. Formalin:Formic:Acetic	1.8:3.5:1.8	7.1	22.5
15. Formalin:Formic:Acetic	3.5:1.8:1.8	7.1	22.5
16. Formalin:Acetic:Propionic	1.8:1.8:3.5	7.1	22.5
17. Formalin:Acetic:Propionic	1.8:3.5:1.8	7.1	22.5
18. Formalin:Acetic:Propionic	3.5:1.8:1.8	7.1	22.5
19. Formalin:Formic:Propionic	1.8:1.8:3.5	7.1	22.5
20. Formalin:Formic:Propionic	1.8:3.5:1.8	7.1	22.5
21. Formalin:Formic:Propionic	3.5:1.8:1.8	7.1	22.5
22. Formic:Acetic:Propionic	1.8:1.8:3.5	7.1	22.5
23. Formic:Acetic:Propionic	1.8:3.5:1.8	7.1	22.5
24. Formic:Acetic:Propionic	3.5:1.8:1.8	7.1	22.5
25. Formalin:Formic:Acetic:Propionic	1.8:1.8:1.8:1.8	7.2	22.5

*DM grass = 31.6%

7. Method of additive application. A suitable additive concentration was applied to chopped grass in a plastic basin. The additive was contained in a total volume of 1 ml and was sprayed over the grass using a 1 ml pipette with a fine nozzle. The chopped grass was then mixed and packed into 40 g laboratory silos before sealing. All operators wore plastic gloves. To check that the additive was evenly dispersed, the treated grass was spread in a monolayer over an area of 0.36 m x 0.36 m which was divided into squares 0.09 m x 0.09 m. The grass from each square was macerated with water and the pH measured. The dispersion of formic acid at an application rate of 1.3 g kg^{-1} was tested in this manner and the mean pH for the set of 9 readings was 4.9 with a standard error of the mean of ±0.03. This was taken as an indication of the even dispersion of the additive by this method.

8. Ensiling Period. In Trial 1, the ensiling period was 63 days while the ensiling time for Trial 2 was 109 days.

Results

Trial 1

Formalin, formic acid, acetic acid and propionic acid were each applied to mixed ryegrass at two concentrations equivalent to 1.1 cm^3 kg^{-1} and 4.5 cm^3 kg^{-1}, and in pairs at two levels, 2.2 cm^3 kg^{-1} and 9.0 cm^3 kg^{-1}. Additive concentrations were expressed in g per kg fresh herbage ensiled. The compositions of the silages produced are given in Table 5.

Table 5

Silage compositions in Trial 1

Additive treatment Total applied in g kg^{-1} fresh wt	Initial* pH	Silage pH	% DM_o	% WSC in DM
Ryegrass as ensiled 19.6.72	6.8	-	15.2	19.8
Control silage	6.8	4.0 ± 0.03	13.0 ± 0.2	1.0 ± 0.1
1. Formalin 1.2	6.7	4.0 ± 0.2	15.2 ± 0.9	2.0 ± 0.5
2. Formalin 4.9	6.7	6.3 ± 0.03	15.2 ± 0.4	15.4 ± 3.0
3. Formic acid 1.3	4.9	3.9 ± 0.07	13.8 ± 0.6	4.4 ± 0.1
4. Formic acid 5.5	3.9	4.2 ± 0.03	15.5 ± 0.5	18.2 ± 1.6
5. Acetic acid 1.2	5.4	3.9 ± 0.03	14.7 ± 0.4	1.5 ± 0.3
6. Acetic acid 4.7	4.8	3.8 ± 0.03	14.7 ± 0.3	1.6 ± 0.4
7. Propionic acid 1.1	5.7	3.8 ± 0.03	14.0 ± 0.2	1.4 ± 0.5
8. Propionic acid 4.5	5.0	3.9 ± 0.07	14.2 ± 0.1	2.1 ± 0.4
9. Formalin:Formic 2.5	5.1	4.7 ± 0.03	11.7 ± 1.3	5.1 ± 3.2
10. Formalin:Formic 10.4	5.1	4.1 ± 0.03	14.8 ± 1.7	18.6 ± 1.7
11. Formalin:Acetic 2.4	5.6	4.1 ± 0.03	14.4 ± 0.4	6.2 ± 1.6
12. Formalin:Acetic 9.6	4.9	4.8 ± 0.03	15.9 ± 0.08	21.5 ± 0.5
13. Formalin:Propionic 2.3	5.8	4.0 ± 0.04	14.2 ± 0.1	3.4 ± 1.3
14. Formalin:Propionic 9.4	5.1	5.0 ± 0.06	15.8 ± 0.2	16.4 ± 3.2
15. Formic:Acetic 2.5	4.9	3.9 ± 0.02	14.5 ± 0.2	2.2 ± 0.1
16. Formic:Acetic 10.2	4.1	4.1 ± 0.06	16.0 ± 0.2	15.8 ± 2.3
17. Formic:Propionic 2.4	5.0	3.9 ± 0.02	15.1 ± 0.15	3.1 ± 0.6
18. Formic:Propionic 10.0	4.0	4.0 ± 0.03	15.6 ± 0.5	13.7 ± 0.8
19. Acetic:Propionic 2.3	4.9	4.1 ± 0.3	14.0 ± 0.8	1.7 ± 0.5
20. Acetic:Propionic 9.2	4.5	3.8 ± 0.03	15.1 ± 0.18	1.1 ± 0.2
Control silage	6.6	4.0 ± 0.03	15.1 ± 1.1	1.1 ± 0.3

Results expressed as the mean and standard error of the mean of results of the three replicates per treatment.

* pH estimated on the macerate of a bulked sample taken from each of the replicates.

Trial 2

Formalin, formic acid, acetic acid and propionic acid were applied to wilted grass (DM 31.6%) at a maximum rate of 7.1 g kg^{-1} which is approximately equal to a rate of application of 4.5 g kg^{-1} for grass with a dry matter of 20%. Additives were applied singly, and in combinations of three. The compositions of the silages produced are shown in Table 6.

Table 6

Silage compositions in Trial 2

Additive treatment Total applied in g kg^{-1} fresh weight	Initial pH	Silage pH	% WSC in DM*
Ryegrass as ensiled 18.4.73 DM_o 31.6%	6.2	-	10.3
Control silage	6.2	4.3	3.3
1. Formalin 1.8	6.0	4.3	4.4
2. Formalin 3.5	6.2	4.3	11.1
3. Formalin 6.9	6.0	5.3	25.9
4. Formic Acid 1.8	4.8	4.3	9.0
5. Formic Acid 3.5	4.1	4.2	14.5
6. Formic Acid 6.9	4.2	4.2	21.9
7. Acetic Acid 1.8	5.6	4.1	4.3
8. Acetic Acid 3.5	4.6	4.1	4.3
9. Acetic Acid 6.9	4.3	4.1	5.8
10. Propionic Acid 1.8	5.7	4.1	4.3
11. Propionic Acid 3.5	5.1	4.0	4.3
12. Propionic Acid 6.9	5.1	4.0	9.0
13. Formalin 1.8 Formic 1.8 Acetic 3.5	5.1	4.3	17.8
14. Formalin 1.8 Formic 3.5 Acetic 1.8	4.4	4.4	22.8
15. Formalin 3.5 Formic 1.8 Acetic 1.8	4.8	4.6	15.9
16. Formalin 1.8 Acetic 1.8 Propionic 3.5	4.8	4.4	9.6
17. Formalin 1.8 Acetic 3.5 Propionic 1.8	4.4	4.2	11.0
18. Formalin 3.5 Acetic 1.8 Propionic 1.8	4.8	4.5	16.4
19. Formalin 1.8 Formic 1.8 Propionic 3.5	4.2	4.3	17.0

(cont.)

Table 6
(cont.)

20. Formalin 1.8 Formic 3.5 Propionic 1.8	4.4	4.4	18.9
21. Formalin 3.5 Formic 1.8 Propionic 1.8	4.6	4.5	18.5
22. Formic 1.8 Acetic 1.8 Propionic 3.5	4.2	4.0	19.9
23. Formic 1.8 Acetic 3.5 Propionic 1.8	4.3	4.1	10.9
24. Formic 3.5 Acetic 1.8 Propionic 1.8	3.8	4.2	14.6
25. Formalin 1.8 Formic 1.8 Acetic 1.8 Propionic 1.8	4.0	4.3	10.0

*Silage DM assumed to be 31.6% since there was no effluent loss.

Results expressed as the mean of values for the two replicates per treatment.

Discussion

Additives were considered to have influenced the course of fermentation within the silo if an increased retention of WSC was observed in the treated silage when compared with the corresponding control. A silage in which a secondary, clostridial fermentation had taken place would have been characterised by a low WSC content and pH above 5.5, indicating that the lactic acid produced in fermentation had been metabolised, but in both screening trials, such a silage was not encountered. The WSC contents and the pH values for each of the silages produced in Trial 1 and Trial 2 are summarised in Figure 3.

Formalin

Formalin was tested as a silage additive at five application rates ranging from 1.2 to 6.9 g kg^{-1}. At the two lowest rates of application the fermentation proceeded almost to the same extent as the control as seen by residual WSC values of 2.0% DM and 4.4% DM compared with respective control values of 1.0% DM and 3.3% DM. As the concentration

of the formalin added to the grass was increased, so the level of residual WSC increased until at the 6.9 g kg^{-1} rate of application, the WSC content of the silage was 25.9% DM (see Figure 3).

When Wilkins, Wilson and Woolford (1973) added formalin to ryegrass at a range of application rates up to 18.3 g kg^{-1}, the low application rate of 2.3 g kg^{-1} resulted in a silage which displayed signs of a secondary, clostridial fermentation with high pH (5.5), high ammonia nitrogen content (36.3% TN) and high levels of acetic and butyric acids (5.8% DM and 7.8% DM respectively). As the concentration of formalin added was increased, the WSC content of the silages also increased. At the application level of 9.1 g kg^{-1} and above, the silages showed complete conservation of WSC with attendant high pH values. It is possible that in the silage made with the low application rate of 2.3 g kg^{-1} formalin, the organisms which initiate the silage fermentation had been inhibited. This would mean that the required "preservation pH" was not reached and thus clostridial growth could occur. As the application rate of formalin was increased, the inhibition of microbial activity and indeed plant enzyme activity was also increased until at levels of approximately 9 g kg^{-1} there was no plant or microbial activity in the silage and so a "non-fermented" silage was produced.

Therefore, at low levels of application, formalin has very little effect on the course of silage fermentation and indeed may have an adverse effect by promoting a clostridial fermentation. According to Wilkins et al. (1973), an application rate of 4.5 g kg^{-1} resulted in a partially fermented silage with a residual carbohydrate content of 5.3% DM and an application rate of 9.1 g kg^{-1} was necessary for

complete conservation of water soluble carbohydrate. Bearing in mind interactions of additive application with the dry matter content of the crop to be ensiled, it would appear that less formalin was necessary to produce a non fermented silage made with wilted ryegrass than with unwilted material. With the wilted material used in Trial 2 an application rate of 7 g kg^{-1} was required to produce silage with WSC 25.9% DM.

When the rate of lactic acid production in a variety of formalin treated silages was examined, it was seen that as the rate of formalin application was increased, the rate of lactic acid production decreased. The three species ensiled, ryegrass, alfafa and lucerne all showed similar trends in production of lactic acid when formalin was included (see Figure 4).

Formic acid

Formic acid was used as a silage additive at five concentrations ranging from 1.3 to 6.9 g kg^{-1}. The DM values of the grass used in Trial 1 and Trial 2 were different, 15.2% and 31.6% respectively. This difference is reflected in the WSC of the control silages which were 1.0 and 3.3% DM respectively. With the wilted silages an application rate of 1.8 g kg^{-1} resulted in a silage with 9.0% WSC in DM while a similar application rate of 1.3 g kg^{-1} resulted in a silage with 4.4% WSC in DM. Thus, less formic acid is required to conserve the same amount of WSC when wilted material is used rather than unwilted material. When a crop has been wilted, fermentation will be inhibited at a higher pH value than if a fresh crop had been ensiled (McDonald and Whittenbury, 1967).

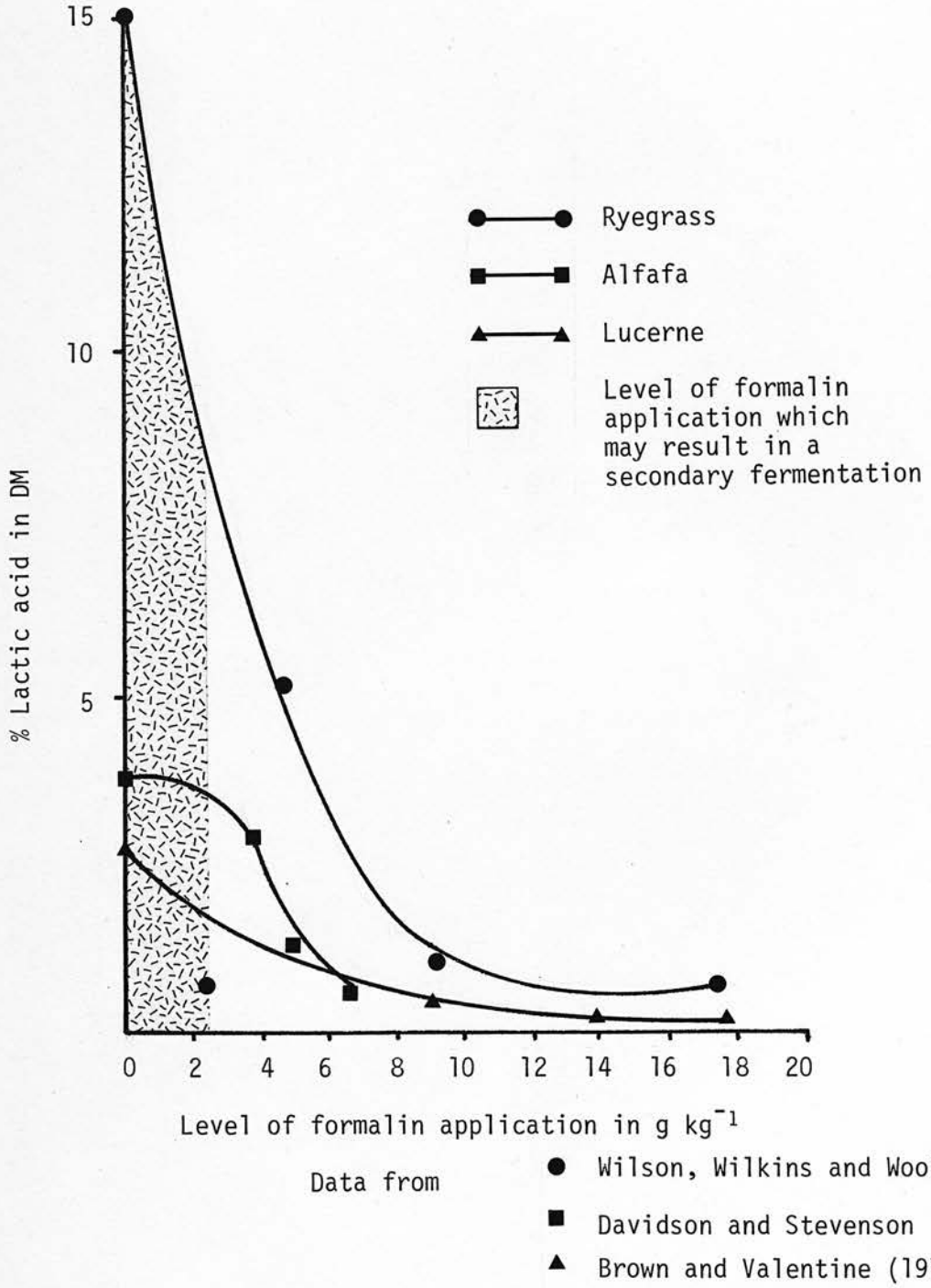


FIGURE 4 Effect of varying rates of application of formalin on lactic acid production in ensilage of ryegrass, alfafa and lucerne.

Table 7 summarises results reported by several authors for WSC contents of formic acid treated silages.

Table 7

Reported levels of % WSC in DM in some formic acid treated silages

	Rate of application of formic acid in g kg^{-1}	% WSC in DM	
		Treated silage	Control silage
1.	2.3	4.6	0.6
2.	2.2	0.7	0.7
	3.4	6.2	2.3
3.	3.3	15.3	4.6
4.	5.0	10.1	1.9
5.	10.0	10.0	2.0

Data from 1. McDonald and Henderson (1974)
2. Henderson and McDonald (1971)
3. Henderson, McDonald and Woolford (1972)
4. Waldo, Keys, Smith and Gordon (1971)
5. Wilkins, Cook and Wilson (1972)

In all cases with the exception of the application of 2.2 g kg^{-1} formic acid (Henderson and McDonald, 1971), formic acid treated silages had increased water soluble carbohydrate levels compared with their respective controls. This indicates that bacterial activity had been restricted during the ensiling period.

Acetic acid

Acetic acid was applied to ryegrass in Trial 1 at 1.2 and 4.7 g kg^{-1} and in Trial 2 at 1.8, 3.5 and 6.9 g kg^{-1}. All levels of application apart from the highest failed to preserve the WSC fraction

of the grass ensiled, but at the 6.9 g kg^{-1} rate, a slight retention of WSC was observed, 5.8% DM compared with 3.3% DM in the control. De Vuyst, Arnould, Moreels and Romedenne (1973) added acetic acid at rates of 3 g kg^{-1} and 4 g kg^{-1} to ryegrass at ensiling. The extent of fermentation which took place in the silages can be judged in the light of the amount of lactic acid present. At the 3 g kg^{-1} rate the lactic acid content was 2.6% (fresh weight) compared with 3.5% (fresh weight) in the control, while at the 4 g kg^{-1} level, the lactic acid contents of the acetic acid treated silage and the control were the same, 2.1% fresh weight. McDonald and Henderson (1974) obtained a similar result when acetic acid was added to Lolium multiflorum at a rate of 3 g kg^{-1} in that there was no WSC retention in the treated silage compared with the control.

It therefore appears that acetic acid has little effect in suppressing fermentation in silage during the ensiling period since neither WSC was retained nor lactic acid production was markedly restricted in the silages examined above and the recommendation of De Vuyst et al. (1973) to use acetic acid as a silage additive particularly on the basis of cost does not seem feasible.

Propionic acid

When propionic acid was tested as a silage additive at the lower levels of application no influence on WSC retention was observed. However, at the 6.9 g kg^{-1} rate, WSC retention in the silage was equivalent to that seen with formic acid treated silage at an application rate of 1.8 g kg^{-1}.

Slight restriction of fermentation in propionic acid treated silages has been noted by McDonald and Henderson (1974) and De Vuyst, Arnould, Vanbelle and Moreels (1972). McDonald and Henderson (1974) noted increased WSC content in a silage which had been treated with 3.7 g kg^{-1} propionic acid compared with a control (WSC 1.6% DM and 0.6% DM respectively). De Vuyst et al. (1972) applied propionic acid to ryegrass at a rate of 1.5 g kg^{-1} and obtained silage which showed evidence of slight restriction of fermentation. The lactic acid content was slightly lower than the control (2.96% and 3.15% on fresh weight basis) and the ammonia nitrogen level was also lower than that of the control.

However, when Cottyn, Boucque and Buysse (1972) added propionic acid (4 g kg^{-1}) to 3rd cut Italian ryegrass, the resulting silage showed evidence of a more active fermentation when results were compared with the control. The treated silage had a higher lactic acid content (2.31% and 2.05% on a fresh matter basis) and the ammonia nitrogen, acetic acid and butyric acid contents were higher than those of the control while the pH of the treated silage was similar to that of the control 4.0 and 4.1.

The application of propionic acid to grass to be ensiled, therefore, has little effect on the course of fermentation within the silo. In comparing the activity of propionic acid with acetic acid there is evidence to suggest that although both are relatively ineffective as additives at the levels of application which would be economically feasible i.e. at 4.5 g kg^{-1} or below, at higher levels of application addition of propionic acid leads to increased restriction of fermentation compared with acetic acid application at the same rates.

The effect of additives combined in pairs on the silage fermentation

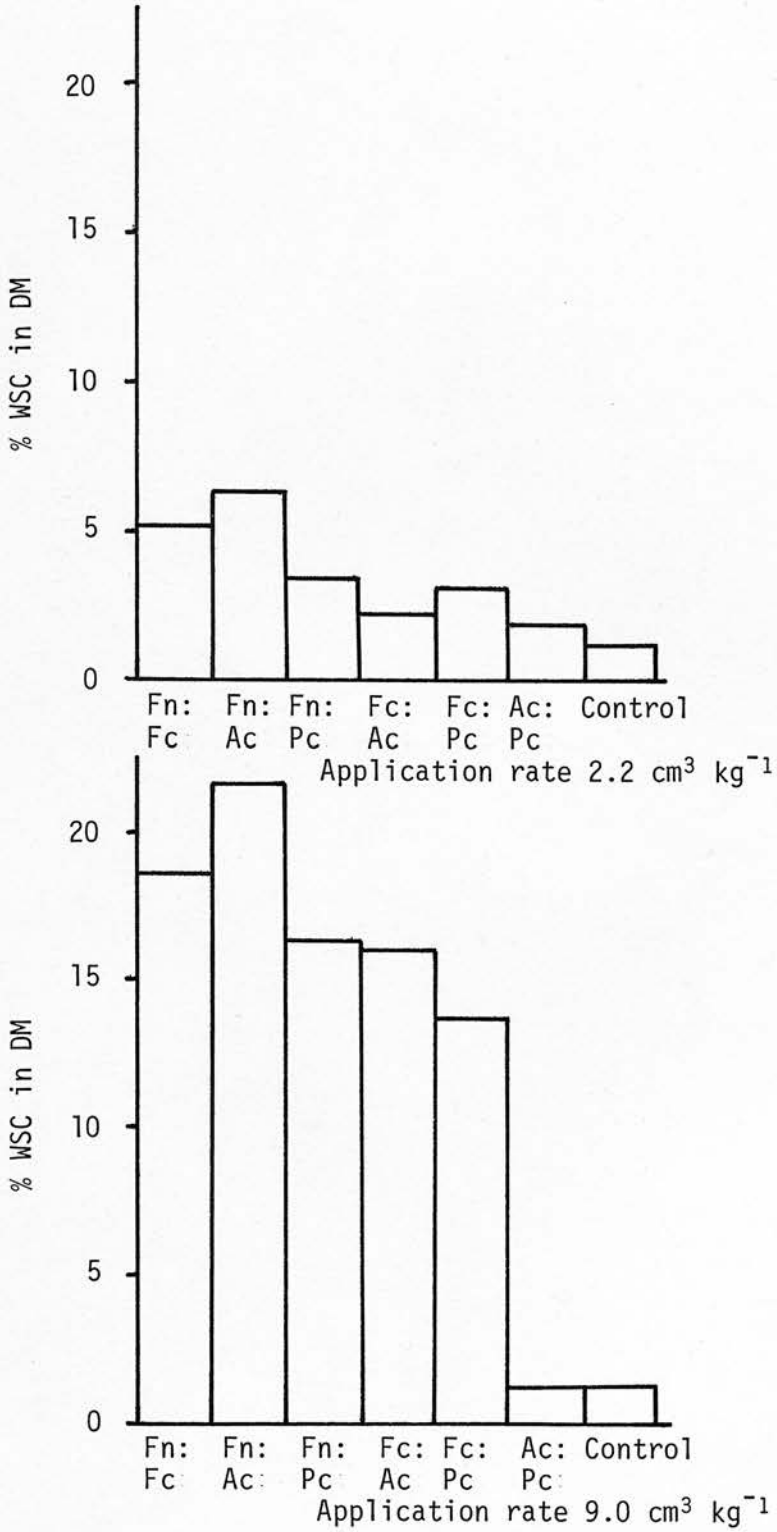
The effect of formalin, formic acid, acetic acid and propionic acid applied in pairs on the WSC content of the resulting silage is summarised in Figure 5.

There was little advantage to be gained from applying the additive pairs at the 2.2 cm^3 kg^{-1} rate though mixtures containing formalin at this application level did show increased WSC level when compared with the control and other mixtures. Of the formalin containing mixtures formalin/acetic acid resulted in silage with the highest WSC value, 6.2% DM, though all the formalin mixtures resulted in silages with higher WSC values than that obtained when formalin alone was used as a silage additive at a rate of 1.2 g kg^{-1}. This effect can possibly be attributed to greater efficiency of formalin as a fermentation inhibitor at lower pH values.

Acetic acid and propionic acid when combined at both 2.3 and 9.2 g kg^{-1} application rates were not effective as silage additives in that the silages produced did not have increased WSC values compared with the control. Thus there was no apparent advantage in applying this particular combination of additives.

At the higher level of application, mixtures containing formalin or formic acid resulted in silages with WSC values ranging from 13.7 to 21.5% DM. Again, silage made with the formalin/acetic acid mixture showed the highest WSC retention 21.5% DM and indeed this was the only mixture in which the silage produced had a higher WSC value than that of the silages made with the addition of each of the components of the mixture.

FIGURE 5 Formalin, formic acid, acetic acid and propionic acid applied in pairs, their effect on the WSC of silage.



Fn formalin
Fc formic acid
Ac Acetic acid
Pc propionic acid

The effect of additives combined in mixtures of three on the silage fermentation

Formalin, formic acid, acetic acid and propionic acid were applied to grass to be ensiled in mixtures of three, and the effect of the combined additives on the course of fermentation was assessed by measuring the WSC retention. The silage with the highest WSC content (22.8% DM) had been treated with a mixture of formalin 1.8 formic acid 3.5 acetic acid 1.8 g kg^{-1}.

The additive mixtures containing both formalin and formic acid with either acetic or propionic acid as the third component all resulted in silages with high WSC values, the formalin/formic/acetic treated group of silages having a WSC range from 15.9 to 22.8% DM while the formalin/formic/propionic group had a WSC range from 17.0 to 18.9% DM.

On the other hand, silages which had been treated with the mixtures which contained both acetic and propionic acid with formalin or formic acid as the third component had lower WSC ranges. The formalin/acetic/propionic acid additives gave rise to silages with WSC values ranging from 9.6 to 16.4% DM and the formic/acetic/propionic acid treated silages had WSC values in the range 10.4 to 19.9% DM.

The lower WSC values in silages which had been treated with mixtures containing both acetic and propionic acids is to be expected since the relative inefficiency of the two acids as silage additives has been demonstrated when they were applied singly. Though the additive mixtures containing both formalin and formic acid were more efficient in preserving the WSC fraction of the silages, compared

with the application of formalin or formic acid alone at the 6.9 g kg^{-1} rate there was little advantage in applying additive mixtures. The formalin and formic acid treated silages had WSC values of 25.9% DM and 21.9% DM and a 6.9 g kg^{-1} rate of application is not commercially prohibitive. However, there are indications that such silages may not be stable when unloaded from the silo in which case application of an additive mixture may be more desirable. A detailed discussion of the aerobic stability of such additive treated silages is included in Section C.

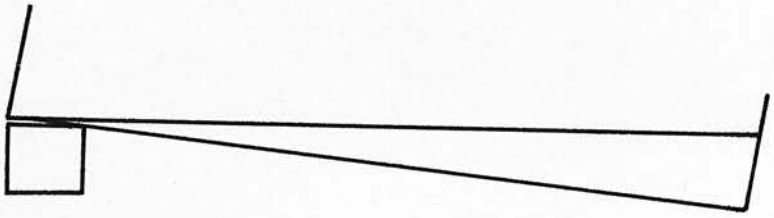
ADDITIVE EFFECT ON SILAGE MICROORGANISMS

Formalin, formic acid, acetic acid and propionic acid were assessed for their antimicrobial activity against microorganisms isolated from silage using the gradient plate technique first described by Szybalski and Bryson (1952).

