Microbial approach to improving aerobic stability of silage

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Target Audience: *Researchers, Animal scientists, Livestock producers, Agronomists*

Abstract

Aerobic deterioration of silage is a major challenge in the feed value chain. This review discusses microbial mitigations to improving aerobic stability of silages. Early detection of silage deterioration at the face of the silo is important to reduce further ingress of spoilage organisms into deeper layers of the silage. The traditional use of thermocouples to measure spot-temperature in aerobically-exposed silages is laborious. Thermal cameras are now used to instantly visualize heat distribution over the silo face by examining thermographs generated in real-time. Microbial mitigation of aerobic silage deterioration remains the safest and most reliable strategy of reducing losses during feed-out. Three generations of microbial additives that have been used to improve the aerobic stability of silages include first generation (1 G), second generation (2 G) and third generation (3 G) silage inoculants. Homolactic fermentation by 1 G produces the highest concentration of lactic acid but 1 G are less capable of reducing aerobic spoilage. Second generation and 3 G both have a dual benefit of improving aerobic stability, and ruminal fibre digestion and growth performance. Although heterolactic fermentation by 2 G and 3 G may cause minimal dry matter losses, improvements in fibre hydrolysis during ensiling and reduction in aerobic spoilage may outweigh these losses. Treatment of tropical forages which have higher concentrations of recalcitrant fibres, with 3 G inoculants could have benefits on ruminal fibre digestion. Development of fourth generation inoculants, possessing 3 G properties but capable of thriving intra-ruminally should be the focus of future research on silage inoculants.

Key words: *aerobic stability, infrared thermographs, silage inoculants, third-generation inoculants*

Description of Problem

Losses due to aerobic deterioration of silage constitute a significant constraint to the operationalization of the feed value chain. The magnitude of these losses is even higher in tropical regions where higher environmental temperatures favour the proliferation of spoilage yeasts (1) and bacilli (2), and give them a competitive advantage over lactic acids bacteria. Time of determining aerobic stability of silages is therefore critical for decision-making in silage-making operations. Traditionally, aerobic stability of silage is defined as the number of hours that the temperature of a silage exposed to air, remains $1^{o}C$ (3) to $2^{o}C$ (4) below ambient temperature.

Losses due to deterioration of aerobically-exposed silage can be huge with significant effects on profit margins. Of the losses associated with silage production, those that occur as a result of aerobic deterioration constitute the greatest percentage, accounting for 10-30% of silage DM (5; 6). The feed-out phase is the phase when the silage is opened during fed-out. It is the phase characterized by re-activation of spoilage organisms and the decomposition of the silage. The biochemistry of silage deterioration involves the metabolism of water-soluble carbohydrates (WSC) and lactic acid, into carbon dioxide, water and

ethanol, leading to a loss of feedable nutrients (6; 5). The predominant microorganisms of aerobic deterioration of silage include yeasts, moulds and other bacteria such as bacilli and enterobacteria (6; 7; 8). In an experiment to determine the origin of spoilage organisms in aerobically deteriorating silage, silage exposed to sterilized air still supported the growth of yeasts and moulds, suggesting that these microorganisms were inherent in the silage itself and survived the ensiling process (5). This implies that epiphytic spoilage organisms influence the stability of the silage upon feed-out.

The principal factors affecting aerobic deterioration of silage are the population and type of yeasts present in the silage, the concentration of residual WSC and lactic acid that serve as readily available substrates for spoilage microorganisms and the concentration of inhibitory organic acids such as acetic, propionic and butyric acids (9; 6). In most silages, deterioration starts when the population of yeasts reaches a threshold of 10^5 to 10^8 CFU g^{-1} DM (6; 8). Yeasts isolated from deteriorating silages can be grouped into lactic acid-utilizers and WSC-utilizers. The extent of susceptibility of silages to deterioration upon exposure to air depends on which of these two is predominant (6). Unlike the activities of WSC-utilizing yeast (e.g. *