Experimental

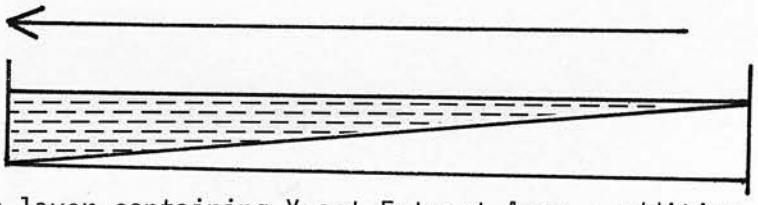
1. Preparation of the Gradient plate. Petri dishes were prepared containing two layers of agar. The first layer was made by pouring 10 cm^3 molten agar into the plate which was pitched at such an angle that the agar just covered the bottom. After the lower layer had set in a wedge, the second layer containing the required concentration of the test additive was poured and set in the horizontal position (see Figure 6). The agar surface was then streaked with test organism in the same line as the slope of the agar and incubated. During incubation the test additive diffused vertically through the plain agar to give a gradient concentration on the plate.

FIGURE 6 Preparation of the Gradient Plate.



lower layer containing Yeast Extract Agar

(I) Stage 1 in preparation of gradient plate in which lower layer is made.



upper layer containing Yeast Extract Agar + additive

(II) Prepared plate showing direction of concentration gradient from zero to concentration of additive used.

The medium used was Yeast Extract Agar. In practice it was found convenient to prepare the agar in 9 cm^3 aliquots and immediately before plate preparation, 1 cm^3 sterile water or 1 cm^3 additive was added aseptically to each aliquot.

2. Test Strains. The following strains were assayed by the gradient plate method.

Lactobacillus buchneri	NCIB 8837
Lactobacillus casei	NCIB 4113
Lactobacillus plantarum	NCIB 8299
Leuconostoc mesenteroides	NCIB 3351
Leuconostoc dextranicum	NCIB 9312
Pediococcus acidilactici	NCIB 6990
Streptococcus faecalis	NCIB 2707
Streptococcus durans	NCIB 2702

Bacillus subtilis	T18	isolated from silages deteriorating under aerobic conditions (see Section C)
Bacillus subtilis	T20	
Bacillus pumilis	R23	
Bacillus pumilis	K2	
Bacillus brevis	R45	
Hansenula subpelliculosa	M56	
Hansenula anomila	T42	
Candida intermedia	Q44	
Torulopsis etchellisii	S15	

NCIB - National Collection of Industrial Bacteria, Torry Research Station, Aberdeen.

3. Additives. Formalin, formic acid, acetic acid and propionic acid were assayed by the slope plate technique. The additives were included in the top layer of the gradient plate at a concentration calculated on the basis of an application rate of 4.5 cm^3 additive

per kg grass ensiled. Correction was made for the dry matter difference between grass (DM assumed to be 20%) and the medium. The DM of Yeast Extract Agar was calculated to be 2.7%. The volume of additive was kept constant. Thus the volume of additive included in each of the slope plates was 0.006 cm^3 additive per 10 cm^3 medium. In practice additives were diluted to the required concentration in 1 cm^3 of sterile water and were added aseptically to 9 cm^3 medium. Sterilisation of the additives was unnecessary.

4. Results. Results were expressed as the percentage of the additive concentration at which the test strains were inhibited. The application rate was equivalent to 4.5 cm^3 additive per kg grass ensiled. The perpendicular bisector of the concentration gradient was marked on the Petri dish. The length of growth of the test strain along the concentration gradient was measured from the perpendicular bisector. This point was assumed to be the point of the gradient at which the additive concentration was 50% of the maximum and the point of inhibition was calculated accordingly.

Results

The effect of formalin, formic acid, acetic acid and propionic acid on pure cultures of microorganisms occurring in silage is shown in Table 8.

Table 8

The effect of formalin, formic acid, acetic acid and propionic acid applied at 4.5 cm^3 kg^{-1} rate on test strains

Test Strain	% inhibition of strain at application level equivalent to 4.5 cm^3 kg^{-1}			
	Formalin	Formic acid	Acetic acid	Propionic acid
L. buchneri	100	62.2	0	0
L. casei	100	53.7	0	0
L. plantarum	100	69.5	0	0
Leuc. mesenteroides	100	100	0	0
Leuc. dextranicum	100	100	0	0
P. acidilactici	100	100	2.1	2.6
Strep. faecalis	100	100	0	2.2
Strep. durans	100	100	2.1	2.1
B. subtilis T18	100	0	0	0
B. subtilis T20	100	0	0	0
B. pumilis R23	25.6	0	0	0
B. pumilis K2	31.7	0	0	0
B. brevis R45	100	0	0	0
H. subpelliculosa M56	100	100	100	100
H. anomila T42	74.4	25.6	0	0
T. etchellisii Q44	57.3	23.2	0	0
C. intermedia S15	80.5	54.9	31.7	56.1

100% inhibition - no growth along gradient

0 inhibition - growth along entire gradient

Discussion

The microorganisms which were assayed by the gradient plate technique fell into two distinct groups, those involved in the silage fermentation and those involved in the aerobic deterioration process discussed in Section C.

The lactic acid bacteria were all found to be completely inhibited by formalin applied at a rate equivalent to 4.5 cm^3 kg^{-1}. This result compared favourably with the results of Woolford (1974) which indicated that application of 0.2 cm^3 kg^{-1} formalin would be sufficient to inhibit lactic acid bacteria in pure culture studies. However, although very low quantities of formalin are sufficient to inhibit lactic acid bacteria in the assay situation, evidence from experimental silages would indicate that high levels of formalin in the region of 9 cm^3 kg^{-1} are required to produce silage in which fermentation has been completely restricted (Wilson et al., 1971: Wilkins et al., 1973). This was also seen in experiments with laboratory silages though an application rate of approximately 7 g kg^{-1} was thought to be sufficient to produce silage in which the fermentation had been severely restricted (see Screening Trials 1 and 2). Wilkins et al. (1973) have advanced several reasons for the discrepancy between the inhibitory effect of low levels of formalin in pure culture studies compared with the high levels required to restrict fermentation in silage. Among the explanations advanced were loss of formalin when applied at harvesting, inactivation of formalin by forage components and increased tolerance to formalin in bacteria involved in the silage fermentation compared with the test strains. It is unlikely that at application almost 100% of the formalin additive is lost and the discrepancy cannot be explained by this factor. Also it would seem

unlikely that a natural "wild" population of lactic acid bacteria would be significantly more tolerant to formalin than the corresponding laboratory population. The major contribution to the discrepancy between pure culture studies and experimental studies with respect to rate of formalin application would seem to be by combination of formalin with forage components since formalin can combine with many of the cell components including proteins and nucleic acids (Hoffman, 1971).

The effect of formic acid on the lactic acid bacteria is less uniform than that of formalin. The strains of Leuconostoc, Pediococcus and Stretptococcus tested were all completely inhibited by 4.5 cm^3 kg^{-1} formic acid. However the Lactobacillus strains tested L. buchneri (heterofermentative), L. casei and L. plantarum (homofermentative) were not 100% inhibited at this level and were able to grow at concentrations of additive below an application rate of 1.8 cm^3 kg^{-1}. This capacity to grow at levels of formic acid application which approach the commercial application rate of 2.5 cm^3 kg^{-1} formic acid explains why formic acid treated silages frequently have higher lactic acid contents than the corresponding control silages (see Section B).

Both acetic acid and propionic acid were not effective in inhibiting the growth of lactic acid bacteria at the level of application tested. According to the results of Woolford (1974) who made an extended study of the effect of additives on silage microorganisms in vitro, application rates in the region of 18 cm^3 kg^{-1} (4 gallons additive per ton grass) would be required before acetic and propionic acids would be effective silage additives in restricting fermentation.

The effect of formalin, formic acid, acetic acid and propionic acid on pure cultures of lactic acid bacteria explained the results obtained with the laboratory silo experiments in that formalin and formic acid were both effective fermentation restrictors when applied at suitable concentrations, but acetic and propionic acids had very little effect on the course of the silage fermentation.

The effect of the silage additives on the Bacillus species and on the yeasts was examined since both types of microorganisms were isolated from deteriorating silage (see Section C). All the Bacillus strains were inhibited by formalin applied at the 4.5 $cm^3\ kg^{-1}$ rate, though B. pumilis was not inhibited to the same extent as B. subtilis or B. brevis. All Bacillus strains grew in the presence of 4.5 $cm^3\ kg^{-1}$ formic acid, acetic acid and propionic acid. The implications of these results are that provided the Bacillus species can survive the ensiling process, silages made with the additives excluding formalin will be subject to proteolytic attack by Bacillus when the silage is exposed to air. This aspect is discussed more fully in Section C.

Yeasts are also closely involved in the aerobic deterioration process of silage. At the 4.5 $cm^3\ kg^{-1}$ level of application all the yeasts tested were inhibited, though as a group they were more tolerant of formalin application than the lactic acid bacteria. The relative tolerance also to formic acid explains the high yeast counts obtained in formic acid treated silages. The level of additive application was generally lower than that necessary to inhibit yeast growth and indeed Woolford (1974) has demonstrated that a formic acid level of 9.2 $cm^3\ kg^{-1}$, acetic acid 9.2 $cm^3\ kg^{-1}$ and propionic acid 6.5 $cm^3\ kg^{-1}$ levels of application were necessary to inhibit yeast growth in pure culture.

SECTION B

FIELD STUDIES

FIELD STUDIES

This section is concerned with an examination of the microflora of grass and silages made under field conditions on the Edinburgh School of Agriculture farms.

Experimental

1. Sampling. The standing crop was sampled by cutting with sterile scissors and collecting the samples in sterile polythene bags. The sites of sampling were determined by pacing the field in a "W" pattern and taking samples every 40 paces. Samples were bulked for each traverse of the field, and three bulk samples were obtained in this manner starting at a different corner of the field each time.

With mown grass in swaths, samples were taken by inverting a sterile polythene bag over the hand, picking up the sample and pulling the bag back over the sample before sealing. The changes in the microflora of cut grass over the wilting period were monitored in a section of 12 swaths lying adjacent to each other. Samples were obtained by pacing the length of the swaths in a zigzag manner and taking a sample every 25 paces.

Forage harvested material was sampled using the inverted bag technique or by using sterile forceps to transfer the material into the container. Material was sampled immediately after forage harvesting from the trailer attached to the harvester.

When silos were being filled, bulk samples were taken from the top layer of grass several times during filling, to give an analysis of the microflora at different levels of the silo. At the time of unloading, samples were taken from the silos throughout the emptying period. In experimental silos of 500 kg capacity and 2 Mg capacity samples were taken from the exposed silage face at various times during emptying and bulked samples were also taken from the heap of unloaded silage. In the case of the large experimental silos, where silage was being unloaded daily for feeding trials with steers, a bulk sample of about 1 kg was taken from the silage face when approximately half of the silage had been unloaded. All samples were transported to the laboratory for processing in polystyrene boxes filled with crushed ice.

2. Microbiological Analysis. Total count, numbers of lactic acid bacteria, yeasts and moulds were recorded. Results were expressed in numbers per g DM of sample.

3. Silages Examined. Additive treated silages in Groups I, II, III and IV were examined. The detailed compositions are given in Appendix 2. The treatments were as follows.

Group I Date of ensiling: 23.8.72

Additive treatments: Control

Formalin/H_2SO_4	5.25/1.75 g kg^{-1}
Formalin/formic acid	8.2/2.1 g kg^{-1}
Formalin	9.7 g kg^{-1}

The grass was unwilted.

Group II Date of ensiling: 28.8.72

Additive treatments: Control

Formalin/acetic acid 4.5/4.5 g kg^{-1}

Formic acid/acetic acid 4.2/1.4 g kg^{-1}

The grass was unwilted.

Group III Date of ensiling: 18-20.7.72

Additive treatments: Control DM 16.4%

Fresh grass + formic acid 3.6 g kg^{-1}

Wilted DM 31.7%

Wilted + formic acid 6.3 g kg^{-1}

Group IV Date of ensiling: 11-15.6.73

Additive treatments: Control

Formic acid 5 g kg^{-1}

Formic acid/formalin 3.4/3.4 g kg^{-1}

Formic acid/formalin/ propionic acid 1.8/1.8/1.8 g kg^{-1}

Results

1. Group I Silages

Silages were made in 2 Mg capacity plastolene silos. The grass was harvested by double chop forage harvester. Each silo was filled with two trailer loads of grass and initial samples for microbiological analysis were taken from the surface when the first load had been consolidated in the silo.

Table 9

Changes in the microflora of Group I silages

Sample	Total Count	Lactic acid bacteria	Yeasts	Moulds
(i) Standing crop	1.5×10^8	$< 10^2$	$< 10^2$	$< 10^2$
(ii) Control				
Grass as loaded to silo	5.6×10^7	$< 10^2$	$< 10^2$	1.5×10^5
"Silage" unloaded after 5 hrs ensiling	3.8×10^8	7.1×10^4	3.1×10^4	$< 10^2$
(iii) Control				
Grass as loaded to silo	4.2×10^7	3.5×10^3	$< 10^2$	2.0×10^6
Silage unloaded. Bulked	3.1×10^7	8.9×10^7	8.0×10^4	$< 10^2$
Bulked	2.2×10^7	2.4×10^7	6.1×10^5	$< 10^2$
Bulked	7.0×10^7	1.1×10^8	1.1×10^5	$< 10^2$
Bulked	1.7×10^7	4.3×10^7	8.0×10^7	$< 10^2$
Silage surface	4.7×10^8	1.7×10^8	8.0×10^7	$< 10^2$
Silage Middle layer	4.7×10^7	9.8×10^7	8.4×10^3	$< 10^2$
Bottom layer	3.0×10^7	4.1×10^7	1.4×10^5	$< 10^2$
(iv) Formalin/H_2SO_4 treatment				
Grass as loaded to silo	5.5×10^5	$< 10^2$	$< 10^2$	$< 10^2$
Silage unloaded. Bulked	7.4×10^7	1.6×10^8	9.7×10^3	$< 10^2$
Bulked	2.2×10^7	7.4×10^7	5.5×10^3	$< 10^2$
Bulked	1.6×10^8	2.9×10^8	3.4×10^4	3.2×10^2
Bulked	5.5×10^7	5.5×10^7	1.8×10^4	2.8×10^2
Silage surface	1.6×10^8	2.5×10^8	4.6×10^4	5.1×10^2
Silage Middle layer	1.1×10^8	2.3×10^8	1.8×10^2	$< 10^2$
Bottom layer	8.4×10^7	4.2×10^7	$< 10^2$	$< 10^2$
(v) Formalin/formic acid treatment				
Grass as loaded to silo	5.1×10^6	$< 10^2$	$< 10^2$	$< 10^2$
Silage unloaded. Bulked	2.4×10^8	3.2×10^7	1.3×10^5	$< 10^2$
Bulked	8.0×10^7	1.9×10^7	1.7×10^5	1.1×10^4
Bulked	9.9×10^7	2.2×10^7	1.4×10^6	$< 10^2$
Bulked	3.2×10^8	2.4×10^7	5.2×10^6	$< 10^2$
Silage surface	1.0×10^8	1.2×10^8	1.6×10^7	4.3×10^5
Silage Middle layer	2.5×10^8	4.2×10^6	4.7×10^3	4.3×10^2
Bottom layer	2.8×10^8	3.9×10^9	6.6×10^3	5.2×10^2

(cont.)

Table 9

(cont.)

(vi) Formalin treatment †				
Grass as loaded to silo	1.6×10^4	$< 10^2$	$< 10^2$	$< 10^2$
Silage surface	2.5×10^7	2.6×10^7	9.4×10^2	$< 10^2$
Silage Middle layer	6.3×10^5	1.4×10^6	$< 10^2$	$< 10^2$
Bottom layer	8.4×10^6	1.9×10^5	$< 10^2$	$< 10^2$

All results expressed in numbers per g silage DM.

† Formalin treatment - no bulked samples taken.

2. Group II Silages

Silages were made in 500 kg capacity plastolene silos. Grass, cut by double chop forage harvester was weighed into the silos in 25 kg amounts after being treated with additive. Two silos were filled per treatment.

Table 10

Changes in the microflora of Group II silages

Sample		Total Count	Lactic acid bacteria	Yeasts	Moulds
(i) Standing crop		5.0×10^7	$< 10^2$	10	3.6×10
(ii) Control					
Silo A - grass as	load 1	9.2×10^7	1.0×10^6	$< 10^2$	1.3×10^5
loaded to silo	load 10	4.4×10^9	8.2×10^5	$< 10^2$	3.9×10^5
	load 20	4.7×10^9	1.8×10^6	$< 10^2$	9.8×10^5
Silo B - grass as	load 1	4.1×10^9	2.1×10^6	1.5×10^6	$< 10^2$
loaded to silo	load 10	3.5×10^9	1.7×10^6	8.0×10^5	$< 10^2$
	load 20	1.1×10^{10}	9.8×10^6	1.9×10^6	$< 10^2$

(cont.)

Table 10

(cont.)

Silo A	Silage surface	1.5×10^8	2.2×10^9	3.0×10^7	$< 10^2$
Silage	Middle layer	4.0×10^8	5.0×10^8	3.5×10^4	$< 10^2$
	Bottom layer	2.0×10^8	3.9×10^8	6.7×10^3	$< 10^2$
Silo B	Silage surface	2.3×10^9	2.9×10^9	1.3×10^7	$< 10^2$
Silage	Middle layer	8.2×10^7	1.6×10^8	2.2×10^5	2.4×10^5
	Bottom layer	2.5×10^8	4.4×10^8	$< 10^2$	$< 10^2$
(iii) Formalin/acetic acid					
Silo C - grass as	load 1	1.7×10^9	1.1×10^6	1.9×10^4	2.5×10^3
loaded to silo	load 10	2.3×10^9	$< 10^2$	$< 10^2$	$< 10^2$
	load 20	5.6×10^8	3.0×10^5	7.6×10^3	2.0×10^3
Silo D - grass as	load 1	2.1×10^7	$< 10^2$	1.9×10^5	$< 10^2$
loaded to silo	load 10	1.5×10^8	$< 10^2$	4.9×10^4	$< 10^2$
	load 20	2.9×10^8	$< 10^2$	1.5×10^5	$< 10^2$
Silo C	Silage surface	1.5×10^7	2.4×10^7	$< 10^2$	$< 10^2$
Silage	Middle layer	1.4×10^6	7.9×10^5	5.9×10^4	$< 10^2$
	Bottom layer	2.0×10^6	3.5×10^5	1.5×10^4	$< 10^2$
Silo D	Silage surface	2.6×10^6	$< 10^4$	$< 10^2$	$< 10^2$
Silage	Middle layer	7.4×10^6	$< 10^4$	$< 10^2$	$< 10^2$
	Bottom layer	1.4×10^6	$< 10^4$	$< 10^2$	$< 10^2$
(iv) Formic Acid/acetic acid					
Silo E - grass as	load 1	1.2×10^9	$< 10^3$	1.2×10^6	$< 10^2$
loaded to silo	load 10	6.0×10^8	$< 10^3$	6.5×10^6	$< 10^2$
	load 20	5.5×10^7	$< 10^3$	2.2×10^5	$< 10^2$
Silo F - grass as	load 1	4.0×10^9	1.8×10^6	1.3×10^6	$< 10^2$
loaded to silo	load 10	2.9×10^9	$< 10^3$	1.8×10^6	$< 10^2$
	load 20	† ND	ND	ND	ND
Silo E	Silage surface	1.9×10^7	2.0×10^7	5.7×10^6	$< 10^2$
Silage	Middle layer	4.0×10^7	2.8×10^7	1.5×10^6	$< 10^2$
	Bottom layer	2.3×10^7	1.2×10^8	$< 10^2$	$< 10^2$
Silo F	Silage surface	8.2×10^7	$< 10^4$	9.8×10^6	$< 10^2$
Silage	Middle layer	3.5×10^7	$< 10^4$	3.7×10^4	8.2×10^4
	Bottom layer	8.7×10^6	$< 10^4$	$< 10^2$	$< 10^2$

Numbers expressed per g DM

† ND not determined

3. Group III Silages

Silages were made in 25 Mg capacity vacuum silos. Forage harvested grass was built in a wedge over a filling period of 3 days, the fresh treatments being loaded on days 1 and 2 and the wilted treatments on days 2 and 3. Samples were taken from the surface of the stack after consolidation by tractor.

Table 11

Changes in the microflora of Group III silages

Sample	Total Count	Lactic acid bacteria	Yeasts	Moulds
(i) Standing crop	1.7×10^7	$< 10^2$	$< 10^2$	$< 10^2$
(ii) Control - fresh grass				
Immediately after forage harvesting	1.5×10^9	3.0×10^6	5.9×10^4	$< 10^2$
Grass as - load 2	1.8×10^9	5.1×10^5	$< 10^2$	1.8×10^5
loaded to silo load 3	4.6×10^7	5.4×10^3	$< 10^2$	4.3×10^8
Before closing Day 1	2.3×10^8	7.3×10^4	$< 10^2$	$< 10^2$
After load 1 Day 2	4.3×10^{10}	9.8×10^5	$< 10^2$	$< 10^2$
Silage Sample 1	2.3×10^5	3.9×10^4	2.9×10^3	$< 10^2$
Sample 2	1.0×10^5	2.9×10^4	1.2×10^4	$< 10^2$
(iii) Formic acid - fresh grass				
Immediately after forage harvesting	6.8×10^7	$< 10^2$	$< 10^2$	$< 10^2$
Grass as - load 2	2.6×10^7	9.3×10^2	$< 10^2$	$< 10^2$
loaded to silo load 5	1.3×10^7	1.8×10^3	$< 10^2$	$< 10^2$
Before closing Day 1	4.1×10^7	9.3×10^2	$< 10^2$	$< 10^2$
After load 1 Day 2	6.9×10^7	1.3×10^5	$< 10^2$	$< 10^2$
Silage Sample 1	3.9×10^5	8.9×10^4	$< 10^2$	$< 10^2$
Sample 2	2.5×10^5	$< 10^2$	$< 10^2$	$< 10^2$

(cont.)

Table 11
(cont.)

(iv) Control - wilted					
* Grass	after mowing	1.2×10^7	3.2×10^3	ND	ND
	after crimping	4.4×10^7	7.4×10^3	2.4×10^4	3.1×10^3
	wilting 3 hrs	7.6×10^6	4.8×10^3	$< 10^2$	$< 10^2$
	wilting 23 hrs*	5.6×10^7	3.0×10^3	8.2×10^3	$< 10^2$
Before forage harvesting		2.2×10^8	1.7×10^4	1.9×10^3	$< 10^2$
After forage harvesting		1.4×10^8	3.7×10^4	1.3×10^4	$< 10^2$
Grass as loaded at silo -	load 1	1.4×10^9	1.9×10^5	6.6×10^4	1.1×10^4
At closing	Day 2	8.5×10^8	$< 10^5$	9.9×10^4	$< 10^2$
load 1	Day 3	2.3×10^8	2.0×10^6	5.1×10^3	$< 10^2$
load 2		8.6×10^{10}	3.5×10^4	$< 10^2$	$< 10^2$
load 4		8.2×10^{10}	1.9×10^5	$< 10^2$	$< 10^2$
Silage	Sample 1	1.4×10^7	1.2×10^7	$< 10^2$	2.2×10^3
	Sample 2	1.0×10^6	1.6×10^6	$< 10^2$	5.7×10^3
(v) Formic acid - wilted					
Before forage harvesting		2.3×10^8	2.9×10^4	3.1×10^3	$< 10^2$
After forage harvesting		7.3×10^6	5.8×10^3	$< 10^2$	$< 10^2$
Grass as loaded at silo -	load 1	3.3×10^8	6.7×10^4	$< 10^2$	$< 10^2$
	load 2	2.4×10^7	1.0×10^4	$< 10^2$	$< 10^2$
At closing	Day 2	5.6×10^7	2.2×10^5	1.7×10^3	$< 10^2$
Day 3	load 1	5.2×10^6	9.1×10^4	$< 10^2$	$< 10^2$
	load 2	4.5×10^9	1.9×10^4	$< 10^2$	$< 10^2$
	load 3	1.0×10^{11}	4.5×10^5	$< 10^2$	$< 10^2$
Silage	Sample 1	1.3×10^5	$< 10^4$	$< 10^2$	$< 10^2$
	Sample 2	2.1×10^6	4.5×10^4	$< 10^2$	$< 10^2$

Results expressed as numbers per g DM

*Results expressed as numbers per g fresh weight

4. Group IV Silages

Silage was made in 100 Mg concrete bunker silos. The material used was wilted grass which was consolidated by tractor during filling and covered by plastic sheeting.

Table 12

Changes in the microflora of Group IV silages

Sample		Total Count	Lactic acid bacteria	Yeasts	Moulds
(i) Standing crop		2.9×10^7	$< 10^2$	$< 10^2$	$< 10^2$
Post mowing *DM	19.5%	6.6×10^7	9.2×10^2	2.2×10^4	1.6×10^4
Wilting 2 hrs	21%	2.7×10^7	$< 10^2$	$< 10^2$	4.5×10^2
4 hrs	22.5%	1.7×10^7	$< 10^2$	$< 10^2$	1.0×10^3
11 hrs	24.5%	3.5×10^7	$< 10^2$	$< 10^2$	4.1×10^2
23 hrs	29.5%	3.4×10^7	$< 10^2$	2.4×10^3	2.1×10^3
(ii) Control					
Preharvesting from swath		8.6×10^6	9.5×10^4	$< 10^2$	$< 10^2$
		5.0×10^7	4.4×10^5	$< 10^2$	$< 10^2$
Postharvesting		1.7×10^7	2.8×10^6	3.3×10^4	$< 10^2$
		8.6×10^7	1.4×10^7	1.0×10^4	$< 10^2$
Loading at silo		3.8×10^8	4.5×10^7	2.9×10^3	2.2×10^3
		1.4×10^8	6.9×10^7	3.6×10^2	4.4×10^2
Silage		7.4×10^6	1.5×10^7	4.0×10^4	$< 10^2$
(iii) Formic acid					
Preharvesting from swath		4.1×10^7	$< 10^2$	6.4×10^2	$< 10^2$
		2.9×10^7	$< 10^2$	2.0×10^2	$< 10^2$
		2.5×10^7	$< 10^2$	1.8×10^2	$< 10^2$
Postharvesting		4.1×10^6	$< 10^2$	$< 10^2$	$< 10^2$
		6.7×10^6	$< 10^2$	$< 10^2$	$< 10^2$
		4.4×10^6	$< 10^2$	$< 10^2$	$< 10^2$
Loading at the silo		1.3×10^7	$< 10^2$	$< 10^2$	$< 10^2$
		1.7×10^8	$< 10^2$	$< 10^2$	$< 10^2$
		3.5×10^7	$< 10^2$	$< 10^2$	$< 10^2$
Silage		2.9×10^7	1.2×10^7	1.4×10^6	$< 10^2$

(cont.)

Table 12

(cont.)

(iv) Formic acid + formalin				
Preharvesting from swath	4.1×10^7	$< 10^2$	2.9×10^3	1.3×10^3
	7.9×10^6	$< 10^2$	5.9×10^3	2.3×10^4
Post harvesting	8.8×10^6	$< 10^2$	$< 10^2$	$< 10^2$
	5.1×10^6	$< 10^2$	$< 10^2$	$< 10^2$
Loading at silo	2.4×10^6	$< 10^2$	$< 10^2$	$< 10^2$
	2.7×10^6	$< 10^2$	$< 10^2$	$< 10^2$
	7.3×10^6	$< 10^2$	$< 10^2$	$< 10^2$
Silage	9.9×10^7	2.6×10^7	2.1×10^6	$< 10^2$
(v) Formic acid + formalin + propionic acid				
Preharvesting from swath	7.2×10^7	$< 10^2$	$< 10^2$	$< 10^2$
	1.4×10^7	$< 10^2$	$< 10^2$	$< 10^2$
Post harvesting	pu	$< 10^2$	$< 10^2$	$< 10^2$
	pu	$< 10^2$	$< 10^2$	$< 10^2$
Loading at the silo	6.0×10^6	$< 10^2$	$< 10^2$	$< 10^2$
	3.9×10^7	4.8×10^3	$< 10^2$	$< 10^2$
	4.5×10^5	4.2×10^2	$< 10^2$	$< 10^2$
Silage	5.2×10^7	3.7×10^7	5.8×10^6	2.7×10^6

All results expressed as numbers per g DM

* Dry matter data from Clark (1974)

pu plates uncountable

Discussion

Control silages, unwilted and wilted

In silage made without an additive, the development of the microflora follows a well established pattern (Whittenbury, 1968). The incidence of lactic acid bacteria on the standing crop is low as demonstrated by Stirling and Whittenbury (1963), and shown in the counts of the lactic acid bacteria obtained for each of the grass crops used

in the four experiments. The total count of microorganisms was in the region of 10^7 - 10^8 organisms per g DM, but lactic acid bacteria were not detected in levels above 10^2 per g DM for each of the standing crops examined.

Henderson, McDonald and Woolford (1972) showed that after forage harvesting, the lactic acid bacteria increased on grass. In Group III control silage (fresh grass) the samples taken before forage harvesting and immediately after forage harvesting showed an increase in total count from 1.7×10^7 to 1.5×10^9 per g DM and the numbers of lactic acid bacteria increased from less than 10^2 to 3.0×10^6 per g DM.

In the case of wilted silages, Groups III and IV, a small inoculum of lactic acid bacteria was introduced by the mower as the grass was cut to lie in swaths in the field. In the control wilted silage of Group III, the number of lactic acid bacteria on the grass was increased from less than 100 to 3.2×10^3 per g fresh weight by mowing. Crimping or conditioning the grass further increased this number to 7.4×10^3 per g fresh weight. Similarly in Group IV, the sample taken immediately after mowing shows an increase from less than 100 to 9.2×10^2 lactic acid bacteria per g DM.

The mower cuts the grass at one point leaving the grass blade intact with the minimum of bruising, whereas the forage harvester chops and lacerates the grass liberating plant juices which provide a suitable growth medium for lactic acid bacteria. Stirling and Whittenbury (1963), in their examination of plants for lactic acid bacteria, found that the great majority of the colonies which developed

were located on sheath material at the base of grasses and on partially withered and decaying blades of grass. By mowing, the grass is cut, with the subsequent liberation of sap, at the part of the grass blade where lactic acid bacteria may be expected to be found, but the grass juice liberated may only be sufficient to maintain a small lactic acid bacteria population. On the other hand, the forage harvester by chopping the plant liberates much larger quantities of grass juice to maintain proportionally larger numbers of lactic acid bacteria. The forage harvester acts as a source of lactic acid bacteria since they must multiply on the machine itself, in the layer of sap coating it. The mower is not such an efficient inoculator, and it is more likely that the increase in lactic acid bacteria numbers can be attributed to local multiplication at the cut ends of the plants.

During the two wilting periods the lactic acid bacteria population remained static. In Group III the numbers of lactic acid bacteria changed from 7.4×10^3 to 3.0×10^3 per g fresh weight over a 23 hour wilting period and in Group IV over the wilting period, lactic acid bacteria were not detected in levels above 10^2 per g DM. Conditions in the field during the two wilting periods studied were not suitable for the development of the lactic population. Indeed, it is unlikely that in favourable wilting conditions the population of lactic acid bacteria would significantly increase as the crop was lying in the field. Conditions would be dry and there would be insufficient seepage of nutrients from the plant to maintain a high population. Forage harvesting of the wilted grass resulted in increased numbers of lactic acid bacteria. (Group III 3.7×10^4 per g DM: Group IV a) 2.8×10^6 b) 1.4×10^7 per g DM.)

After forage harvested grass has been loaded into a trailer in the field, there can be a substantial time lag before the material is loaded into the silo, in Groups III and IV this was approximately 35 minutes. During this time, a further increase in the count of lactic acid bacteria was observed and as the material was loaded into the silo the lactic acid bacteria formed approximately 10% of the total microflora. (See Figure 7 for summary of data.)

Formic acid treated silages

In silages of Group III and IV, formic acid was added at levels of 3.6 and 6.3 g kg^{-1}, and 5 g kg^{-1} respectively. It was applied to the grass at forage harvesting. The treatment reduced the total count of microorganisms after harvesting compared with control, and it also reduced the numbers of lactic acid bacteria present per g DM compared with the control. (See Figure 8.)

This effect was also observed after transportation of the material to the silo. In the untreated controls, by the time the material had reached the silo, the lactic acid bacteria numbers had reached 10^7 per g DM in Group IV though in Group III untreated controls, the numbers were lower ranging from 10^3 to 10^6 per g DM. Formic acid treatment resulted in grass with a lower pH (4.59) and lower levels of lactic acid bacteria being loaded into the silos. In Group III the lactic acid bacteria were in the range 10^4 - 10^5 per g DM in the unwilted control, while the unwilted, formic acid treated grass was being loaded into the silo with a level of lactic acid bacteria between 10^2 - 10^3 per g DM. Similarly in the wilted treatments in Group III, the treated material had a lactic acid bacteria count of 10^4 - 10^5 bacteria per g DM while the range was 10^4 - 10^6 in the control. In

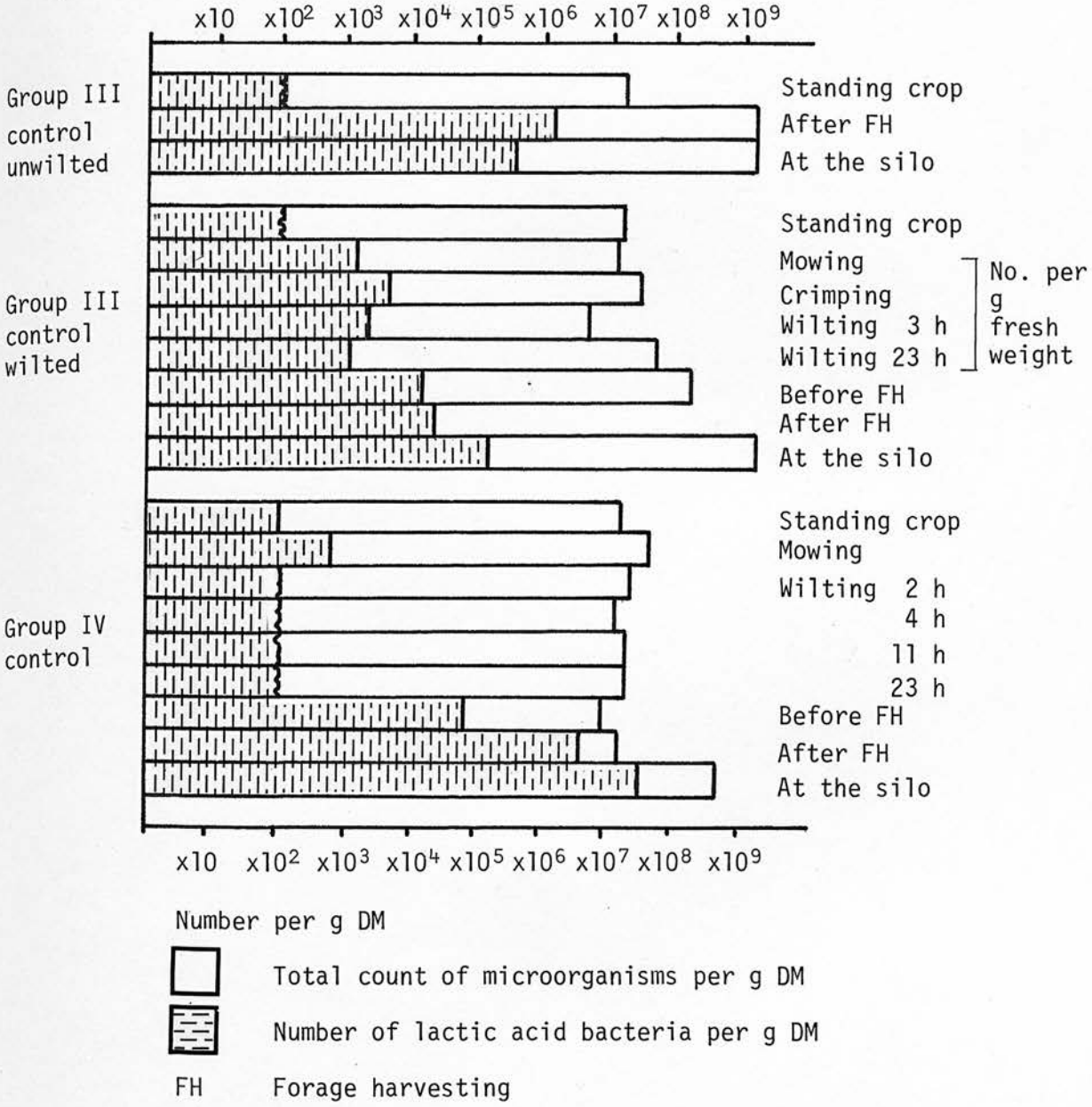


FIGURE 7 The effect of mowing, crimping, wilting and forage harvesting on the microflora of grass cut for ensiling.

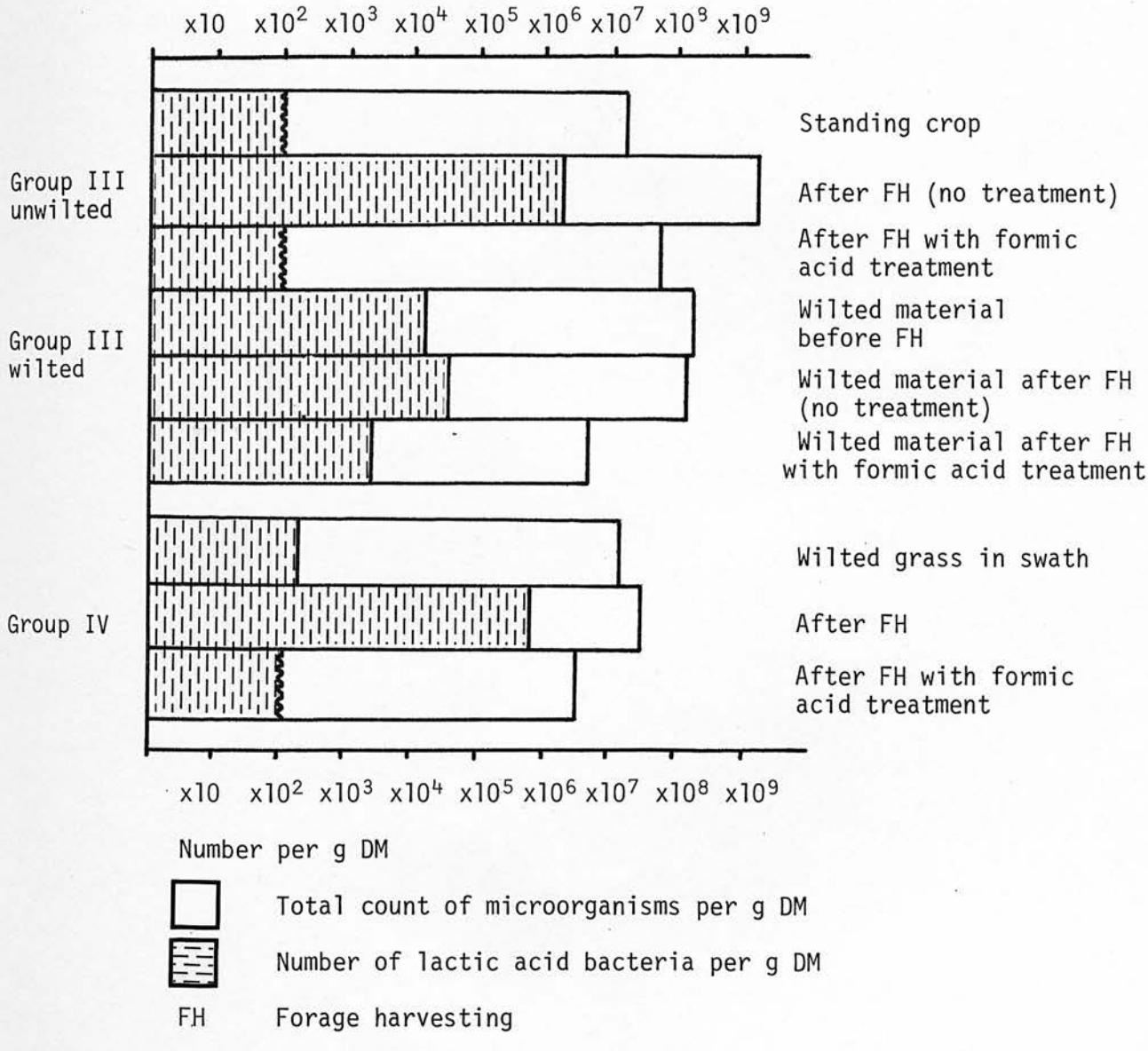
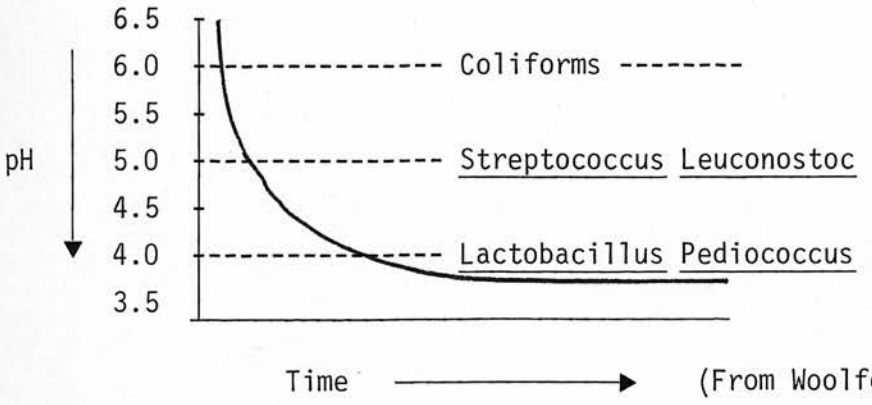


FIGURE 8 The effect of forage harvesting with formic acid treatment on the microflora of unwilted and wilted grass to be ensiled.

Group IV the formic acid treated material loaded at the silo had counts of lactic acid bacteria less than 100 per g DM.

The initial stages of fermentation within the silo are carried out by the following sequence of microorganisms.



(From Woolford, 1974)

Formic acid applied at the commercially recommended level of 2.3 g kg^{-1} fresh grass reduces the pH of grass at ensiling to pH 4.6 - 4.9 and effectively excludes the coliform and Streptococcus/Leuconostoc stages of the fermentation i.e. the activity of these groups in reducing the pH is not necessary. However, in addition to a pH effect, Woolford (1974) has demonstrated that coliforms are inhibited by lower concentrations of formic acid than inhibit lactic acid bacteria, the test being carried out at pH 5. Beck (1968) has also shown that formic acid has little effect on the growth of L. plantarum but a marked inhibitory effect on the growth of butyric acid bacteria and coliforms.

At the end of the ensiling period, in Group III, the formic acid treated silage showed no marked difference from the unwilted control in total count, though one formic acid treated sample had a

low count of lactic acid bacteria (less than 100 per g DM). With the wilted silages of this group, formic acid treatment reduced both the total count and the lactic acid bacteria count compared with the control. In Group IV, formic acid treatment gave silage with a higher total count than the control, 2.9×10^7 and 7.6×10^6 per g DM respectively, but there was no difference between the numbers of lactic acid bacteria, 1.2×10^7 and 1.5×10^7 per g DM respectively. (See Figure 9.)

The information in Table 13 has been abstracted from published results and compares the effect of formic acid treatment on microbiological counts of silages.

Table 13

Comparison of the numbers and types of microorganisms isolated from formic acid treated silage

Ensiling in Days	Treatment	Total Count	Lactic acid bacteria	Yeast	Mould
1. 244	Formic acid 2.7 g kg^{-1}	1.8×10^9	1.6×10^9	ND	ND
244	Control	2.2×10^{10}	2.0×10^9	"	"
183	Formic acid 2.7 g kg^{-1}	8.4×10^6	6.0×10^5	"	"
183	Control	2.0×10^8	2.1×10^9	"	"
2. 149	Formic acid 2.7 g kg^{-1}	7.6×10^5	4.9×10^7	1.9×10^4	
156	Control	8.4×10^6	3.9×10^6	< 10	
3. * 62	Formic acid 2.5 g kg^{-1}	1.5×10^8	1.6×10^7	0	9.5×10^3
62	Control	1.1×10^9	1.1×10^9	2.9×10	9.5×10^3

Results corrected to numbers per g silage DM

ND - Not determined

1. From Taylor and Phillips (1970)
2. From Henderson, McDonald and Woolford (1972)
3. From Pedersen, Olsen and Guttormsen (1973)

* Results abstracted from graphs. Dry matter figures of silages not given in text, assumed value 19%.

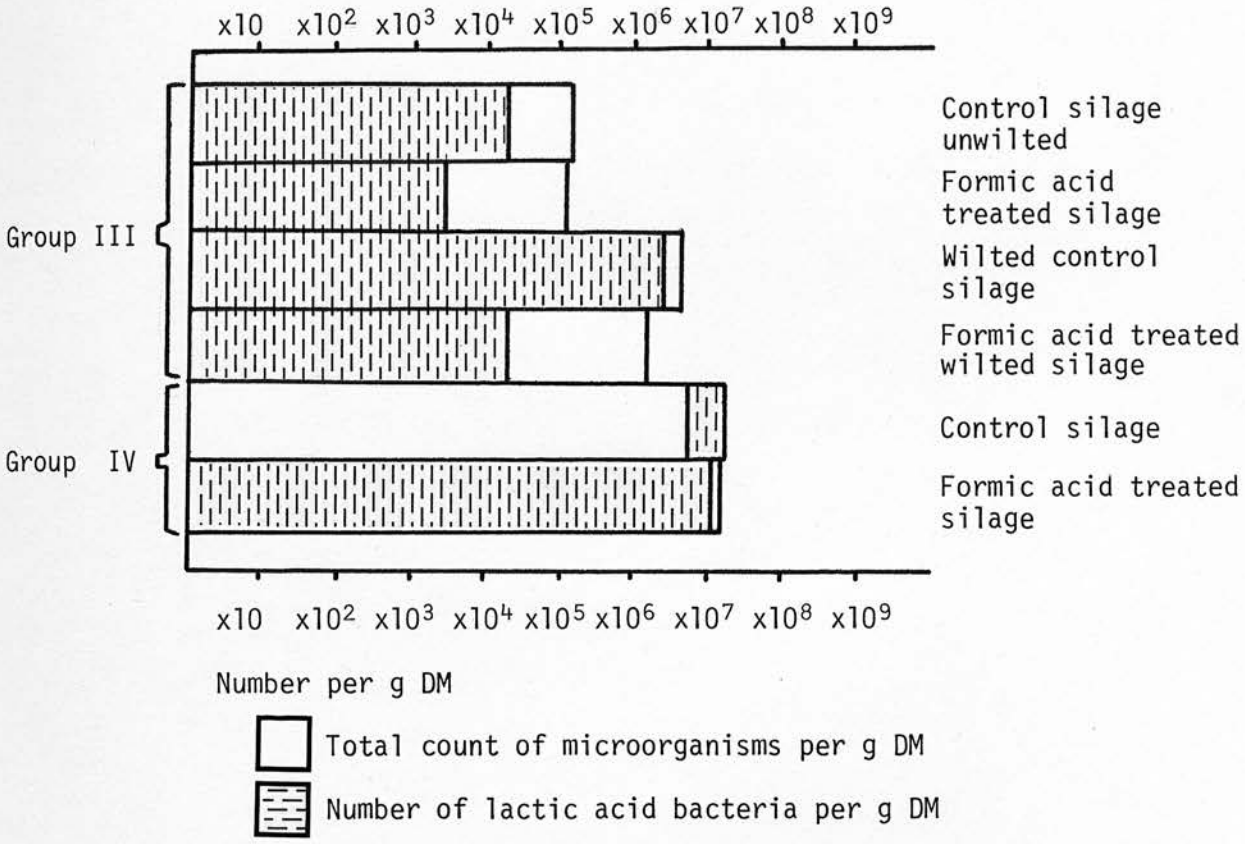


FIGURE 9 The effect of formic acid application at ensiling on the microflora of the silage at opening.

Taylor and Phillips (1970) and Pedersen et al. (1973) both recorded decreases in total count and decreases in lactic acid bacteria counts in formic acid treated silages compared with control silages. Henderson et al. (1972) showed that although total count was reduced by formic acid treatment, the number of lactic acid bacteria was in fact greater in the additive treated silage than in the control. (However the lactic acid bacteria count may have been exaggerated since there was a high yeast population present. Yeasts are capable of growing on the selective counting medium used for the lactic acid bacteria.)

Henderson and McDonald (1971) and Henderson et al. (1972) first postulated, then showed increased yeast populations in formic acid treated silages. This effect was not observed in Group III, but in Group IV, the formic acid treated silage had a yeast count of 1.4×10^6 per g DM compared with 4.0×10^4 per g DM in the control. Pedersen et al. (1973) did not detect yeasts in formic acid treated silage, but recorded a population of 2.9×10^3 yeasts per g DM in the control silage.

The reason for the high yeast populations found in formic acid treated silage has not been fully investigated. However Woolford (1974) has shown that formic acid is less inhibitory to yeasts than it is to lactic acid bacteria and this was also observed in the pure culture studies reported in Section A. Thus in a formic acid treated silage the growth of yeasts would be favoured.

Silages treated with formalin mixtures

The purpose of including formalin in an additive, is to produce silage with reduced levels of fermentation acids, thereby increasing the voluntary intake by animals, (Wilkins and Wilson, 1974). Fermentation in the silo is virtually eliminated by the addition at ensiling of formalin at 7-9 g kg^{-1} (Wilkins, Wilson and Woolford, 1973). However, at this level, Wilkins (1973) has postulated that formalin may have an adverse effect on the rumen microflora. Non fermented silages can be made by the use of low quantities of formalin in mixtures with acids. On the other hand, Ewart (1974 pers. comm.) has demonstrated that formalin in mixtures with organic acids, when used as an additive, produces silage which may be more detrimental to the rumen microflora than when formalin is used alone.

In Group I silages, the additive treated (formalin/H_2SO_4, formalin/formic acid and formalin) grasses all had low counts of lactic acid bacteria ($<10^2$ per g DM) when loaded into the silo. The inhibitory effect of the additives was also reflected in decreased total counts, the formalin treatment reducing the total count to 1.6×10^4 per g DM. (See Figure 10.)

In Group II, the additives tested were formalin/acetic acid and formic acid/acetic acid mixtures. The additives were not applied at forage harvesting, but were applied to 25 kg batches of grass before consolidation in the silo. Both the controls had lactic acid bacteria populations of 10^6 per g DM throughout the loading period. Formalin/acetic acid treatment tended to reduce the level of lactic acid bacteria, the three samples taken from silo D having less than 100 per g DM, though in silo C two samples had counts of 1.1×10^6

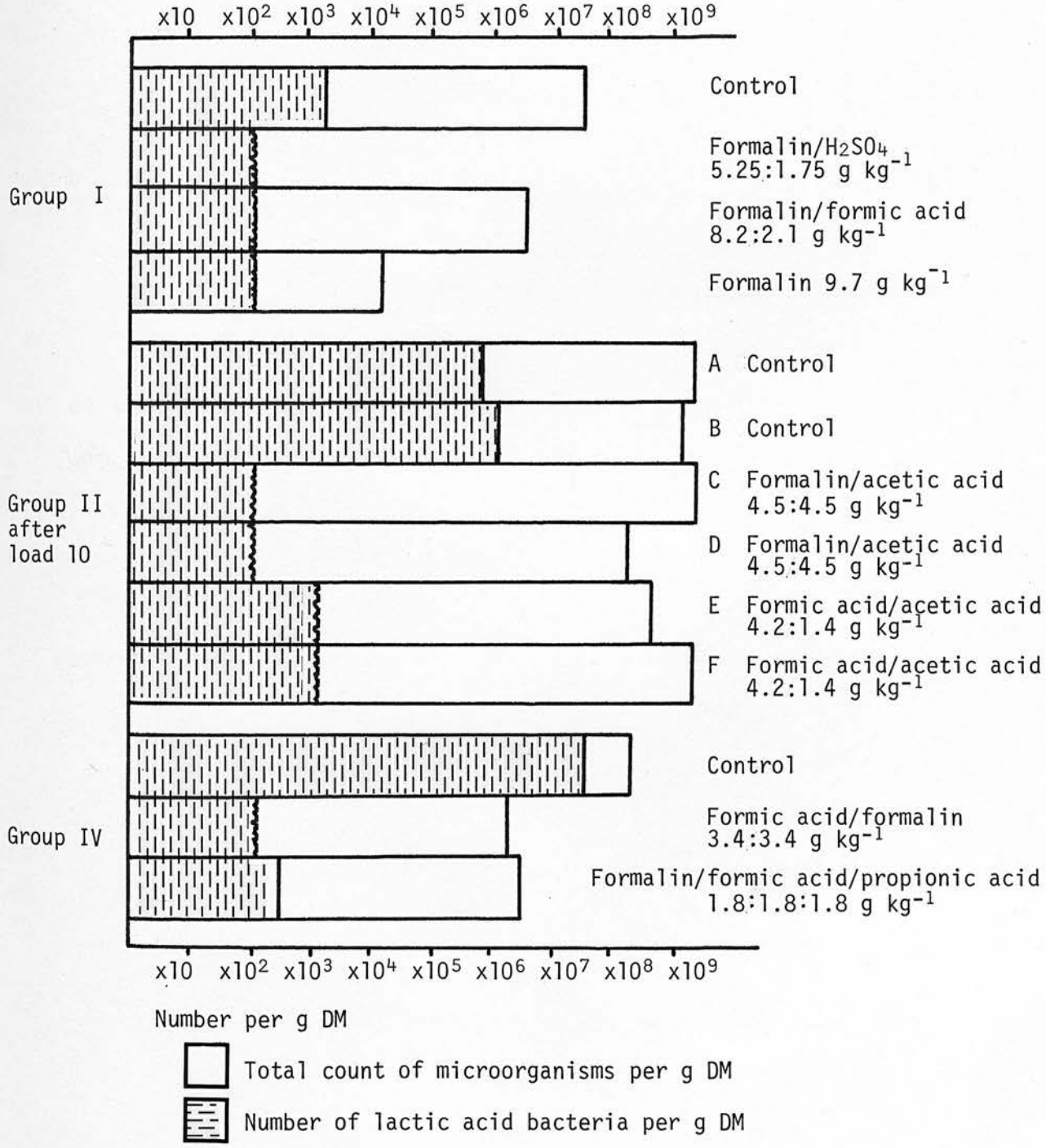


FIGURE 10 The effect of the application of additive mixtures at forage harvesting on the microflora of the grass being loaded into the silo.

and 3.0×10^5 per g DM. Formic acid/acetic acid treatment had a similar effect on the lactic acid bacteria population. (See Figure 10.)

The yeast counts, however, show that the yeast population was not affected to the same extent by the application of the two mixtures. Though the control A showed yeast counts of less than 100 per g DM, this silo was filled first and consequently there had been no time for the population to develop. When the formalin/acetic acid mixture was applied to the grass, the yeast count on the grass being loaded into silo D was 10^5 per g DM compared with 10^6 per g DM in silos E and F the formic/acetic acid mixture, and 10^6 per g DM in the control silo B.

The additive mixtures were sprayed on to forage harvested grass and were not applied at the time of forage harvesting as were the additives used in the other silage experiments. Consequently the additives of Group II were not in contact with the grass for such a long period before sampling as were the others. Nevertheless they had a marked inhibitory effect on lactic acid bacteria, though the total counts were not so affected.

In Group IV silages, the application of the formalin/formic acid mixture reduced the total count after harvesting, and no increase in total count and number of lactic acid bacteria was observed from swath to silo. When the formalin/formic acid/propionic acid mixture was used, there was a slight increase in lactic acid bacteria by the time the grass had reached the silo (4.8×10^3 per g DM). In neither of these treatments was yeast detected above 100 per g DM.

The effect of additive mixtures on the development of the microflora of grass harvested for ensiling is summarised in Figure 10.

Comparing the effect of formic acid and mixtures containing formalin as silage additives at the harvesting and ensiling stages, it would appear that formic acid exerts a specific inhibitory effect on the organisms which initiate the fermentation and by immediately lowering the pH in the silo, it also achieves conditions normally attained by fermentation with the conservation of a proportion of the available sugars. Formalin does not change the pH of the harvested crop and the effect of this additive applied at high levels is to sterilise the grass to be ensiled. Acid mixtures containing formalin at lower levels combine the effects of pH reduction and inhibition of the microorganisms.

In Group I, the unloaded silages all had total counts in the region of 10^7 - 10^8 per g DM for the bulked samples (bulked samples were not taken for the formalin treatment). The formalin/formic acid treatment had the lowest range of lactic acid bacteria counts (excluding the formalin treatment) and this is reflected in a high level of WSC, 13.3%, coupled with a final pH of 5.05. The yeast counts for the bulked samples from the formalin/formic acid treatment ranged from 1.3×10^5 to 5.2×10^6 per g DM compared with a range of 5.5×10^3 to 3.4×10^4 per g DM for the formalin/H_2SO_4 treatment and the wider range of 8.0×10^4 - 8.0×10^7 for the control. Formalin treated silage showed a lower range of total count, 6.3×10^5 - 2.5×10^7 per g DM, lower lactic acid bacteria numbers and lower yeast counts all indicating that fermentation had been suppressed in the silo. (See Figure 11.)

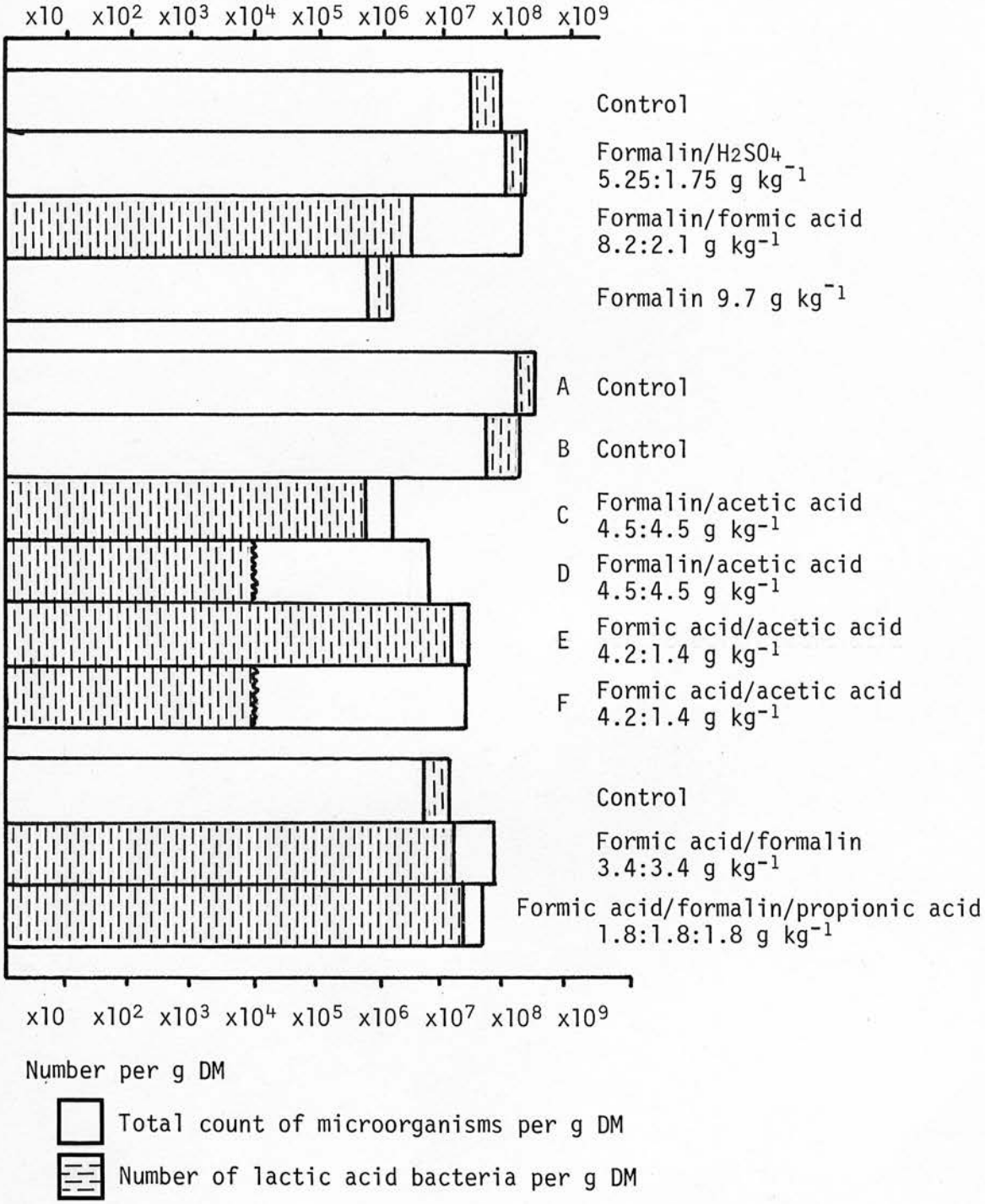


FIGURE 11 The effect of the application of additive mixtures at ensiling on the microflora of the silage so produced.

The unloaded silages of Group II were examined in layers as the silos were being opened. The control silage had counts in the range 8.2×10^7 to 4.0×10^8 per g DM while the formalin/acetic acid treated silage had total counts in the range 1.4×10^6 - 1.5×10^7 per g DM. In the control and formalin/acetic acid treatments, the numbers of lactic acid bacteria were in the ranges 8.7×10^6- 8.2×10^7 and $<10^4$ - 2.4×10^7 per g DM respectively. (See Figure 11.)

The silages of Group IV showed little difference between total counts, the control being lower than the formic acid/formalin treatment and the formic acid/formalin/propionic acid treatments. (7.4×10^6, 9.9×10^7 and 5.2×10^7 per g DM respectively.) The numbers of lactic acid bacteria were all similar, in the region of 10^7 per g DM but the yeast count was lower in the control compared with the two additive treatments. Pedersen et al. (1973) found that formalin/formic acid treated silage had a similar final total count to the control silage, but the coliforms showed a marked increase. Butyric acid bacteria were found more frequently in formalin/formic acid treated silage. (A comparison of the total counts and numbers of lactic acid bacteria for the additive treatments discussed is shown in Figure 11.)

Gibson and Stirling (1959) were of the opinion that bacteriological analysis of mature silage was not a good index of silage fermentation quality since lactic acid bacteria could dominate some silages and be absent in others irrespective of quality. Analysis of additive treated silages has shown that such silages have different microbial profiles from the control silages. With reference to Figures 9 and 11 it can be seen that formic acid treated silages have lower lactic acid bacteria counts than their respective controls, though there is

not such a marked difference in total count. This was also observed by Taylor and Phillips (1970) and Pedersen et al. (1973). (See Table 13.) Additive mixtures containing formalin do not show such a specific effect though formalin treatment itself depresses both total and lactic acid bacteria counts.

The silos used for Groups I and II were of the bag type and there was a stratification effect seen for yeast counts for samples taken at different levels from the silos. (See Figure 12.) The yeast count at the silage surface was higher than that for the middle or lower layers in all cases except Group II, silo C, formalin/acetic acid treatment. This stratification of the yeast population reflects the degree of anaerobiosis achieved in the silo, the layers of silage near the top of the silo being less anaerobic than the bottom layers. Thus the ideal silo is one which has a very small surface area, thus reducing the aerobic layers of silage and therefore reducing the stratification effect which was observed in both additive treated and control silages.

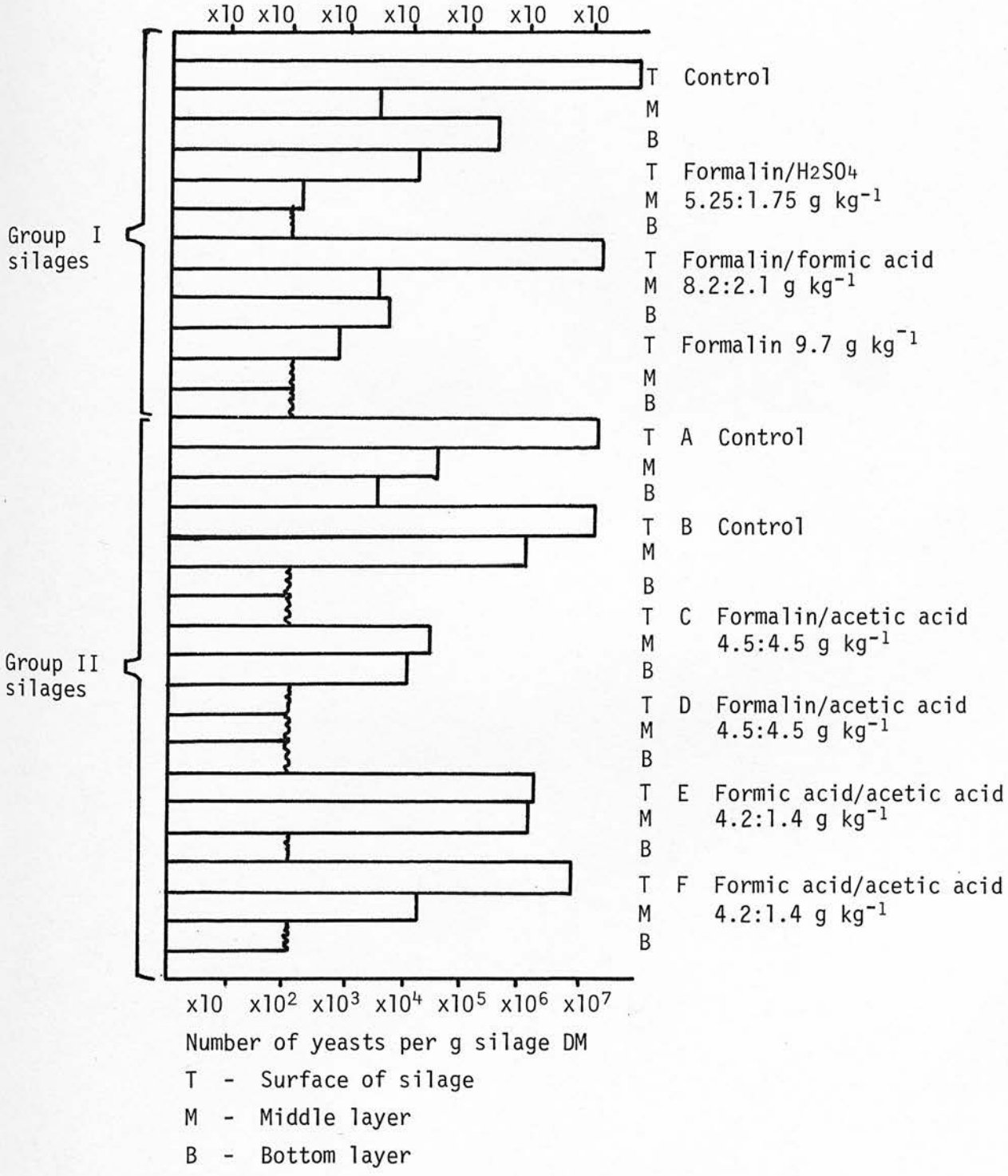


FIGURE 12 Stratification of yeasts within the silo (silos used - plastolene bag silos, see Methods).

SECTION C

AEROBIC DETERIORATION STUDIES

AEROBIC DETERIORATION STUDIES

Introduction

Silage, when it is removed from the silo, is subject to a change in environment from the anaerobic stable situation to a more aerobic state. Thus anaerobic conditions, the prime objective of the silage-making process, are removed. In this state, silage may be susceptible to the so-called "after-fermentation" or "after-heating" defined by Daniel, Honig, Weise and Zimmer (1970) as aerobic metabolism of lactic acid and residual sugar in silage by yeasts. This leads to a temperature rise in the silage which is dependent on the specific heat of the material. The pH of the silage also rises. Gross and Beck (1970) have stated that all silages with high contents of lactic acid or residual sugar are susceptible to yeast attack on unloading while acetic and butyric acid rich silages are at very little risk.

An essential part, therefore, of an additive screening programme is the assessment of the stability (or keeping properties) of silage as it is unloaded from the silo and exposed to aerobic conditions. A rapid method of assessing silage stability is to monitor the temperature changes in silage over the period it is exposed to air. This has been successfully done by Cook (1973) in assessing the effect of additives Pro-sil* and propionic acid on the stability of maize silage. Both additives improved the stability and reduced the temperature increase in the unloaded maize. Temperature change as a measure of silage stability and extent of yeast activity in unloaded silage has also been used by Schukking (1972) and Ohyama and Masaki (1971).

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* Pro-sil - a suspension of minerals and ammonia in molasses.

Accordingly apparatus was developed to enable the temperature change in 14 silage samples to be simultaneously and continuously monitored and compared with ambient temperature or a selected reference temperature. This apparatus was called the Thermolog.

The Thermolog

The Thermolog is a recording thermistor thermometer with 14 thermistors mounted on electrical leads attached to the control panel by means of plugs and sockets. Each thermistor can measure absolute temperatures by comparison with a fixed resistor at one of a series of values (10°C, 20°C, 30°C and 40°C). Each thermistor can also measure differential temperatures from ambient by comparison with a reference thermistor measuring ambient temperature. In the differential mode, fluctuations in ambient temperature are automatically damped.

Each thermistor can be selected manually or automatically. Edge operated digital switches are used for manual selection of thermistors. When the Thermolog is used automatically, a programmable drum selector operates a series of microswitches in sequence. Between readings, the meter is grounded at zero.

The thermistor readings of temperature were measured on a meter on the control panel of the Thermolog and on an associated chart recorder. Temperatures were logged on a single channel recorder by means of a striker bar and pressure sensitive paper. A typical recording cycle consisted of 14 temperature readings each separated by a pulse at zero, a reading of ambient (reference) temperature and a

pulse to indicate the end of the cycle. It took 4 hours to complete a set of readings and each cycle was recorded on a digital counter on the panel of the Thermolog. Full details of the theoretical background, construction and maintenance of the Thermolog are given in Appendix 3 and Plates 1 and 2 show the Thermolog apparatus and the Control Panel respectively.

Silage deterioration studies

The examination of the effect of an aerobic storage period on the unloaded silage was divided into two parts. The first part consisted of the temperature profiles of unloaded additive treated silages in Groups I, II and III (described in Section B and Appendix 2) and the second part was a more detailed consideration of the deterioration processes in silages of Group IV (described in Section B and Appendix 2) including temperature profiles and daily monitoring of chemical and microbiological changes in the silage over the period it was exposed to air.

Experimental

1. Temperature measurement. The Thermolog apparatus was used to monitor temperature changes in silages of Groups I, II, III and IV over a 120 hour aerobic storage period. The silages were placed in 500 g amounts in polystyrene boxes and thermistors were placed in the centre of the sample, one per box. The external dimensions of the polystyrene containers were:- width 15 cm; length 23 cm; depth 7 cm; thickness of polystyrene walls 1.5 cm. The samples were covered by a polystyrene lid of similar dimensions (see Plate 1). Reference (ambient) temperature was measured in a polystyrene box filled with polystyrene granules to reduce the cooling effects of air currents.

2. Silages. Silages of Groups I, II, III and IV were examined for stability on exposure to air. Detailed compositions are given in Appendix 2. Yeast counts for each of the silages were abstracted from microbiological data reported in Section B.

3. Microbial changes in Group IV silages. Silages of Group IV were examined over an aerobic storage period of 96 hours from unloading to monitor the successive changes in the respective microflorae. Each silage was sampled every 24 hours including the time of unloading. Approximately 100 g silage was aseptically sampled daily from each silage monitored, the sample being a bulk sample from 7 replicate polystyrene boxes containing silage which were simultaneously being monitored in the Thermolog. Each sample was examined for total number of microorganisms, number of lactic acid bacteria and number of yeasts using Yeast Extract Agar, Tween Acetate Agar and acidified Malt Extract Agar respectively.

For a more detailed examination of the components of the microflorae of Group IV silages, 25 colonies were picked at random from the total count plates for each silage on each day of the deterioration period. A grid was marked on the back of the Petri dish, each square in the grid was numbered in sequence, and all colonies in a successive series of squares, selected by random number tables, were subcultured into Yeast Extract Broth and incubated at 30°C for 3 days. The isolates were then subcultured on to Yeast Extract Agar, incubated at 30°C for 5 days and examined for purity. Preliminary examination of colony morphology, microscopal appearance, Gram reaction and catalase production was done at this stage. On this basis, the isolates were divided into groups, lactic acid bacteria (Gram + rods or cocci, catalase -), yeasts (colony morphology, microscopal appearance), Bacillus species (sporeforming rods) and other isolates.

To maintain the isolates prior to more detailed characterisation, lactic acid bacteria were transferred to Cooked Meat Medium, yeasts were subcultured to Wort Agar slopes, Bacillus species were subcultured on Nutrient Agar slopes and other isolates were maintained on Yeast Extract Agar slopes.

Lactic acid bacteria were identified according to the scheme of Sharpe, Fryer and Smith (1966). The yeasts were identified using the morphological and biochemical keys of Beech, Davenport, Goswell and Burnett (1968). Bacillus strains were identified using the scheme outlined by Wolfe and Barker (1968) and incorporating the key of Knight and Proom (1950). (Details of identification methods are described in Methods.)

Yeasts were tested for their ability to assimilate lactate and acetate using the auxanographic method of Smith (1969). The growth temperature range of the isolates was recorded by incubating the yeasts at 0°C, 10°C, 20°C, 30°C and 37°C for periods up to 4 weeks on Wort Agar and recording the growth or no-growth response.

4. Chemical changes in Group IV silages. In Group IV every 24 hours over the 96 hour deterioration period, the pH and buffering capacity of the samples were determined on the same bulk sample used for the microbiological analysis.

Results

1. Temperature profiles of silages on exposure to aerobic storage conditions

Silages of Groups I, II and III were unloaded from their respective silos and subjected to a storage period of 120 hours in aerobic conditions. The temperature changes of the silage samples compared with ambient temperature were continuously monitored over the storage period in the Thermolog. The compositions of the silages as unloaded at time zero (DM, pH, BC, WSC, lactic acid and yeast count) are reported in Table 14, along with the mean temperature differences from ambient over the storage period and the corresponding ambient temperatures.

2. Temperature, chemical and microbiological changes in Group IV silages over a 96 hour aerobic storage period

Silages of Group IV had the following treatments:- Control, formic acid 5.3 g kg^{-1}, formic acid, formalin (1:1) mixture 7.6 g kg^{-1} and formic acid, formalin and propionic acid (1:1:1) mixture 5.7 g kg^{-1}. The silages were monitored over a 96 hour aerobic storage period for temperature change. Chemical and microbiological analyses were done on a bulk sample taken every 24 hours from the time of unloading. The temperature changes, pH, buffering capacity and microbiological data are shown in Table 15. Details of silage compositions are given in Appendix 2.

Table

The composition of silages of Groups I, II and III and

		Silage composition						
	Additive Treatment	Application rate in g kg^{-1}	% DM_t	pH	BC in m. equiv. per 100 g DM	% WSC in DM	% Lactic acid in DM	Yeast count per g DM
I	Control	-	21.38	4.10	96	4.1	6.5	2.3×10^5
	Formalin/H_2SO_4	5.25/1.75	21.75	3.98	82	6.4	10.6	1.7×10^4
	Formalin/Formic	8.2 /2.1	21.15	5.05	56	13.3	3.8	1.7×10^6
	Formalin	9.7	21.33	4.90	64	15.1	1.6	$< 10^3$
II	Control	-	19.41	4.08	114	2.85	ND	3.5×10^4
	Formalin/Acetic	4.5/4.5	20.17	4.57	86	14.28	ND	$< 10^2$
	Formic/Acetic	4.2/1.4	19.44	4.16	83	11.82	ND	1.5×10^6
III	Control	-	20.90	3.80	111	2.24	8.4	7.5×10^3
	Formic	3.6	21.40	3.85	102	4.50	3.6	$< 10^2$
	Wilted	-	32.50	4.19	85	6.84	4.6	$< 10^2$
	Formic	6.3	33.10	4.19	73	19.6	3.9	$< 10^2$

* Ambient Temperature in °C.

† Thermolog malfunction

14

temperature changes over a 120 hour aerobic storage period

Temperature profile					
Mean temperature change from ambient: Confidence limits shown at P = 0.05					
Time in hours from unloading					
0	24	48	72	96	120
0 ± 0	1.5 ± 1.2	1.5 ± 1.1	2.0 ± 1.7	3.2 ± 2.6	3.2 ± 2.7
0 ± 0	3.0 ± 1.5	4.7 ± 2.1	9.7 ± 3.8	13.7 ± 1.3	13.3 ± 1.8
0 ± 0	2.0 ± 0.6	4.0 ± 2.1	6.7 ± 3.1	10.9 ± 1.7	12.3 ± 1.8
0 ± 0	3.5 ± 1.1	13.5 ± 1.5	7.3 ± 0.6	10.1 ± 2.9	11.2 ± 1.7
19.5	18.5	20.0	20.5	20.0	20.0 *
0 ± 0	-1.1 ± 1.0	1.0 ± 0.5	0.5 ± 0.2	1.8 ± 1.1	3.1 ± 1.2
0 ± 0	0 ± 0	0.6 ± 0.3	0.2 ± 0.1	0.8 ± 0.5	1.6 ± 0.5
0 ± 0	0 ± 0	1.6 ± 1.1	1.0 ± 0.4	1.8 ± 0.6	2.1 ± 0.7
18.5	17.6	17.6	17.8	17.9	18.1 *
6.0 ± 2.6	17.1 ± 3.3	18.8 ± 3.2	13.9 ± 2.3	- †	12.8 ± 2.0
0 ± 0	1.6 ± 0.5	4.3 ± 1.2	20.6 ± 1.9	-	15.2 ± 2.9
4.2 ± 2.9	14.7 ± 5.6	23.9 ± 6.8	21.4 ± 5.9	-	23.4 ± 3.1
0.6 ± 1.5	5.8 ± 4.0	7.8 ± 5.0	14.4 ± 6.7	-	21.7 ± 3.3
17.8	16.2	16.0	16.1	-	20.3 *

Temperature readings given as mean values

Group I - 3 replicates
Group II - 4 replicates
Group III - 6 replicates

Table 15

The changes in temperature, microbial flora, pH and BC of Group IV silages measured over a 96 hour aerobic storage period

Treatment	Time in h from unloading	Temp. * increase from ambient	Chemical			Microbiological †		
			pH	% DM_o	BC m equiv/ 100 g DM	Total count	Lactic acid bacteria	Yeasts
Control	0	0 ± 0.2	4.3	30.5	67.9	1.6×10^7	1.6×10^7	3.7×10^5
	24	0 ± 0.4	4.3	32.5	70.0	4.0×10^6	4.0×10^6	2.2×10^4
	48	0 ± 0.3	4.3	35.0	52.6	1.6×10^7	1.6×10^7	2.7×10^4
	72	0 ± 0.4	ND	ND	ND	ND	ND	ND
	96	0.2 ± 0.2	4.4	41.2	65.9	1.9×10^5	1.9×10^5	5.5×10^4
Formic	0	3.4 ± 0.1	4.6	30.5	44.6	3.1×10^7	1.3×10^7	1.5×10^6
acid	24	5.0 ± 0.8	4.8	36.9	36.4	1.4×10^8	9.8×10^6	3.8×10^6
5.3 g kg^{-1}	48	5.6 ± 0.6	6.0	38.4	38.3	2.3×10^8	1.4×10^7	7.3×10^7
	72	6.3 ± 0.6	ND	ND	ND	ND	ND	ND
	96	8.2 ± 0.7	5.3	39.1	32.7	1.7×10^8	7.6×10^6	5.6×10^6
Formic	0	3.6 ± 0.3	4.6	33.1	37.8	9.3×10^7	2.5×10^7	2.0×10^6
acid +	24	3.7 ± 0.6	4.9	39.3	35.6	7.1×10^6	5.4×10^6	1.3×10^6
Formalin	48	4.9 ± 0.2	5.3	37.5	43.2	3.2×10^7	3.5×10^6	5.9×10^6
7.6 g kg^{-1}	72	6.2 ± 0.6	5.8	37.1	33.2	4.9×10^7	4.3×10^6	1.9×10^7
1:1	96	5.7 ± 0.7	5.9	47.3	43.3	2.3×10^8	3.2×10^6	3.8×10^7
Formic acid	0	0.7 ± 0.6	4.7	34.5	45.5	4.9×10^7	3.4×10^7	5.5×10^6
+ Formalin	24	0.1 ± 1.1	4.6	33.9	45.7	8.6×10^8	5.7×10^6	2.8×10^8
+ Propionic	48	1.4 ± 1.6	5.7	33.5	31.0	8.1×10^8	5.4×10^6	2.5×10^8
acid	72	1.5 ± 1.4	7.9	35.0	13.7	1.3×10^9	3.2×10^6	1.3×10^9
5.7 g kg^{-1}	96	1.6 ± 1.6	6.0	49.0	30.4	9.4×10^8	1.3×10^5	1.5×10^9
1:1:1								

* Temperature increase from ambient expressed as the mean of 7 replicates. Confidence limits at P = 0.05 given.

† Microbiological counts expressed as numbers per gram silage DM.

ND Not determined.

3. Identification of microorganisms isolated from Group IV silages over 96 hour aerobic storage period

During the 96 hour aerobic storage period, 25 isolates were made daily from each of the silages. The microorganisms were isolated from the total count plates by a random method. The microorganisms isolated and identified from Group IV silages are shown in Tables 16, 17, 18 and 19.

Table 16

Number of each type of microorganism isolated from control silage over 96 hour aerobic storage period

Time after unloading in hours	Number of isolates *				
	1	2	3	4	5
0	24	1	0	0	0
24	21	0	3	0	1
48	15	4	1	0	5
96	12	6	0	4	3
Total	72	11	4	4	9
Per cent †	79	12	4	4	-

* Isolates: 1. Lactobacillus plantarum 2. Bacillus species
3. Pediococcus cerevisiae 4. Yeast species
5. Unidentified isolates

† Per cent of the identified isolates

Table 17

Number of species of microorganism isolated from formic acid treated silage over 96 hour aerobic storage period

Time in hours after unloading	Isolate *										
	1	2	3	4	5	6	7	8	9	10	11
0	7	0	0	2	0	0	0	0	1	6	9
24	0	8	1	5	0	0	2	1	0	3	5
48	1	0	10	2	2	5	0	0	0	3	2
96	0	0	16	7	0	0	0	0	0	2	0
Total	8	8	27	16	2	5	2	1	1	14	16
Per cent †	10	10	32	19	2	6	2	1	1	16	-

* Isolates: 1. Lactic acid bacteria 2. Lactobacillus plantarum
3. Bacillus species 4. Hansenula anomila
5. Hansenula subpelliculosa 6. Brettanomyces bruxellensis
7. Candida tropicalis 8. Candida pseudotropicalis
9. Torulopsis candida 10. Yeast species
11. Unidentified isolates

† Percent of the identified isolates

Table 18

Number of species of microorganism isolated from silage treated with formic acid/formalin over 96 hour aerobic storage period

Time in hours after unloading	Isolates*													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	17	1	5	1	0	0	0	0	0	0	0	0	1	0
24	0	0	3	0	0	0	1	0	0	0	0	0	9	12
48	1	0	1	2	3	1	0	1	0	1	0	0	3	12
72	3	1	0	1	5	0	0	1	1	0	1	1	5	6
96	0	0	3	0	0	0	0	0	0	0	0	0	3	19
Total	21	2	12	4	8	1	1	2	1	1	1	1	21	49
Per cent †	20	2	12	4	8	1	1	2	1	1	1	1	-	47

* Isolates: 1. G+, catalase +, fermenting cocci 2. Bacillus brevis
3. Bacillus pumilis 4. Bacillus species
5. Hansenula anomila 6. Hansenula mrakii
7. Hansenula minuta 8. Brettanomyces bruxellensis
9. Candida melinii 10. Hansenula subpelliculosa
11. Torulopsis candida 12. Torulopsis ernabii
13. Unidentified isolates 14. Yeast species

† Per cent of the identified isolates

Table 19

Number of each type of organism isolated from formic acid/formalin/ propionic acid treated silage over 96 hour aerobic storage period

Time in hours from unloading	Isolates *											
	1	2	3	4	5	6	7	8	9	10	11	12
0	0	2	14	1	0	1	0	0	0	0	0	7
24	2	0	8	0	2	2	1	0	0	0	0	10
48	0	2	16	0	0	0	0	0	0	0	0	7
72	0	0	0	0	0	3	0	1	1	1	1	18
96	0	1	10	4	0	5	0	0	0	0	0	5
Total	2	5	48	5	2	11	1	1	1	1	1	47
Per cent †	3	6	62	6	3	14	1	1	1	1	1	-

* Isolates: 1. g + fermentative catalase + cocci 2. Bacillus brevis
3. Bacillus pumilis 4. Bacillus subtilis
5. Bacillus lichenformis 6. Hansenula anomila
7. Candida intermedia 8. Brettanomyces bruxellensis
9. Torulopsis ernabii 10. Torulopsis etchellisii
11. Torulopsis versitalis 12. Unidentified isolates

† Per cent of identified isolates

4. Assimilation of lactic acid and acetic acid by yeasts isolated from Group IV silages

Yeasts isolated from the additive treated silages of Group IV were tested for their ability to use lactic acid and acetic acid as sole source of carbon. Of 79 yeast strains tested, 76 could use lactic acid as sole carbon source. Similarly 26 out of 79 yeasts could use acetate as sole carbon source.

5. Growth temperature range of yeasts isolated from Group IV silage

Yeasts isolated from Group IV additive treated silages were tested for growth at 0°C, 10°C, 20°C, 30°C and 37°C on Wort Agar. Results were scored as growth or no growth (+ or -) and the tests were duplicated at different times. Table 20 shows the growth temperature ranges of 106 yeasts tested.

Table 20

Growth temperature ranges of yeasts isolated from Group IV silages

Temperature in °C	Growth Response	
	+	-
0	3	103
10	104	2
20	105	1
30	106	0
37	45	61

Discussion

The processes which can occur under aerobic conditions, when silage is removed from the silo, and are characterised by increased temperature of the silage have been referred to by Daniel et al. (1970) and Gross and Beck (1970) as "after fermentation" or "after heating". The term "secondary fermentation" has also been used in this context, but conventionally is used to describe clostridial fermentation during

the ensiling period. Since the silage is exposed to air, the degradation process is oxidative and so the application of the term "fermentation" is incorrect. Instead, the term "aerobic deterioration" is preferred and has been used subsequently in this context.

Aerobic deterioration of silage is rapid involving high nutrient losses, but the nature of the process has been little investigated. According to Gross and Beck (1970) nutrient losses can be of the order of 20-30% within a few days of exposure to aerobic conditions. A distinction may be drawn between surface waste and aerobic deterioration of silage, though both are caused by access of air to the silage. The former is essentially a localised area of waste silage caused by access of air through inadequate sealing of the silo, and the amount of waste is limited by the extent of air penetration, whereas the latter is a rapid breakdown of silage components after the silage has been removed from the silo.

Gross and Beck (1970) concluded that the nutrient energy of silage was being converted into heat energy by the action of yeasts on lactic acid and the residual carbohydrates in silage and this was the cause of the aerobic deterioration process. The potential heat energy available in silage can be calculated assuming complete oxidation of residual WSC and lactic acid. The calorific values of glucose and lactic acid were assumed to be 673 and 326 k cal per mole (McDonald and Whittenbury, 1973). The available heat energy in 1 kg silage was calculated as follows.

$$H_A = \frac{D}{10}\left(\frac{673}{180} \times \%\ \text{WSC in DM} + \frac{326}{90} + \%\ \text{lactic acid in DM}\right) \quad \text{-----} \quad (1)$$

Where H_A = Available heat energy in 1 kg silage in k cal.

D = Dry matter of silage.

For example H_A for Group I Control silage is calculated thus:-

$$H_A = \frac{21.4}{10}\left(\frac{673}{180} \times 4.1 + \frac{326}{90} \times 6.5\right)$$

$$= 83.2 \text{ k cal.}$$

The amount of energy required to raise the temperature of the silage mass can be calculated from the following equation.

$$H_T = \Delta TS \quad \text{-----} \quad (2)$$

Where H_T = number of k cal required to raise the temperature of 1 kg silage by T C^o

ΔT = total temperature increase over aerobic storage period

S = specific heat of silage (fresh weight).

The specific heat of herbage dry matter is 0.45 $cal^{-1}\ g^{-1}\ deg^{-1}$ (McDonald and Whittenbury, 1973).

$\therefore$ S = 1-0.0055 D

Where D = dry matter silage as % fresh weight

$\therefore$ $H_T = \Delta T\ (1-0.0055\ D)$

For example the number of k cal required to raise the temperature of 1 kg Group I control silage through 3.2 C^o is calculated as 2.8 k cal.

Table 21 shows the potential heat energy available in, and the number of k cal required to raise the temperature of 1 kg silage.

Table 21

The available amount of heat energy in k cal in 1 kg silage assuming complete oxidation of residual WSC and lactic acid compared with the number of k cal required to raise the temperature of 1 kg silage through T C^{o}

Silage Treatment	Available amount of heat energy in k cal kg^{-1} H_A	Amount of energy in k cal required to raise the temp of 1 kg silage H_T
Control	83.1	2.8
Formalin/H_2SO_4	135.5	12.1
Formalin/formic	134.3	10.8
Formalin	149.8	11.9
Control	20.7	3.8
Formalin/acetic	107.7	1.4
Formic/acetic	85.9	1.9
Control	81.1	11.3
Formic acid	63.9	18.2
Wilted	137.3	16.2
Wilted + formic acid	289.4	17.3

The amount of heat energy used in raising the temperature of 1 kg silage over the aerobic deterioration period represents approximately 10% of the potential heat energy in the silage assuming complete oxidation of the residual sugar and lactic acid. Loss of heat through imperfect insulation of the experimental system was not taken into account and also as residual WSC and lactic acid were not measured at the end of the aerobic storage period, it was not possible to determine the number of

k cal which were used in the deterioration process and therefore the remaining available energy could not be determined. Thus temperature increase per se cannot be stated to be an absolute measure of silage deterioration under aerobic conditions, though it was considered that it provided a convenient means of comparing the stabilities of silages.

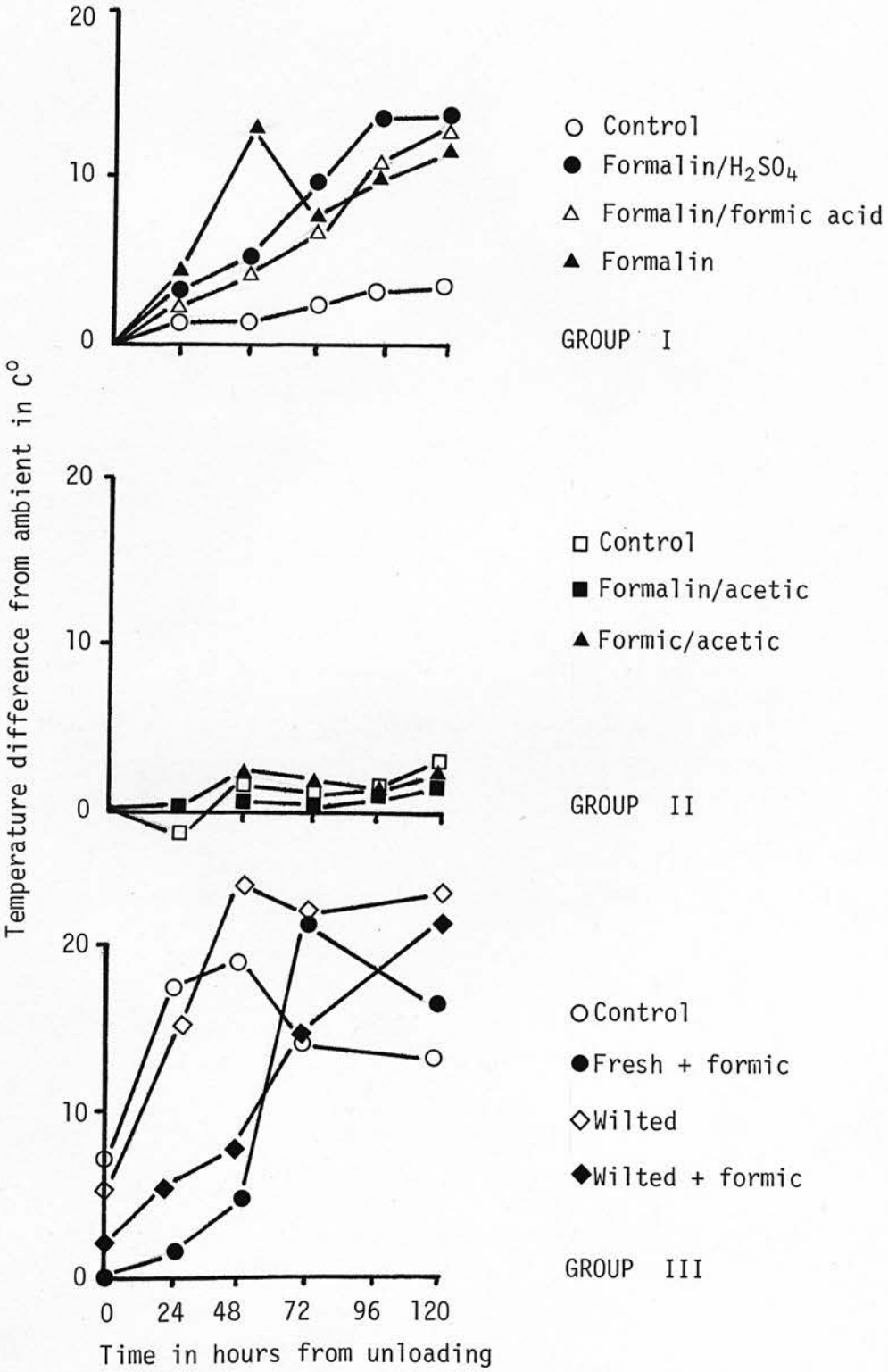
The available amount of heat energy in a wilted silage will tend to be high since the WSC levels of wilted silages are higher than those of unwilted silages and the DM of the silage is high. The wilted, formic acid treated silage of Group III had an available heat energy of 289.4 k cal. According to Gross and Beck (1970) one of the factors influencing silage stability is the specific heat of the silage. However, the extent of aerobic deterioration within a silage is not dependent on the specific heat of the silage per se but on the amount of the available energy sources and the dry matter of the silage. Applying equation (2) $H_T = \Delta T (1-0.0055\ D)$ it can be seen that the number of k cal required to raise the temperature of 1 kg silage through $10C^{o}$ can be calculated as 8.9 k cal for a silage of 20% DM and 8.4 k cal for a silage of 30% DM. However, applying equation (1)

$$H_A = \frac{D}{10}\left(\frac{673}{180} \times \%\ \text{WSC in DM} + \frac{326}{90} \times \%\ \text{lactic acid in DM}\right)$$

the available heat energy in 1 kg silage containing 10% WSC in DM and 1% lactic acid in DM is 82.0 k cal if the DM is 20% and 123.0 k cal if the DM is 30%.

Silages of Groups I, II and III were stored under aerobic conditions for 120 hours after unloading and the temperature changes from ambient of the samples were monitored in the Thermolog. The temperature profiles of each of the silages examined are summarised in Figure 13.

FIGURE 13 Summary of the temperature change from ambient in silages of Groups I, II and III under aerobic storage conditions for 120 hours.



The control silages of Groups I and II remained stable with regard to temperature change over the aerobic storage period. Both controls had undergone lactic acid fermentation since the silages had low pH (4.1), high BC (Group I 96 m equiv per 100 g DM: Group II 114 m equiv per 100 g DM) and low WSC levels (Group I 4.1% DM: Group II 2.85% DM).

The temperature profile of the formalin treated silage of Group I showed an initial rapid temperature increase which reached a peak after 48 hours. Fermentation had been restricted in this silage consequently it had a high pH (4.9), high WSC level (15.1% DM) and a low BC (64 m equiv per 100 g DM).

The combination of formalin with formic acid or sulphuric acid when used as additives at rates of 7.0 and 9.7 g kg^{-1} respectively produced silages with different compositions but with similar temperature profiles. Formalin/sulphuric acid treated silage had a low pH with a high lactic acid content (10.6% DM), 6.4% residual WSC and a BC of 82 m equiv per 100 g DM, while the formalin/formic acid treated silage had a high pH (5.05) with a low lactic acid content (3.8% DM), 13.3% residual WSC and a buffering capacity of 56 m equiv per 100 g DM. The temperature profiles of the silages were similar, showing a steady increase to 13.3 ± 1.8C° and 12.3 ± 1.8C° above ambient at the end of the aerobic storage period.

According to Gross and Beck (1970) silages with high contents of residual sugar or high lactic acid levels are susceptible to aerobic deterioration on exposure to aerobic conditions. The formalin/sulphuric

acid and the formalin/formic acid treated silages both were in this category the former having a high lactic acid content and the latter having a high WSC content. Though the compositions of these silages differed, the available heat energy in 1 kg silage for each of the silages was approximately equal (135.5 and 134.3 k cal respectively).

Inclusion of acetic acid in the additive treatments produced silages which were stable with regard to temperature increase over the 120 hour aerobic storage period. This was in agreement with the results of Gross and Beck (1970) who concluded that acetic acid or butyric acid rich silages were stable on exposure to air.

One of the main disadvantages of formalin treated silages is their instability in aerobic conditions. The formalin treatment of Group I was the most unstable of the silages which included formalin in the additive treatment. The initial temperature peak, not observed with other silages, on the temperature profile of the formalin treated silage was a result of initial rapid oxidative activity within the unloaded silage. According to Daniel et al (1970), silages which have a tendency to deteriorate on unloading have yeast counts above 10^5 per g. The yeast count of the formalin treated silage was below 10^3 per g silage. The findings of Daniel et al. (1970) can therefore not be applied to additive treated silages which may have yeast counts below the critical level of 10^5 per g but may be unstable on exposure to air. In such silages where microbial activity has been restricted there may be a lack of competition for the available energy sources on exposure to air to the benefit of yeasts.

The buffering capacity of a silage may be an indication of its stability. According to Playne and McDonald (1966), the buffering capacity of silage can be attributed to the formation of lactates and acetates. A silage with a low buffering capacity will tend to be unstable on unloading since the acetate level may not be sufficiently high to exert a retarding influence on the aerobic deterioration process. The converse is not however true. A silage with a high buffering capacity may not be stable on exposure to air. Such silages are Group I, formalin/sulphuric acid treatment and all Group III silages. High buffering capacity may be caused to a large extent by the presence of high levels of lactic acid with very small quantities of acetic acid and a silage of this type tends to be unstable.

In Group III, none of the silages was stable with regard to temperature increase during the aerobic storage period. Both control silages, unwilted and wilted showed temperature increases to 17.1 ± 3.3C^{o} and 14.7 ± 5.6C^{o} above ambient within 24 hours of unloading. With both unwilted and wilted silages, the application of formic acid retarded the initial deterioration process until 72 hours from unloading when the temperatures of the silages reached 20.6 ± 1.9C^{o} and 14.4 ± 6.7C^{o} above ambient in the unwilted formic acid treatment and the wilted formic acid treatment respectively. In this case, residual formic acid seems to be retarding the initial temperature increase in both treated silages (Formic acid levels:- control 0.14% DM: wilted, 0.18% DM: unwilted formic acid treated, 0.55% DM: wilted formic acid treated, 1.07% DM). The inhibition of the aerobic deterioration process could not be attributed to the acetic acid contents of the silages since the levels were in fact higher for untreated silages (1.65 and 1.53% DM) than for the formic acid treated silages (0.94 and 0.86% DM).

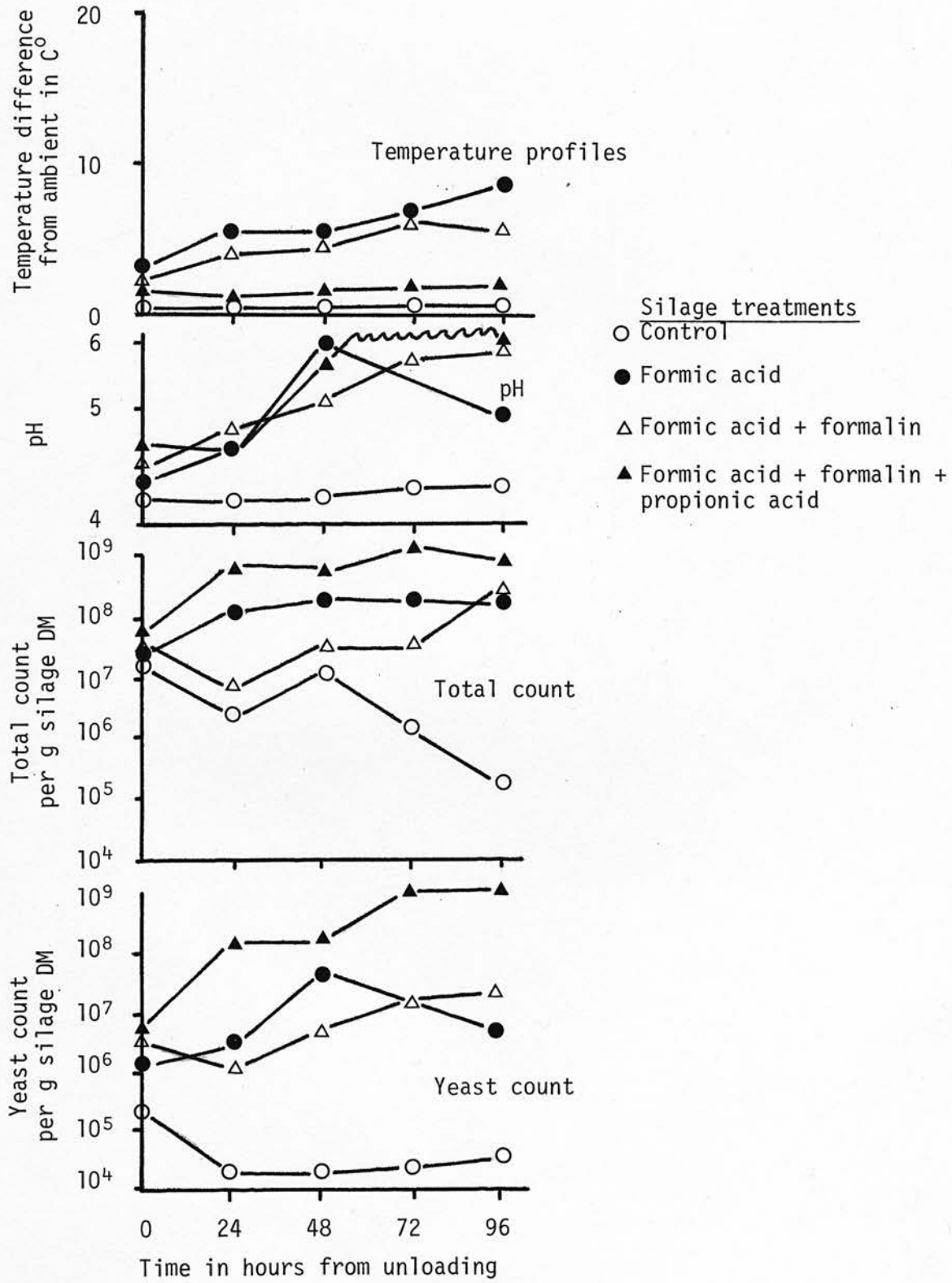
The microbiological changes associated with the aerobic storage period

In an investigation into the prevention of aerobic deterioration processes in maize silage by propionic acid, Gross and Beck (1970) reported two types of yeast were involved, lactic acid and acetic acid assimilating yeasts such as Candida krusei, Pichia fermentans and Hansenula anomila and sedimentary yeasts, primarily of the genus Torulopsis.

The changes in temperature, pH, total count and yeast count in Group IV silages over the 96 hour deterioration period are summarised in Figure 14. Group IV silages were wilted to DM 34% approximately. The control silage was stable with respect to temperature increase over the 96 hour period. This is reflected in the pH values which remained stable within the range 4.3 - 4.4. The total count of microorganisms per g DM tended to decrease over the 96 hour storage period and the yeast population decreased from the time of unloading. It was concluded from these observations that there was little microbial activity in the control silage over the aerobic storage period. This can be verified with reference to the microflora of the silage over the aerobic storage period (Table 16). L. plantarum dominated the microflora of the silage as it was unloaded but after 96 hours it formed only 50% of the microflora. Yeasts were not isolated until the end of the aerobic storage period when the total count had fallen to 1.9×10^5 per g DM and the yeast count was 5.5×10^4.

Compared with the restricted microflora of the control silage, Group IV additive treated silages had more varied populations at opening. The formic acid treated silage and the formic acid/formalin treated silage had similar temperature profiles and were considered to be unstable

FIGURE 14 Summary of changes in temperature pH, total count and yeast count in Group IV silages over 96 hour aerobic storage period.



under aerobic conditions. The total counts of microorganisms in both silages did not decrease as did those of the control silage, but increased from 3.1×10^7 to 1.7×10^8 per g DM in the formic acid treated silage and 9.3×10^7 to 2.3×10^8 per g DM in the formic acid/formalin treated material. The yeast counts in both silages were maintained between 10^6 and 10^7 per g DM over the deterioration period.

Additive treated silages in general have different microbial florae at opening compared with silages made without additives. This factor may influence the pattern of microbial change over the deterioration period and so aerobic deterioration of additive treated silages may not be typical of the aerobic deterioration process in silage made without additives.

The initial microflora of the formic acid treated silage contained 28% lactic acid bacteria and 42% yeast. Over the aerobic storage period of 96 hours, Bacillus species increased to 64% of the total population. Of the yeast species isolated, Hansenula anomila was the most commonly encountered forming 16% of the total identified isolates. H. anomila was also isolated from the formic acid/formalin treated silage forming 8% of the total identified isolates. Burmeister and Hartman (1966) found that the species of yeasts which predominate after the 12th day of ensilage of high moisture corn were H. anomila (66% of the isolates) and Candida krusei (26% of isolates). Lacey (1971) isolated H. anomila from moist barley stored in unsealed silos.

The most readily available energy sources in silage are lactic acid and WSC. The lactic acid levels in both silages were 0.9% and 1.9% DM while the WSC values for the formic acid treated and the formic

acid/formalin treated silages were 15.6 and 15.7% DM respectively. However the majority of yeasts isolated from Group IV silages were able to assimilate lactic acid as sole carbon source.

Bacillus species were isolated from the silages over the aerobic storage period. In the formic acid treated silage, 27% of the identified isolates were Bacillus while in the formic acid/formalin treated silage, Bacillus formed 18% of the identified isolates, the main species isolated being B. pumilis. In pure culture, Bacillus strains were inhibited by an application rate of 4.5 cm^3 kg^{-1} formalin while in formic acid at the same application rate, the growth was not limited (see Section A). Thus the percentage of Bacillus strains isolated from formic acid treated silage was greater than the percentage isolated from formalin/formic acid treated silage. Stirling (1953) isolated B. pumilis from 6 month old silage and stated that it would not be expected to increase in silage, but its capacity for survival through the ensilage process was demonstrated.

The identification scheme here used included amylase production. Since there is little, if any, starch present in grass silage, the possession of the amylase enzyme is not a critical factor for the survival of a particular strain throughout the ensiling period. B. pumilis has been considered to be a variant of B. subtilis which lacked amylase. Stirling (1953) also isolated B. subtilis from silage. Allen, Harrison, Watson and Ferguson (1937) found high levels of B. subtilis in silage taken from large concrete silos.

Bacillus can therefore survive the ensiling process in spore form and may germinate when the silage is unloaded and stored in aerobic

conditions. The contribution of Bacillus to the deterioration process would be largely proteolytic though B. pumilis forms acids from glucose, fructose and sucrose. It is likely that in an aerobic storage situation this contribution to carbohydrate breakdown is not significant compared with the rate of carbohydrate breakdown by yeasts.

The group of microorganisms classified as Gram-positive, catalase-positive fermenting cocci, isolated from the silage treated with formic acid/formalin mixture, are distinguished from the streptococci, pediococci and leuconostocs by the positive catalase reaction. Such Gram-positive, catalase-positive cocci belong to the genus Staphylococcus or the genus Micrococcus. Since the modified Hugh and Leifson test was not used to determine the oxidative/fermentative reaction of the isolates, assignment to one or other of the genera was not possible (Baird-Parker, 1966).

Stirling (1953) found that micrococci did not become the dominant organisms at any stage of the silage fermentation. However catalase-positive cocci were isolated from AIV silage (Cunningham and Smith, 1939). Though the Gram-positive, catalase-positive cocci made up 68% of the identified isolates from the formic acid/formalin treated silage at opening, they were not isolated in such great numbers on subsequent days of the aerobic storage period, since they were rapidly overgrown by yeasts. The importance of Gram-positive, catalase-positive cocci in silage has been little investigated as they are not isolated in great numbers from silages made without additives but the role of such microorganisms in additive treated silage requires further investigation.

Though the formic acid/formalin/propionic acid treated silage was stable with respect to temperature increase, it was highly unstable

with regard to yeast activity over the 96 hour deterioration period. Again H. anomila was the dominant yeast species isolated. The yeast counts were the highest of the four silages examined yet the temperature of the silage remained stable. In this case, the temperature measurements over the deterioration period were not a reliable measure of the stability of the silage. Therefore the recommendation must be made that aerobic deterioration of silage should not be measured by one parameter alone, but by a variety of parameters, the more important being loss of WSC and lactic acid, changes in pH, changes in the microbial flora and temperature changes.

McDonald and Whittenbury (1967) in summarising losses during ensilage have stated that to keep losses down to a minimum, crops of DM above 30% should be ensiled, anaerobic conditions should be maintained throughout. Consequently, the only losses encountered should be those resulting from fermentation and a small amount of respiration. In high dry matter silages, the lactic acid fermentation will be inhibited at a higher pH than if a fresh crop had been ensiled. Therefore, if due attention is paid to keeping the losses in the silo as low as possible, there is a considerable risk that such a silage will be subject to aerobic deterioration with attendant high losses.

On the other hand, McDonald, Henderson and Ralton (1973) have questioned the validity of using dry matter losses in expressing silage efficiency and have suggested that changes in the gross energy might be a more meaningful description. Energy changes in the aerobic deterioration process should be monitored, and detailed investigations into the significance to the animal should be undertaken.

SECTION D

THE SELECTION OF A SUITABLE SILAGE ADDITIVE

THE SELECTION OF A SUITABLE SILAGE ADDITIVE

This concluding experiment was designed to provide information about the most suitable additive or mixture of additives to use in order to obtain a silage of high WSC and low lactic acid contents, and one which was relatively stable on exposure to aerobic conditions. Selection of additives was restricted to the commercially available volatile fatty acids, formic, acetic and propionic acids, and formalin.

Experimental

1. Grass. Italian ryegrass, cut from Botany Plots, Bush House, Midlothian in the morning of 3.9.73 was ensiled in 2.5 kg quantities in plastic bag silos (see Methods). The grass had the following composition:- DM 23.2%; WSC 16.5% in DM; pH 5.98; crude protein 18.6%; cellulose 22.9%; crude fibre 19.1%.

2. Experimental design. Formalin, formic acid, acetic acid and propionic acid were used singly, in pairs and in combinations of three and four at a constant application rate of 4.5 g kg^{-1}, keeping the weights of the proportional parts of the mixtures constant. Each treatment was replicated four times.

3. Chemical analysis of grass and silage. The extent of fermentation in the silages was monitored by measuring the parameters:- pH, DM_o, % WSC in DM, % TSN in TN, % VN in TN.

4. Microbiological analysis. Silages were examined for total viable count and yeast count at unloading by routine methods.

5. Monitor of aerobic deterioration. The Thermolog was used to monitor temperature changes in the additive treated silages over a 96 hour period of aerobic storage after the silage was unloaded from the silo.

6. Period of ensiling. The ensilage period was 161 days.

Results and Discussion

The values of pH, WSC, VN and TSN, temperature change in 36 hours from unloading silage, total count of microorganisms at unloading and total count of yeasts per g silage DM at unloading were recorded. The results were analysed statistically and the standard error of difference between two means was computed. Full results are given in Appendix 4.

Since the aim of the experiment was to produce silage in which fermentation had been restricted, the parameters pH and WSC were examined in detail for each additive treated silage. The parameters VN and TSN as % TN were also measured but as no silages were produced with abnormally high values of either, i.e. the silages did not show extensive signs of proteolysis or deamination, these two parameters are not discussed in such detail. The tables of means for all parameters are given in Table 22.

All additive treatments tested fulfilled one of the criteria for the selection of a suitable silage additive, they all restricted fermentation. All had significantly higher WSC contents than the control (%WSC in DM = 5.1) and with the exception of the silage treated

Table 22

Tables of means and the standard error of difference between two means (SED) for chemical and microbiological parameters monitored

Treatment 4.5 g kg^{-1} fresh wt.	pH	% WSC in DM	% TVN in TN	% TSN in TN	Temp change 36 h C°	Log_e Total count per g DM	Log_e Yeast count per g DM
Control	4.42	5.1	8.5	67	8.87	19.3	6.5
Formalin	4.83	13.3	4.2	50	10.37	15.0	10.7
Formic acid	4.74	14.8	4.1	65	2.44	15.7	7.2
Acetic acid	4.14	8.3	3.9	70	3.17	15.0	5.9
Propionic acid	4.29	10.1	3.7	64	5.13	13.8	10.0
Formalin/formic	4.98	14.1	4.7	47	14.25	17.0	7.2
Formalin/acetic	4.88	15.2	5.6	55	15.74	17.0	6.5
Formalin/propionic	5.03	13.8	5.7	61	7.25	17.1	10.2
Formic/acetic	4.34	14.4	3.9	64	5.38	16.9	4.6
Formic/propionic	4.90	14.1	6.2	62	6.00	14.5	4.6
Acetic/propionic	4.74	13.7	5.8	64	4.17	15.6	4.6
Formalin/formic/acetic	4.87	14.8	5.5	56	3.13	13.1	5.5
Formalin/formic/propionic	4.97	15.6	6.8	57	5.58	14.0	8.4
Formalin/acetic/propionic	5.20	15.0	6.1	65	7.19	18.1	8.2
Formic/acetic/propionic	4.83	16.7	3.9	64	4.33	15.0	4.6
Formalin/formic/acetic/propionic	5.13	14.9	5.1	58	10.56	14.2	5.2
Grand Mean	4.77	13.4	5.2	61	7.10	15.7	6.9
SED	0.17	1.7	1.1	4	3.07	1.8	1.7

with formalin/acetic acid/propionic acid mixture the total counts for the additive treated silages were significantly lower than the total count of the control silage.

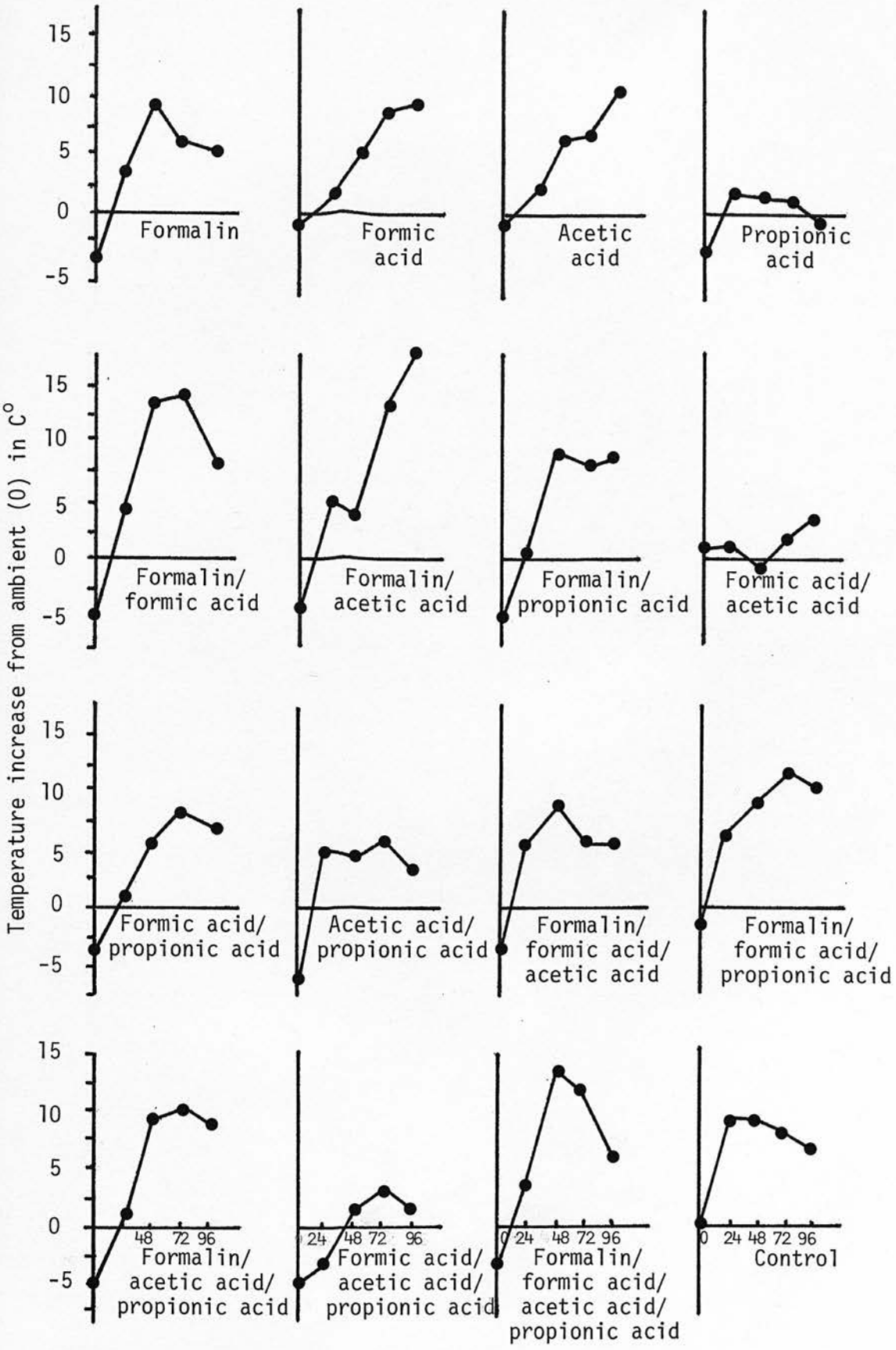
As anticipated from results of previous screening trials, the application of acetic acid alone and propionic acid alone at 4.5 g kg^{-1} resulted in silages with WSC levels which were significantly lower than the WSC contents of the other additive treated silages (8.3% in DM and 10.1% in DM respectively). However, fermentation had been restricted since the WSC contents of the acetic and propionic acid treated silages were significantly higher than that of the control. Neither acetic acid nor propionic acid was effective in inhibiting the growth of lactic acid bacteria when tested in pure culture at a level of application equivalent to 4.5 g kg^{-1} (see Section A). Therefore, the limited restriction of fermentation must be largely attributed to a pH effect. Since the pK_a of acetic acid is 4.64 and the pK_a of propionic acid is 4.70, the application of either of these acids to grass cut for ensiling should lower the pH of the grass to 4.7 approximately. Thus the production of only a small amount of lactic acid should be necessary to lower the pH to a stable level.

There is little practical advantage to be gained by applying acetic acid or propionic acid when results are compared with the addition of similar concentrations of formalin or formic acid. Indeed the value of both formalin and formic acid in restricting fermentation in silage has been well documented. However, when the relative stabilities in aerobic storage conditions of each of the silages were considered, it was seen that formalin treated silage was subject to rapid aerobic deterioration, whereas formic acid, acetic acid and propionic acid treated silages were not subject to initial rapid temperature increase

(see Figure 15). Therefore, although formalin is an effective fermentation inhibitor, its use as a silage additive on its own is not to be recommended from the point of view of the stability of the silage under aerobic storage conditions.

When the efficiency of the additive mixtures was assessed, from the aspect of the silage fermentation, there was no advantage to be gained by combining formic acid with formalin, acetic or propionic acids with respect to WSC retention. There was no significant difference in the WSC contents of each of the silages when compared with the WSC value of formic acid treated silage (WSC 14.8% in DM). However, the importance of establishing whether a silage made with a particular additive is subject to aerobic deterioration was again emphasised for these silages treated with additive mixtures containing formic acid in combination with one of the other additives. Thus formalin/formic acid 1:1 4.5 g kg^{-1} would not be recommended as a silage additive, since although it was effective in the silo in producing high WSC silage, the silage was not aerobically stable (see Figure 15). Similarly the formalin/acetic acid and the formalin/formic acid/acetic acid/propionic acid mixtures were also eliminated as suitable silage additives within the criteria set for the purpose of this experiment. The formic acid/acetic acid treated silage was stable with respect to temperature increase over 72 hours of the aerobic storage period and the silage was more stable than the silages made with addition of either formic acid 4.5 g kg^{-1} or acetic acid 4.5 g kg^{-1}. Original $\log_e$ yeast counts in the silages made with the addition of formic acid and acetic acid were 7.2 and 5.9 respectively whereas in the silage made with formic acid/acetic acid mixture the $\log_e$ yeast count was 4.6 per g DM. Thus the proposal that if the yeast population in a silage was above a certain

FIGURE 15 Temperature Profiles of additive treated silages.



Additives applied at constant rate of 4.5 g kg^{-1} keeping proportional parts of mixtures constant.

critical level, the silage would be subject to aerobic deterioration might be applied to additive treated silages, although the critical yeast content would appear to be lower for additive treated silages than for untreated silages. Daniel, Honig, Weise and Zimmer (1970) suggested that if a silage had a yeast count of above 10^5 yeasts per g (equivalent to 11.5 $\log_e$) it would be aerobically unstable, but this figure was obviously too high for the additive treated silages examined here. The formalin/acetic acid treated silage deteriorated rapidly. From previous results it might have been expected that this silage would have remained stable on exposure to aerobic conditions because of its acetic acid component. However the yeast content of this silage must have been above the critical level discussed above and so the silage deteriorated when opened.

Propionic acid treatment of silage proved an exception. Although the $\log_e$ of the yeast count at unloading was 10.0, the silage remained stable with respect to temperature increase over the aerobic storage period. A similar effect was noted in Group IV silages where the silage treated with formic acid/formalin/propionic acid 1.8:1.8:1.8 g kg^{-1} remained stable with respect to temperature increase, but had high yeast counts (see Section C, p128). It is difficult to offer a reasonable explanation for this anomaly, apparent stability on exposure to aerobic conditions, coupled with high yeast counts at unloading. When yeast is transferred from an anaerobic to an aerobic environment, growth is accelerated while uptake of sugar is diminished, i.e. the Pasteur effect. Sugar is transported into the cell by means of permeases. In yeast, it has been shown that the affinity of glucose for the permease decreases when the yeast is grown aerobically rather than anaerobically. The rate of solute uptake by permeases is decreased with any agent which reacts at the membrane level, (Rose 1968). Propionic acid is thought

to exert its effect here. Thus since the rate of sugar transport profoundly affects the metabolism of the entire organism, it is likely that the rate of transport has been markedly reduced in both aerobic and anaerobic conditions. Under aerobic conditions the rate of permease activity might have been reduced by the propionic acid to such an extent that the yeast population was unable to increase, but in the corresponding anaerobic state, i.e. during the ensiling period, the propionic acid might not have affected the permease activity to such an extent, therefore the yeast population was able to increase.

The additive treatment which had the highest WSC level, 16.7% in DM was formic acid/acetic acid/propionic acid 1:1:1 4.5 g kg^{-1}. This treatment fulfilled all the criteria set in that the silage had a high level of WSC and was stable on exposure to aerobic conditions. The formic acid/acetic acid treatment might also be suitable since the silage was stable when unloaded and also had a high level of WSC - 14.4% in DM. However the pH of this silage was lower than that of the silage treated with formic acid/acetic acid/propionic acid (4.34 and 4.83 respectively). This might be a factor involved in intake of the silage.

The data obtained can be used as a basis for recommending silage additives to fulfill other criteria. For example propionic acid treatment of silage will not affect the course of silage fermentation unduly, but the resulting silage will be remarkably stable under aerobic conditions. Daniel, Honig, Weise and Zimmer (1970) have reported on the use of propionic acid in unloading silage and have found that silages treated with propionic acid at the time of unloading did not deteriorate rapidly when stored aerobically, compared with suitable control silages.

CONCLUDING DISCUSSION

The aim one should try to achieve in ensiling is to prevent fermentation ..... since (it is) the best way to avoid undesirable fermentation.

(O. Hoffart, 1876.)

The purpose of using an additive in silage making has always been to ensure that a clostridial fermentation does not occur. In the past, this has been done in a number of ways including stimulation of lactic acid production and application of acid additives. Indeed the use of the AIV process was based on the fact that proteolytic enzymes are not active below pH 4.0 and AIV acid was applied to reduce the pH of the crop to below this level. Thus clostridial fermentation was inhibited and in fact all microbial fermentation was inhibited.

The AIV process has been recently superseded by the use of organic acids, particularly formic acid, as silage additives. The action of formic acid combined pH reduction of the crop with a selective microbial effect. Coliforms are inhibited by much lower concentrations of formic acid than those required to inhibit the lactic acid bacteria (Woolford, 1974). Since a large proportion of the volatile fatty acids present in a silage which has not undergone clostridial fermentation must have been produced as a result of coliform activity, it follows that a silage made with the inclusion of formic acid will contain large quantities of lactic acid with low levels of other fermentation acids. Such a silage has always been considered to be

a qualitatively "good" silage. However, if the concentration of formic acid is increased, the lactic acid bacteria will be inhibited and the resultant silage will contain a high level of WSC with correspondingly low levels of fermentation acids. A silage treated with a similar level of formalin will also have a high WSC level since formalin inhibits microbial activity within the silo.

Thus the aim proposed by Hoffart can be fulfilled by the use of a suitable additive treatment. Although there can be no argument that restriction of fermentation in the silo is a method of avoiding clostridial fermentation, prevention of fermentation does not guarantee that the silage so produced will be stable when unloaded from the silo and exposed to aerobic conditions.

In 1970, McLeod, Wilkins and Raymond concluded that acids produced during normal silage fermentation could limit intake. They therefore recommended that chemical treatments to conserve the nutrients in grass without the presence or formation of large quantities of acids should be investigated.

It is paradoxical that in striving to improve silage as a feedstuff for animals, silages have been made which may be inherently unstable on exposure to aerobic conditions. As with many foods, departure from traditional processes of preservation may be responsible for spoilage problems which were previously uncommon, (Horner and Anagnostopoulis, 1973).

When an additive is selected, the susceptibility to aerobic deterioration of that silage must be considered. Indeed, additives can be used to tailor silage for particular needs but if such a silage,

however desirable its properties may be, is not stable under aerobic conditions, there is little point in carrying investigations further. It is possible, however, to combine the additive with some compound which will confer stability to the silage. Acetic acid and propionic acid are in this class of silage additive. Neither has a marked effect on the course of silage fermentation when compared with formalin or formic acid. However, the inclusion of acetic acid or propionic acid in an additive generally increases the stability of the silage on exposure to air.

Caution would have to be exercised in the use of acetic acid in view of the suggestion that large amounts of this free acid might depress intake (Hutchinson and Wilkins, 1971). No additive treatment can ever be recommended for use until satisfactory feeding trials have been carried out. The effect of the additive on the rumen microflora should also be assessed using an apparatus such as the Rumenstat (Ewart, 1974).

The technology of silage making is sufficiently advanced that satisfactory silages can be produced. It is possible to manipulate the silage fermentation by judicious use of silage additives, but it is essential that such silages be demonstrated to be nutritionally desirable.

SOME SUGGESTIONS FOR FURTHER STUDY

Although the deterioration process has been investigated in three additive treated silages and their respective control, this is not a suitable base from which to generalise about the aerobic deterioration process. The mechanism of silage deterioration should be thoroughly investigated using silages produced without additive treatment. A full explanation should be sought for the temperature increases in deteriorating silage and it should be established if temperature measurement is a good monitor of the aerobic deterioration process.

The Thermolog is, at present, a restricted piece of apparatus in that there are only 14 temperature probes. This restricts the number of silage samples that can be examined at any one time and since the aerobic deterioration is measured over days rather than hours the limited facility for measuring temperature changes continuously, precludes much replication of samples. Ideally the Thermolog should be of modular construction so that a very large number of temperature probes could be used. It should have provision for portability to enable field work to be done. The data should be logged into a computer to eliminate the reading of the recorder trace and subsequent computations which at present are done manually.

The role of yeasts in the silage fermentation should be fully studied. In the past this area has tended to be overlooked, but in the light of the involvement of yeasts in the aerobic deterioration

process, the importance of these organisms in the silage fermentation should be fully assessed.

The mould microflora of silage has been little investigated. However Penicillium roqueforti is commonly isolated from silage (Smith, 1969). Wei, Still, Smalley, Schnoes and Strong (1973) have extracted a toxin from P. roqueforti isolated from ground mixed grains and corn silage associated with cases of bovine abortion and placental retention. The whole question of toxin production in silage requires further investigation. Fusarium has been isolated from grass loaded into silos and from the corresponding silage at unloading (Mann, unpublished results). Fusarium will grow under conditions of low pH and anaerobiosis as found in a silo.

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APPENDIX 1

SILAGE ADDITIVES IN USE 1949-1970

(Abstracted from:- Silage Additives, Annotated Bibliography 1293(1) and 1293(2), Commonwealth Bureau of Pastures and Field Crops)

	No. of references	Composition
AAZ	4	$HCl + Na_2SO_4$
Acetic acid	1	CH_3COOH
Acetone	1	$(CH_3)\ CO$
Agrocym	4	"A biological product" lactic acid bacteria
AIV	51	Solution of mineral acids
Amasil	7	85% formic acid solution
Amidosulphonic acid	1	
Ammonia solution	12	
Ammonium bicarbonate	1	NH_4HCO_3
Ammonium bisulphate	1	NH_4HSO_4
Ammonium bisulphite	1	NH_4HSO_3
Ammonium chloride	3	NH_4Cl
Ammonium sulphate	4	$(NH_4)_2SO_4$
Amylase	2	
Anthranilic acid	1	$NH_2C_6H_4COOH$
Antibiotics	1	
Arachis oil	1	
Ascorbic acid	1	$OCOC(OH):C(OH)CHCH(OH)CH_2OH$
Atrazine	1	
Avamorin	2	Russian enzyme preparation
Aureomycin	1	
Bacitracin	12	
Bagasse	1	
Baneasa	2	Cellulose/inorganic acid/ macronutrient mix
Barley meal	7	
Beet pulp	9	

	No. of references	Composition
Biomycin	1	
Biuret	3	$NH_2CO\ NH\ CONH_2$
Brewer's grains	2	
Buttermilk	1	
Butyric acid	1	$CH_3CH_2CH_2COOH$
Calcifor	1	
Calcium acetate	1	$CH_3\ [COO]_2\ Ca$
Calcium carbonate	7	$CaCO_3$
Calcium chloride	1	$CaCl_2$
Calcium formate	6	$H(COO)_2\ Ca$
Calcium hypochlorite	1	
Calcium nitrate	3	$Ca(NO_3)_2\ 4H_2O$
Calcium phosphate	1	$Ca_3(PO_4)_2$
Calcium sulphate	1	$CaSO_4$
Captan	1	
Carbonic acid	1	Solid
Carob meal	1	
Cellulose	1	
Cellulolytic enzyme	2	
Cereal grain	1	
Cerelose	2	Maize glucose
Chalk	2	
Chloroform	1	$CHCl_3$
Citric acid	2	$C[OH][COOH][CH_2COOH]_2H_2O$
Citrolas	1	Citrus by-products
Citrus meal	1	
Cyanuric acid	1	$C[OH]:N[COH]:NC[OH]N$
$Ca[H_2PO_4]_2 2CaSO_4$	1	
$Ca[H_2PO_4]_2 2H_2O$	1	
CO_2	4	
DAG salt	1	Sulphamic acid NH_2HSO_3
Dextrin	1	$[C_6H_{10}O_5]_n\ x\ H_2O$
Diammonium phosphate	7	$[NH_4]_2\ HPO_4$
Dicalcium phosphate	2	
Diquat	1	
Dusarit	2	H_2SO_4 absorbed in charcoal, spread as dust

	No. of references	Composition
EKB silosalt	1	
Ensilage phosphate	1	
Ensilan	3	Sodium formate
Ensilan SF2	2	Sodium formate + excess formic acid
Ensilan PQ4	2	Calcium phosphate + sodium nitrate
Ensilit	1	Kofa salt
Ensimalt	1	
Enzyme preparation	1	
Ethanol	2	C_2H_5OH
Formic acid	41	
Formasil	4	
Formalin	5	
Fungal culture	1	
GK powder	1	H_2SO_4 + sawdust
Glucose	6	$O\underline{\underline{[CHOH]_4}}\,CHCH_2OH$
Gluten	3	
Glycollic acid	2	
Grain	2	
Grillosil	1	
Hominy meal	1	
Howden's additive	2	Alkaline liquid, Ca or $NaCO_3$, lactic or benzoic acid or both or salts K or NaCl + maize starch
Hydrochloric acid	4	
HCl + H_2SO_4	2	
HCl + Na_2SO_4	2	
Inoculum	1	
Kofa salt	30	Calcium formate:sodium nitrite 20:3
Kofasil	6	
Kompasil	1	
Konpasil	2	
Kylage	7	Calcium formate + sodium nitrite

	No. of references	Composition
Lactic acid	1	
Lactic acid bacteria	21	
Limestone	12	
Magnesian limestone	1	$MgCO_3$
Magnesium oxide	1	MgO
Maize	18	
Methionine hydroxy analogue	1	$CH_3S[CH_2]_2\ CH[NH_2]\ COOH$
Molasses	70	
M.S.B. Kultur	1	Lactic acid bacteria + sugar
Myosil	2	Formic acid
Molassine meal	1	
Nigrin	1	Russian enzyme preparation
Nisin	2	
Norsil	1	85% Formic acid
Nitrate	3	
Nitrite	2	
Oats	1	
Orizin	1	Russian enzyme preparation
Oleandomycin	1	
Penicillin	2	
Pfeiffer mixture	1	Calcium formate:sodium nitrate 4:1
Phenyl ethyl alcohol	1	$CH_3CH[C_6H_5]OH$
Phosphoric acid	4	HPO_3
Phossilan	4	Phosphoric acid solution 6.7%
Potato	3	
Potassium bisulphite	1	$KHSO_3$
Potassium carbonate	1	K_2CO_3
Potassium metabisulphate	3	$K_2S_2O_5$
Propionic acid	3	$CH_3\ CH_2\ COOH$
Propylene oxide	1	$CH_3\underline{CH\ CH_2O}$
Propylparaben	1	
Protosil	5	H_3PO_4:milled peat:molasses 20:30:50
Pyrosal	2	Sodium pyrosulphate

	No. of references	Composition
Reymersholm's ensiling PO_4	3	$NaH_3[PO_4]_2$ + $NaHSO_4$
Reymersholm's ensiling salt	4	$NaHSO_4$
Rerstorp's ensiling salt	1	Na salts of acetic and formic acids
Rezeptur 2	2	
Rezeptur 3	1	
Rice bran	1	
Silamon	1	$Na_2S_2O_5$ + carbohydrate
Siloferm	2	Lactic acid bacteria
Silofertil	2	
Silosan	1	
Silotex	2	$Na_2S_2O_5$
Silotracin	1	0.22% zinc bacitracin
Sodium bicarbonate	1	$NaHCO_3$
Sodium bisulphate	3	$NaHSO_4$
Sodium bisulphite	12	$NaHSO_3$ (solution of Na metabisulphite)
Sodium carbonate	3	Na_2CO_3
Sodium chloride	12	NaCl
Sodium formate	14	HCOONa
Sodium metabisulphite	78	$Na_2S_2O_5$
Sodium nitrate	1	$NaNO_3$
Sodium nitrite	4	$NaNO_2$
Sodium pyrosulphite	1	$Na_2S_2O_7$
$NaH_5(PO_4)_2$	1	
$NaH_5[PO_4]_2$ $NaHSO_4$	1	
$NaH_5[PO_4]_2$ $2NaHSO_4$	2	
$NaH_5[PO_4]_2$ $3NaHSO_4$	1	
Sopura	1	Ester of monobromeacetic acid
SO_2	18	
Sorbic acid	4	CH_3CH $CHCHCH$ $COOH$
Sovilon	7	Bacteriostatic salt
Soyabean meal	4	
Spurosil	2	62% NaCl
Starch	1	
Steep liquor	1	

	No. of references	Composition
Streptomycin	3	
Sucrose	8	$C_{12}H_{22}O_{11}$
Sugar	20	
Sulphamic acid	2	NH_2SO_3H
Sulphuric acid	1	H_2SO_4
S47	1	
S48	1	
Terramycin	2	
Thiourea	1	NH_2CSNH_2
Trisodium phosphate	1	Na_3PO_4
Tylosin	1	
Ukreasil	1	
Urea	43	NH_2CONH_2
Valeric/isovaleric acid	1	$CH_3CH_2CH_2CH_2COOH/(CH_3)_2CHCH_2COOH$
Vitasan	1	Mono ammonium phosphate
VFA	1	
Walcosil	2	30% Kofa + 70% ground locust beans
Wheat bran	5	
Whey	4	

APPENDIX 2

Compositions of additive treated silages

GROUP I

Ensiled 23.8.72

Harvested from Lower Lambing Field, Boghall Farm

Ensiled in 2 Mg plastolene silos

Treatment	Rate of application g kg^{-1} fresh wt	% DM_t	pH	% WSC in DM	% Lactic acid in DM	BC in m equiv/ 100 g DM
Control	-	21.38	4.10	4.1	6.5	96
Formalin/H_2SO_4	5.25:1.75	21.75	3.98	6.4	10.6	82
Formalin/formic acid	8.2:2.1	21.15	5.05	13.3	3.8	56
Formalin	9.7	21.33	4.90	15.1	1.6	64

Formalin applied as 40% solution of formaldehyde.

H_2SO_4 applied as 20% solution.

Seeding mixture of Lower Lambing field.

2nd year ley.	Leda Italian Ryegrass	4
	Presto Perennial Ryegrass	6
	Barvestra Perennial Ryegrass	6
	Barlatra Perennial Ryegrass	6
	S23 Perennial Ryegrass	6
	Altaswede Red Clover	3
	N.Z. White Clover	2
		33 lb/acre

GROUP II

Ensiled 28.8.72

Harvested from Lower Lambing Field, Boghall Farm

Ensiled in 500 kg plastolene silos.

Treatment	Rate of application g kg^{-1} fresh wt	% DM_t	pH	% WSC in DM	BC in m equiv/ 100 g DM	% TN in DM	% TSN in DM	% VN in DM
Control	-	19.41	4.08	2.90	114	2.7	1.5	0.1
Formalin/acetic acid	4.5:4.5	20.17	4.57	14.28	86	2.7	1.2	0.1
Formic acid/acetic acid	4.2:1.4	19.44	4.16	11.82	83	2.7	1.0	0.1

GROUP III

Ensiled 18-20.7.72

Harvested from Jean Lowrie Field and Howgate Stockyard, Easter Howgate Farm

Ensiled in 25 Mg vacuum silos at Woodhouselea Farm

Treatment		Control	Wilted	Fresh + formic acid	Wilted + formic acid
Rate of additive application g kg^{-1} fresh weight		-	-	3.6	6.3
Grass	pH	6.1	6.1	4.7	4.9
	% DM_o	16.4	31.7	17.2	33.0
	% WSC in DM	19.1	24.4	22.0	23.5
Silage	pH	3.80	4.19	3.85	4.19
	% DM_t	20.9	32.5	21.4	33.1
	% WSC in DM	2.2	6.8	4.5	19.6
	BC m equiv/100 g DM	111	85	102	73
% DM	Formic acid	0.14	0.18	0.55	1.07
	Acetic acid	1.65	1.53	0.94	0.86
	Propionic acid	0.04	0.03	tr	nil
	Butyric acid	tr	tr	nil	nil
	Lactic acid	8.4	4.6	3.6	3.9

Formic acid applied as Add-F.

Seeding mixture of Jean Lowrie Field.

1st year ley EF 486 Dasas Italian Ryegrass 30 lb/acre

Seeding mixture of Howgate Stackyard.

1st year ley EF 486 Dasas Italian Ryegrass 40 lb/acre

GROUP IV

Ensiled 11-15.6.73

Harvested from Hay Knowes and Low Fulford, Boghall Farm

Ensiled in 100 Mg concrete bunker silos at Easter Howgate Farm

Treatment		Control	Formic acid	Formic acid/ formalin	Formic acid/ formalin/ propionic acid
Rate of application in g kg^{-1} fresh wt		-	5	3.4:3.4	1.8:1.8:1.8
Grass	pH	5.97	4.59	4.77	5.06
	% WSC in DM	19.8	20.4	19.4	19.3
	% DM_o	33.8	34.2	34.1	33.5
Silage	pH	4.51	4.78	5.00	4.81
	% DM_t	32.4	32.4	31.4	32.7
	% WSC in DM	5.4	15.6	15.7	15.5
	BC m equiv/100 g DM	70	47	48	51
	% Lactic acid in DM	4.4	0.9	1.9	1.9

Formic acid applied as Add-F.

Seeding mixture of Hay Knowes.

2nd year ley	Presto Perennial Ryegrass	5
	Barvestra Perennial Ryegrass	5
	S23 Perennial Ryegrass	4
	EF 486 Dasas Italian Ryegrass	4
	Manawa HI Ryegrass	5
	Altaswede Red Clover	3
	N.Z. White Clover	2
		28 lb/acre

Seeding mixture of Low Fulford.

1st year ley	Tetila Tetraploid Italian Ryegrass	10
	EF 486 Italian Ryegrass	25
		35 lb/acre

APPENDIX 3

THE THERMOLOG

1. Theoretical Background

The Thermolog is based on a simple thermistor thermometer circuit which involves a resistance bridge arrangement shown in Figure 1. The bridge will be more or less 'in balance' (balance = 0 volts across G) according to the ratios of A:C and H:D. Any imbalance will cause current to flow through the galvanometer, G. Thermistors are temperature sensitive resistors (with a negative temperature coefficient; resistance falls as temperature rises). The value of D will change with temperature, causing a proportional change in the voltage appearing across G.

A thermistor is not, however, a linear device (resistance does not change at the same rate for all ranges of temperature) though an acceptable degree of linearity can be obtained by placing a resistor in parallel with the thermistor. This resistor should have a value close to that of the thermistor at the mid-point in the temperature range over which measurements are to be made.

In the case of the thermistors used with the Thermolog, this value was 1 K ohms. Thermistors are not normally perfectly matched, and small variations in their resistance at a given temperature are encountered. To allow exact matching at the mid-point temperature, the parallel resistance was arranged to be variable by ±100 ohms. (Figure 2).

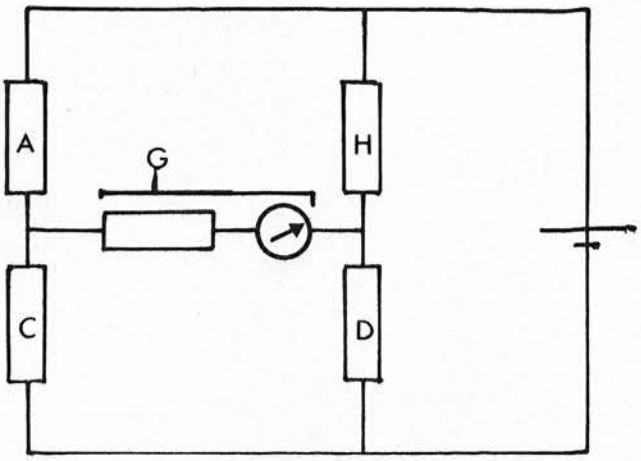


FIG 1 Thermistor resistance bridge
A C H - resistors
D - thermistor
G - galvanometer

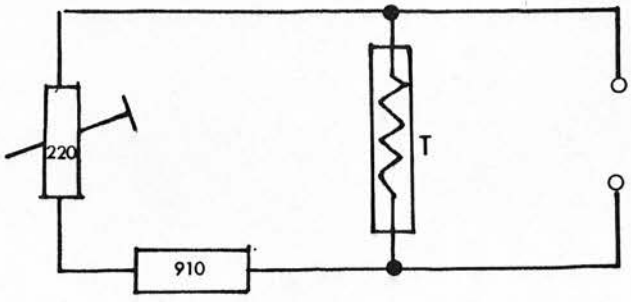


FIG2 Parallel resistance circuit for linearisation and matching.
T- NTC thermistor (ITT type KR222CN)

A further characteristic of thermistors is that, like all resistors, if large currents are passed, heating arises which would distort temperature readings. Unfortunately, reducing the currents employed also reduces the temperature/voltage coefficient and amplification is thus required.

In the Thermolog, the voltage changes are amplified by means of integrated circuit operational amplifiers of the 741 type. These devices have the advantage of ease of use and a voltage gain characteristic which has a low temperature coefficient. Such an operational amplifier is shown schematically in Figure 3.

Two inputs are provided on such amplifiers, the 'inverting input' which gives an output in the opposite sense (i.e. + inputs give - outputs), and the 'non-inverting input' which results in outputs of the same sense. The theoretical or 'ideal' gain of these devices when used in the 'open loop' mode (i.e. without feedback) is infinite. Practical considerations reduce the open-loop gain to about 100 dB (about 200v/mv) but it is usually assumed that 'ideal' gain is achieved when establishing practical circuit parameters. The other ideal property of operational amplifiers is an infinite input impedance, that is, an input which draws no current from the signal.

Clearly the very high 'open loop' gain would be useless in practice and some means of accurately controlling gain is essential. This is achieved by the use of negative feedback; applying the output of the device to the inverting input so that a 'self cancellation' effect arises. If the output is connected directly to the inverting input, then the device will have a gain of one and will 'follow' any

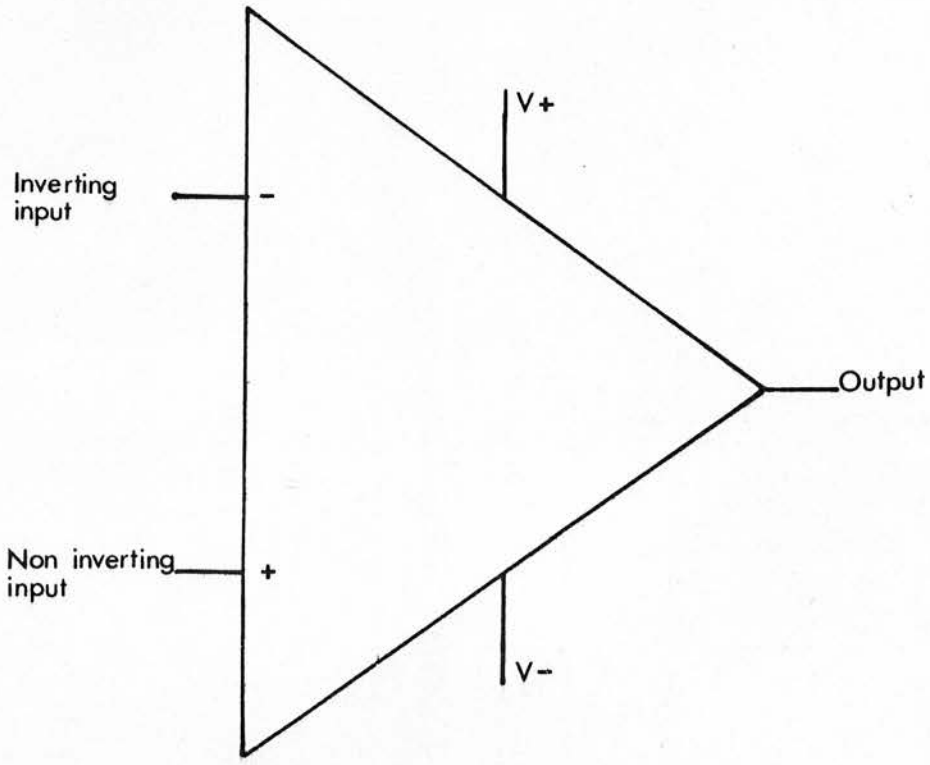


FIG 3 Basic operational ampliflier.

voltage applied to the non-inverting input. This configuration is described as a 'follower' and is used to 'buffer' reference voltages in the Thermolog. (Figure 4).

The inverting amplifier configuration given in Figure 5 allows gains other than one to be achieved. According to Ohm's Law, the current through the input resistor (R_i) must equal the voltage across it, divided by its resistance.

$$I_i = \frac{V_i - V_s}{R_i}$$

Similarly the current through the feed-back resistor is

$$I_f = \frac{V_s - V_o}{R_f}$$

Since the input resistance is regarded as infinite, no current flows to the input. This means that

$I_i = I_f$ and thus

$$\frac{V_i - V_s}{R_i} = \frac{V_s - V_o}{R_f}$$

$$\therefore \quad \frac{V_o}{V_i} = -\frac{R_f}{R_i} = \text{the Gain}$$

The gain is therefore the ratio of the feedback resistor and the input resistor.

Similar arguments apply to the use of the amplifier in the non inverting configuration. (Figure 6).

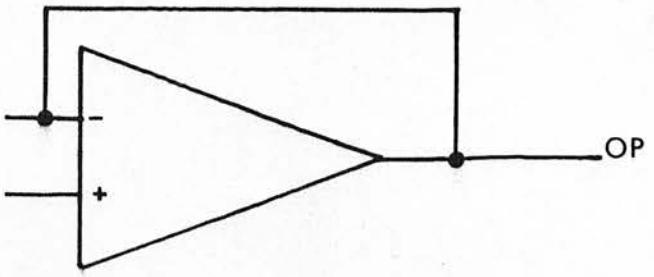


FIG 4 Operational amplifier in follower configuration (Gain = 1)

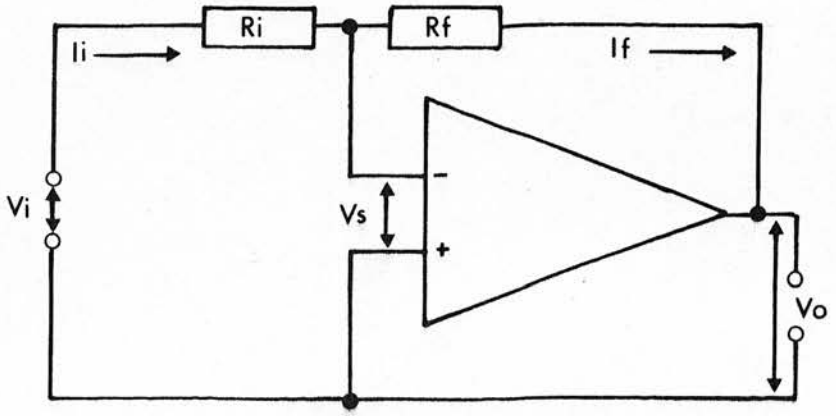
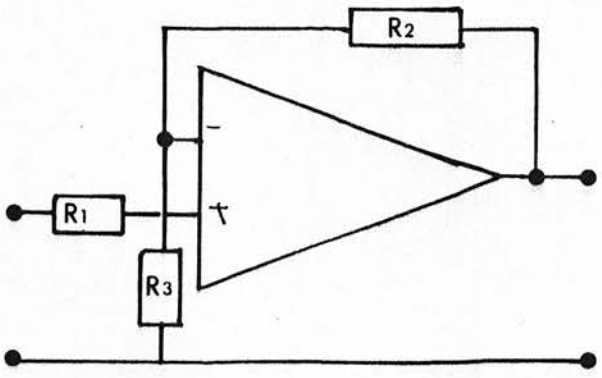


FIG 5 Inverting amplifier configuration



$$V_{out} = V_{in} \frac{R_2 + R_3}{R_3}$$

$$R_1 = \frac{R_2 R_3}{R_2 + R_3}$$

FIG 6 Non inverting ampliflier configuration

The Thermolog is a differential thermometer, however, and the difference between two temperatures is measured. This is accommodated by using the amplifiers in their differential mode (Figure 7). Hence the gain $\frac{R_2}{R_1}$ and the output V is $\frac{R_2}{R_1}(V_1 - V_2)$.

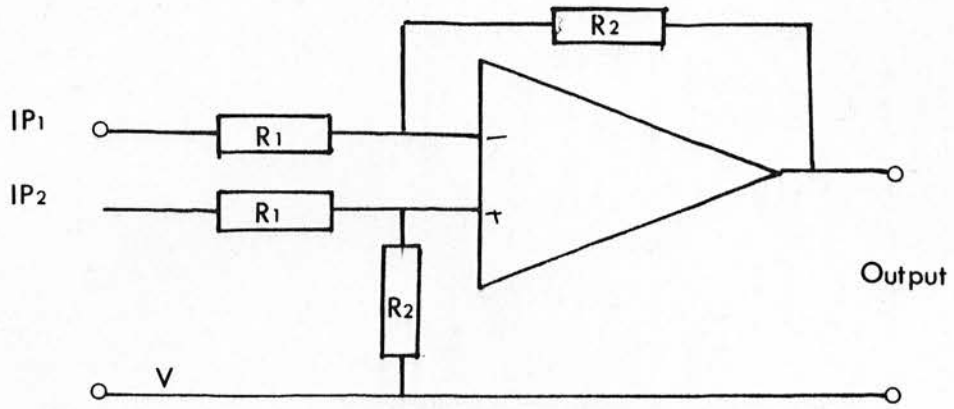
2. Practical Application

a. Temperature measurement. The amplifiers in the Thermolog are all arranged in the differential mode according to Figure 8. Input 1 is the reference input which can be connected by switch selection to either the reference (ambient temperature) thermistor for differential temperature measurements, or to a fixed value resistor having the same value as the reference thermistor at some selected temperature, by switch selection (Figure 9). These two modes of operation are selected by a front panel switch 'Differential/Absolute'.

Thus each thermistor has a trimmer in parallel to allow matching at 20°C, and an amplifier which allows matching at 40°C thus giving good matching throughout the range of operation. Each thermistor may be compared with either a reference thermistor at ambient temperature or a fixed resistor at a value corresponding to any one of a range of temperatures.

b. Temperature recording. Each amplifier output is selectable either manually or automatically. The outputs are then applied to a panel meter on the instrument or to an associated chart recorder.

(i) Automatic recording. A programmable drum selector operates a series of microswitches in sequence. Between readings, the meter is grounded by the 'normally closed' contacts of the microswitches which are connected in series via the common terminal (Figure 10).



$$V = (V_1 - V_2)\frac{R_2}{R_1}$$

FIG 7 Amplifier used in differential mode

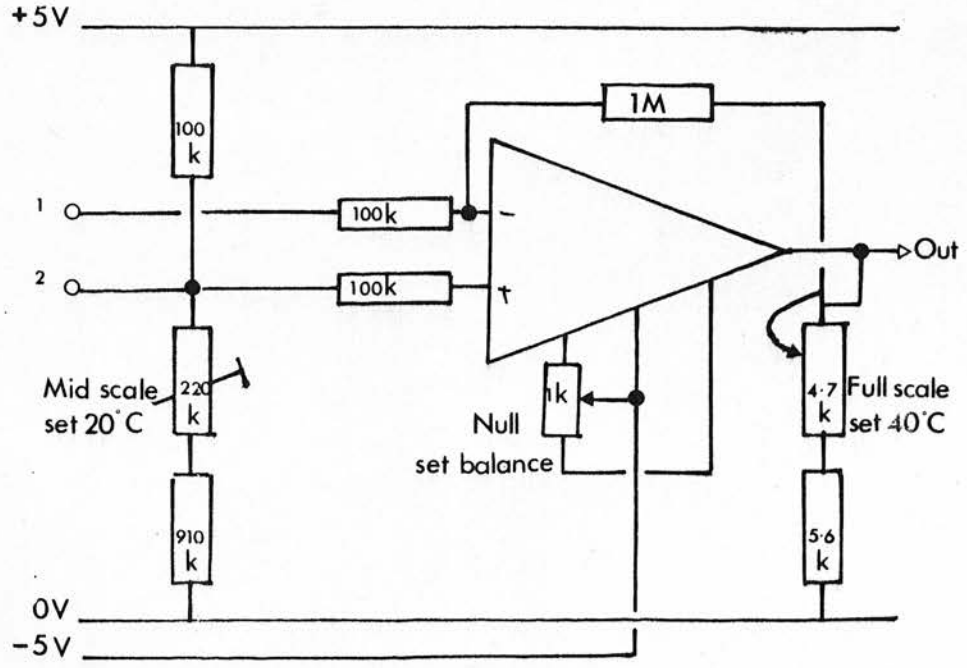


FIG 8 Differential mode arrangement of amplifiers in the Thermolog

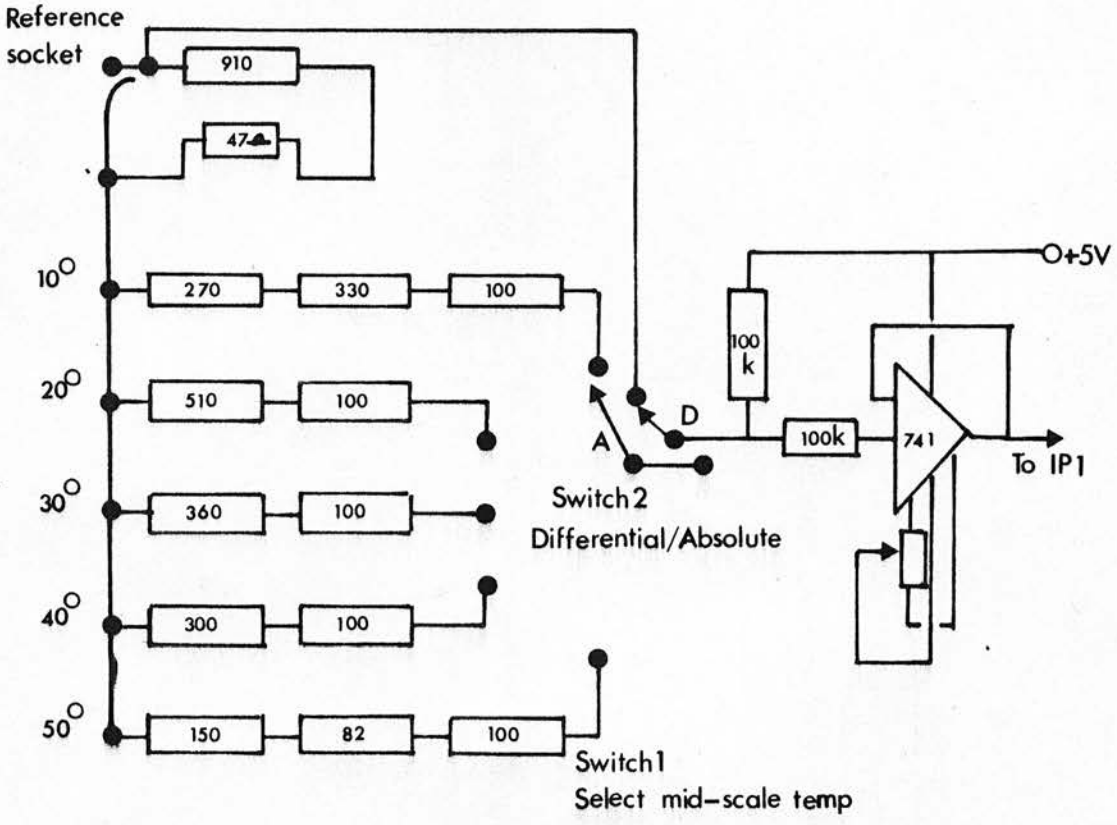


FIG9 Temperature/mode selection circuit

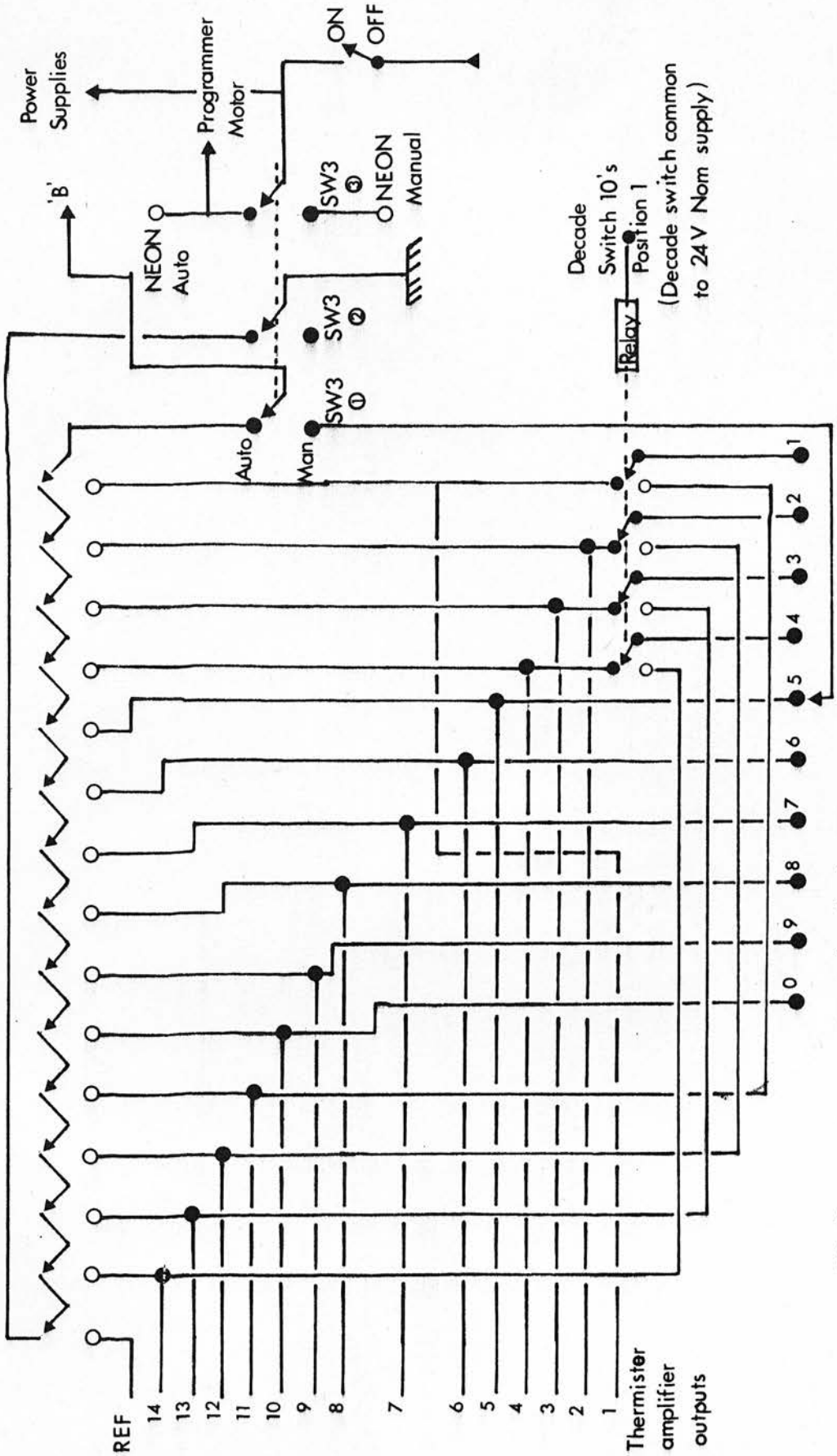


FIG 10 Automatic – Manual selection circuit

(ii) Manual recording. Edge operated digital switches are used for manual selection of thermistors. The first decade, thermistors 1-10 is selected directly by the appropriate switch. For thermistors 11-14, the second decade switch operates a 4 pole change-over relay when digit 1 is selected. This relay selects thermistors 1-4 in the normally closed position and thermistors 11-14 in the normally open position, the common contacts being connected to positions 1-4 on the first decade switch (Figure 10). A further front panel switch allows the selection of either the programmer output 'Auto' or the decade switch output 'Manual'. Selecting these options lights a suitably labelled neon on the panel and the 'Auto' function also starts the programmer motor.

In the manual condition, a separate 24v (nominal) power supply is available for the relay and in the auto mode, this supply operates a digital counter on the panel at the end of each scan (Figure 11). In addition this supply is used to power the recorder driver amplifier which is required to provide a 13v 'swing' (Figure 12).

A multipole connection cable couples the recorder to the main apparatus and in addition to the signal carrying temperature information, the mains supply and a signal to an event recorder. The event recorder takes the form of a trace on the left hand edge of the recorder chart which deviates at the end of each scan. A typical trace is shown in Figure 13.

3. Additional details

i. Power supply. The amplifiers in the apparatus operate on a ±5v supply. The circuit is given in Figure 14.

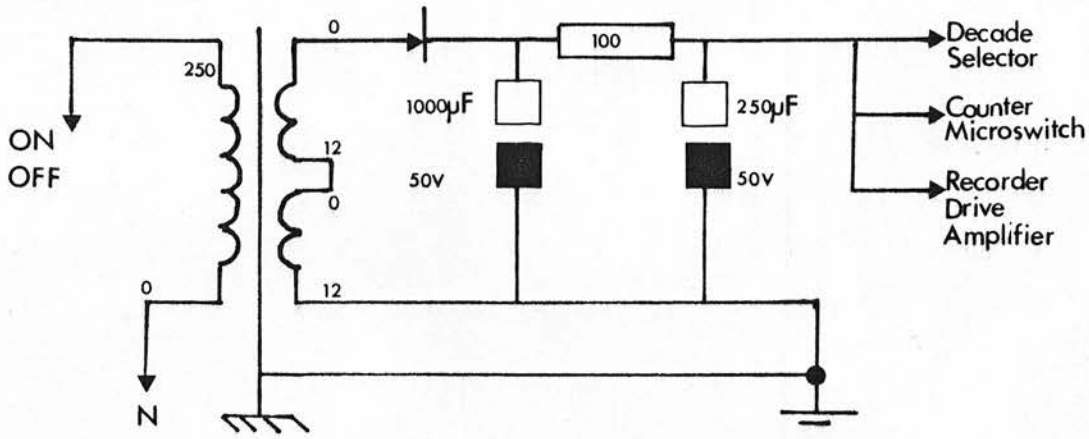


FIG 11 Auxillary supply

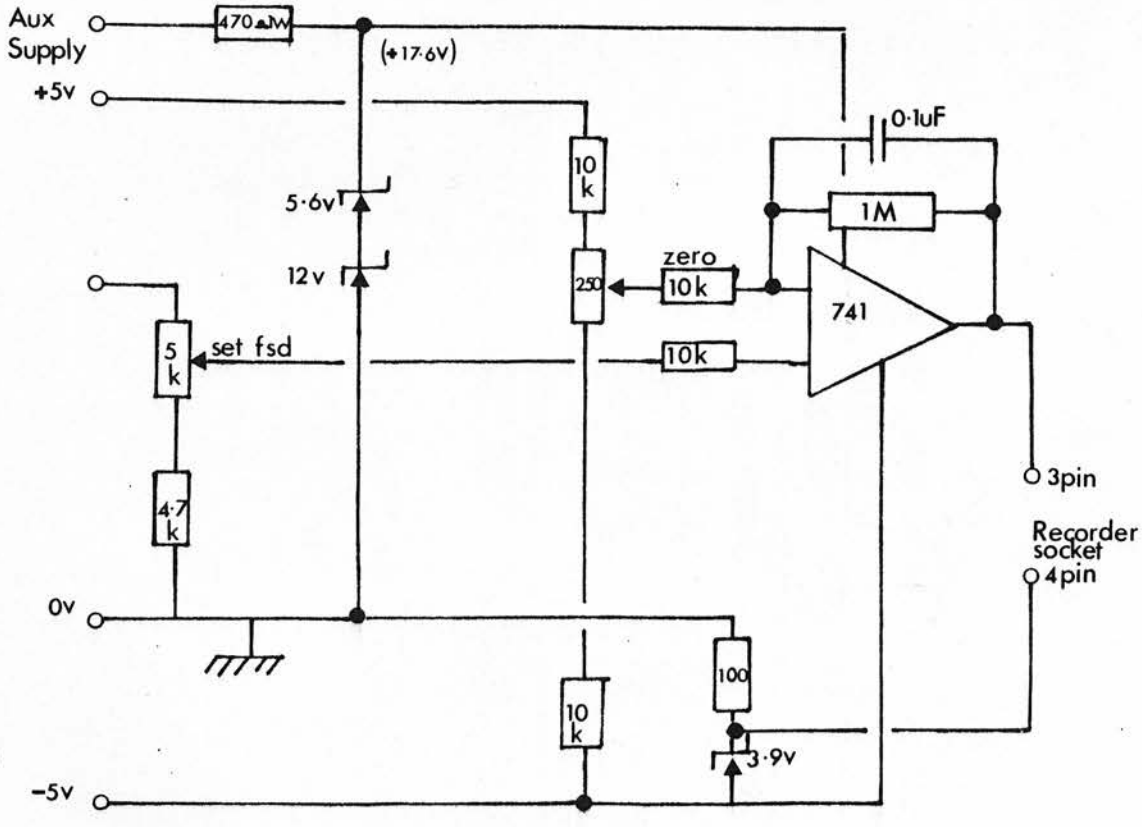


FIG 12 Recorder drive amplifier

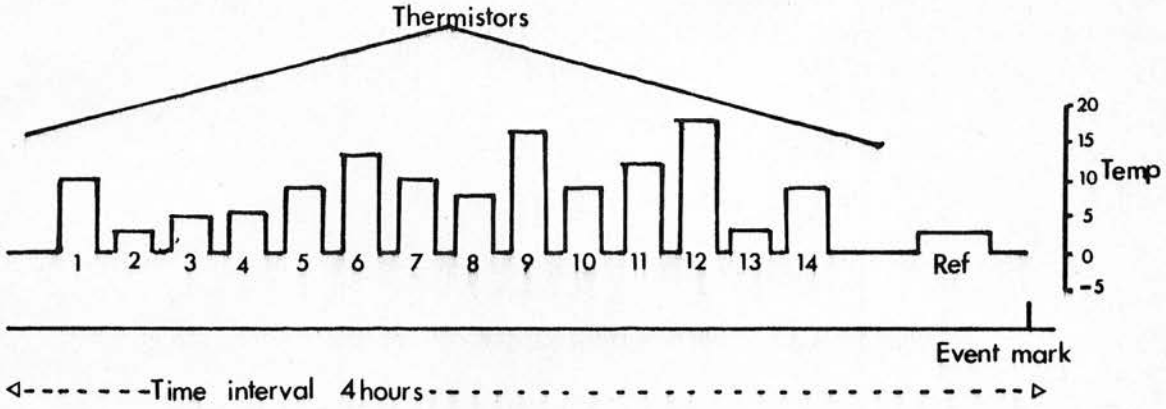


FIG 13 Recorder trace

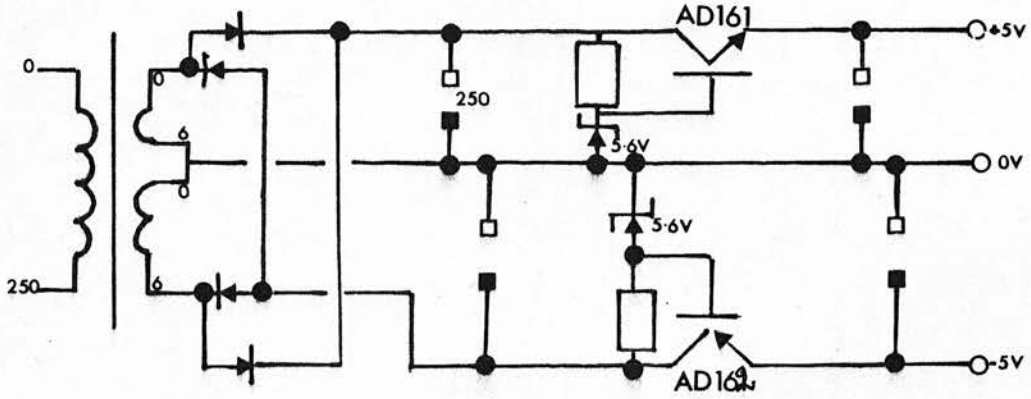


FIG 14 Dual power supply

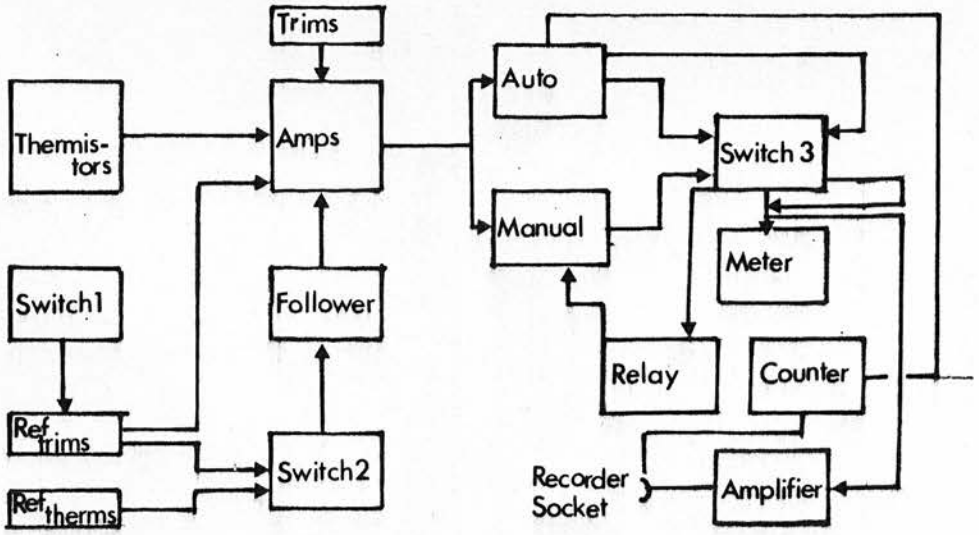


FIG 15 Block diagram

ii. A block diagram shows how the various parts of the apparatus are interconnected. (Figure 15).

iii. Setting-up procedure.

(a) Reference circuit: Plug in reference thermistor and set to 20°C. Measure exact resistance. Set 20°C absolute temperature standard to this value by adjusting 100 ohm trim resistor. Set microswitch 15 on programmer drum. Select auto, select differential. Adjust reference/standard follower offset trimmer for 0°C.

(b) Amplifier null: Manually select and short circuit each channel in turn. Adjust null trimmer (1K) to give 0°C reading.

(c) Amplifier zero: Put all thermistors at 20°C. Select Manual, 20°C Mid Scale Differential. Select thermistors in turn and adjust mid scale trimmers for 0°C.

(d) Amplifier gain: Put all thermistors at 40°C. Select Manual 20°C Mid Scale Absolute. Select in turn and adjust gain (4.7K ohm) trimmer for +20°C.

iv. Adjusting the recorder output.

(a) Switch on apparatus and allow 1 hour to settle.

(b) Select 'set zero' switch and adjust 'zero' control till the recorder reads '0°C'. Switch off 'set zero'.

(c) Select Manual, Absolute and a midscale setting to give a 15°C deflection on panel meter. Adjust 'FSD' till recorder reading agrees with that on panel meter.

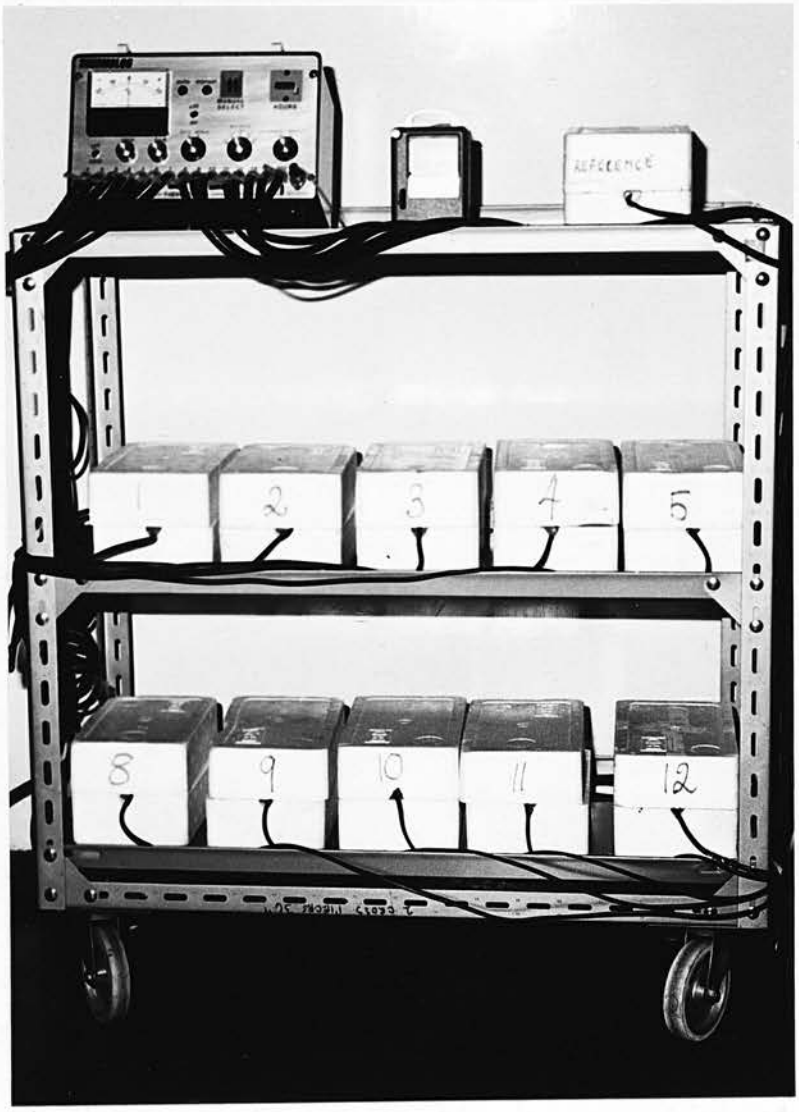


PLATE 1 The Thermolog apparatus showing control panel, recorder and polystyrene boxes containing samples under test.

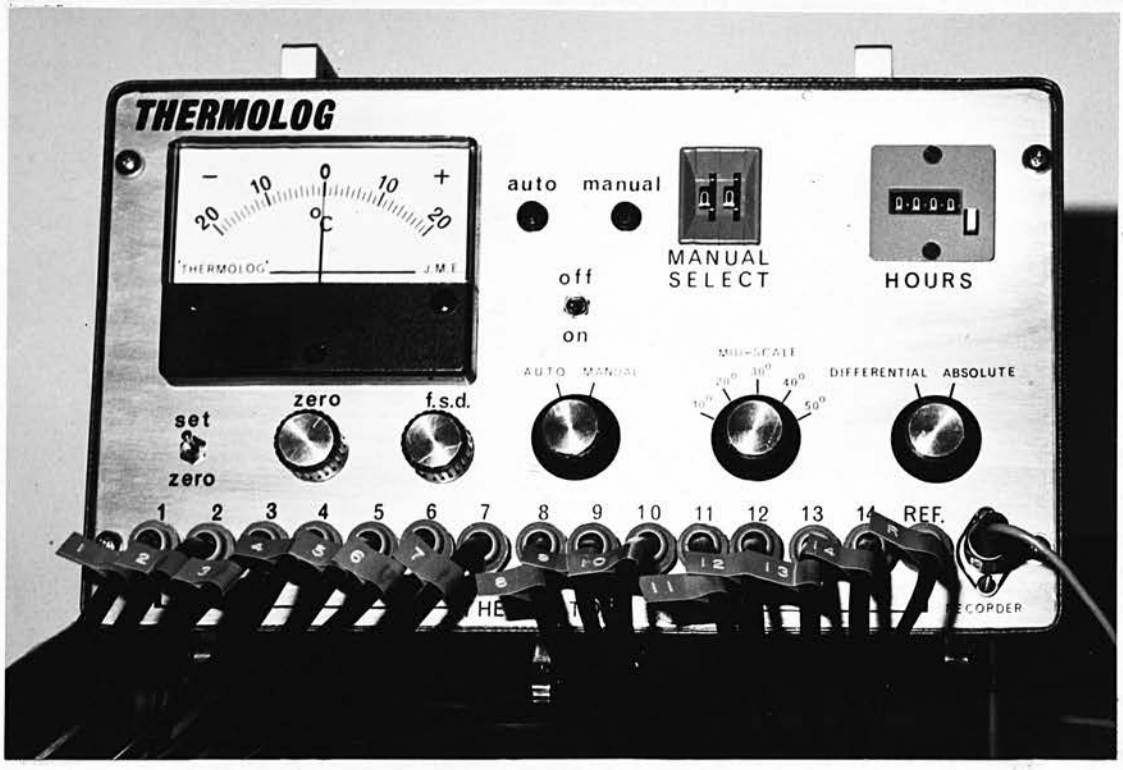


PLATE 2 The control panel of the Thermolog.

APPENDIX 4

The microbiological and chemical composition of additive treated silage including stability under aerobic storage conditions

pH

Treatment 4.5 g kg^{-1} (fresh wt)	Replicate Results				Mean
Control	4.24	4.21	4.30	4.92	4.42
Formalin	4.65	4.78	5.20	4.68	4.83
Formic acid	4.68	4.47	5.00	4.81	4.74
Acetic acid	4.03	3.92	4.45	4.14	4.14
Propionic acid	4.28	4.42	4.15	4.29	4.29
Formalin/formic acid 1:1	5.42	4.90	4.59	5.00	4.98
Formalin/acetic acid 1:1	5.20	4.80	4.94	4.58	4.88
Formalin/propionic acid 1:1	4.66	5.12	5.20	5.12	5.03
Formic acid/acetic acid 1:1	4.44	4.34	4.11	4.42	4.34
Formic acid/propionic acid 1:1	4.99	5.06	4.75	4.81	4.90
Acetic acid/propionic acid 1:1	5.08	4.68	4.40	4.78	4.74
Formalin/formic/acetic 1:1:1	5.00	4.62	4.97	4.88	4.87
Formalin/formic/propionic 1:1:1	5.10	5.20	5.12	4.45	4.97
Formalin/acetic/propionic 1:1:1	5.25	4.92	5.24	5.38	5.20
Formic/acetic/propionic 1:1:1	4.92	5.01	4.87	4.52	4.83
Formalin/formic/acetic/propionic 1:1:1:1	4.88	5.25	5.11	5.28	5.13

Grand Mean 4.77

SED 0.17

SED - standard error of difference between two means

% WSC in DM

Treatment 4.5 g kg^{-1} (fresh wt)	Replicate Results				Mean
Control	7.5	3.6	3.4	5.8	5.1
Formalin	12.2	13.1	15.6	12.4	13.3
Formic acid	16.9	10.1	15.5	16.8	14.8
Acetic acid	7.0	6.4	8.4	11.5	8.3
Propionic acid	7.6	14.3	8.1	10.5	10.1
Formalin/formic acid 1:1	11.0	18.1	11.6	15.5	14.1
Formalin/acetic acid 1:1	16.5	14.8	15.6	13.9	15.2
Formalin/propionic acid 1:1	12.7	14.4	15.0	13.2	13.8
Formic acid/acetic acid 1:1	16.4	18.1	9.1	14.1	14.4
Formic acid/propionic acid 1:1	11.9	15.2	13.8	15.3	14.1
Acetic acid/propionic acid 1:1	14.4	13.5	13.0	13.8	13.7
Formalin/formic/acetic 1:1:1	12.8	13.8	15.8	16.6	14.8
Formalin/formic/propionic 1:1:1	16.7	15.5	17.3	12.7	15.6
Formalin/acetic/propionic 1:1:1	16.2	17.5	10.9	15.4	15.0
Formic/acetic/propionic 1:1:1	18.2	17.1	15.6	15.7	16.7
Formalin/formic/acetic/propionic 1:1:1:1	17.1	16.6	9.6	16.2	14.9

Grand Mean 13.4

SED 1.7

Volatile N as % TN

Treatment 4.5 g kg^{-1} (fresh wt)	Replicate Results				Mean
Control	5.1	7.2	8.3	13.5	8.5
Formalin	3.8	4.8	6.0	2.1	4.2
Formic acid	5.0	2.9	6.4	2.0	4.1
Acetic acid	3.3	3.8	4.0	4.3	3.9
Propionic acid	4.5	4.0	3.0	3.4	3.7
Formalin/formic acid 1:1	4.4	5.3	5.3	3.7	4.7
Formalin/acetic acid 1:1	7.8	5.6	4.6	4.4	5.6
Formalin/propionic acid 1:1	5.9	4.4	4.9	7.6	5.7
Formic acid/acetic acid 1:1	2.9	3.4	3.2	6.1	3.9
Formic acid/propionic acid 1:1	6.9	6.1	6.0	5.7	6.2
Acetic acid/propionic acid 1:1	4.6	6.6	4.9	7.0	5.8
Formalin/formic/acetic 1:1:1	7.9	3.6	4.9	5.7	5.5
Formalin/formic/propionic 1:1:1	6.1	6.9	6.9	7.3	6.8
Formalin/acetic/propionic 1:1:1	3.9	6.3	6.7	7.5	6.1
Formic/acetic/propionic 1:1:1	2.5	4.8	5.6	2.6	3.9
Formalin/formic/acetic/propionic 1:1:1:1	2.9	4.9	7.7	5.0	5.1

Grand Mean 5.2

SED 1.1

Soluble N as % Total N

Treatment 4.5 g kg^{-1} (fresh wt)	Replicate Results				Mean
Control	76	61	58	74	67
Formalin	56	49	46	50	50
Formic acid	69	59	71	63	65
Acetic acid	67	71	71	70	70
Propionic acid	52	69	67	67	64
Formalin/formic acid 1:1	36	54	57	41	47
Formalin/acetic acid 1:1	57	55	54	52	55
Formalin/propionic acid 1:1	64	52	68	66	61
Formic acid/acetic acid 1:1	60	71	64	62	64
Formic acid/propionic acid 1:1	56	68	68	56	62
Acetic acid/propionic acid 1:1	65	69	54	66	64
Formalin/formic/acetic 1:1:1	54	61	57	52	56
Formalin/formic/propionic 1:1:1	58	57	58	55	57
Formalin/acetic/propionic 1:1:1	67	66	62	64	65
Formic/acetic/propionic 1:1:1	62	66	67	59	64
Formalin/formic/acetic/propionic 1:1:1:1	54	62	60	57	58

Grand Mean 61

SED 4

Temperature change in C° in 36 hours from unloading

Treatment 4.5 g kg^{-1} fresh wt.	Replicate Results				Mean
Control	2.25	16.25	8.00	9.00	8.87
Formalin	15.25	15.25	9.75	1.25	10.37
Formic acid	5.00	1.50	3.00	0.25	2.44
Acetic acid	3.17*	1.25	4.75	3.50	3.17
Propionic acid	11.00	4.75	5.25	-0.50	5.13
Formalin/formic acid 1:1	17.25	5.75	22.75	11.25	14.25
Formalin/acetic Acid 1:1	18.00	13.50	15.74*	15.74	15.74
Formalin/propionic acid 1:1	6.50	11.00	7.25*	4.25	7.25
Formic acid/acetic acid 1:1	5.42*	5.42*	5.25	5.42	5.38
Formic acid/propionic acid 1:1	8.50	4.75	5.00	5.75	6.00
Acetic acid/propionic acid 1:1	6.50	4.75	1.25	4.17*	4.17
Formalin/formic/acetic 1:1:1	3.13*	2.00	3.13	4.25	3.13
Formalin/formic/propionic 1:1:1	9.00	3.00	4.75	5.58*	5.58
Formalin/acetic/propionic 1:1:1	2.75	9.50	11.75	4.75	7.19
Formic / acetic/propionic 1:1:1	6.00	5.00	2.00	4.33*	4.33
Formalin/formic/acetic/propionic 1:1:1:1	4.75	9.50	15.25	12.75	10.56

* Estimated value

Grand Mean 7.10

SED 3.07 (not adjusted for missing values)

Log_e total count microorganisms per g silage DM at opening of the silo

Treatment 4.5 g kg^{-1} fresh wt.	Replicate Results				Mean
Control	19.3	19.2	19.4	19.2	19.3
Formalin	12.6	16.8	18.9	11.5	15.0
Formic acid	13.5	21.7	14.3	13.5	15.7
Acetic acid	16.3	11.5	17.0	15.3	15.0
Propionic acid	16.5	12.2	12.9	13.5	13.8
Formalin/formic acid 1:1	17.7	17.8	18.1	14.5	17.0
Formalin/acetic acid 1:1	16.2	17.5	18.1	16.1	17.0
Formalin/propionic acid 1:1	17.0	17.5	18.0	15.9	17.1
Formic acid/acetic acid 1:1	14.3	16.9*	19.6	16.9*	16.9
Formic acid/propionic acid 1:1	19.0	12.2	15.2	11.5	14.5
Acetic acid/propionic acid 1:1	11.5	20.1	14.1	16.8	15.6
Formalin/formic/acetic 1:1:1	11.5	11.5	13.1*	16.4	13.1
Formalin/formic/propionic 1:1:1	14.8	15.2	13.1	13.1	14.0
Formalin/acetic/propionic 1:1:1	17.5	20.2	18.3	16.1	18.1
Formic/acetic/propionic 1:1:1	11.5	17.5	14.8	16.1	15.0
Formalin/formic/acetic/propionic 1:1:1:1	13.3	16.5	15.4	11.5	14.2

* Estimated value

Grand Mean 15.7

SED 1.8 (not adjusted for missing values)

$\underline{\text{Log}_e \text{ Yeast count per g silage DM}}$
$\underline{\text{at time of opening of silo}}$

Treatment 4.5 g kg^{-1} fresh wt.	Replicate Results				Mean
Control	8.7	7.6	4.6	4.6	6.4
Formalin	10.9	9.2	12.1	10.7*	10.7
Formic acid	8.7	10.9	4.6	4.6	7.2
Acetic acid	5.9*	4.6	5.9*	7.3	5.9
Propionic acid	10.7	9.1	8.7	11.5	10.0
Formalin/formic acid 1:1	5.9*	3.9	10.6	5.9*	7.2
Formalin/acetic acid 1:1	8.3	8.6	4.6	12.9	6.5
Formalin/propionic acid 1:1	10.7	7.1	7.2*	4.6	10.2
Formic acid/acetic acid 1:1	4.6	4.6	4.6	4.6	4.6
Formic acid/propionic acid 1:1	4.6	4.6	4.6	4.6	4.6
Acetic acid/propionic acid 1:1	4.6	4.6	4.6	4.6	4.6
Formalin/formic/acetic 1:1:1	4.6	4.6	4.6	8.3	5.5
Formalin/formic/propionic 1:1:1	4.6	12.2	2.1	4.6	8.4
Formalin/acetic/propionic 1:1:1	11.1	12.6	4.6	4.6	8.2
Formic/acetic/propionic 1:1:1	4.6	4.6	4.6	4.6	4.6
Formalin/formic/acetic/propionic 1:1:1:1	4.6	4.6	6.9	4.6	5.2

* Estimated value

Grand Mean 6.9

SED 1.7 (not adjusted for missing values).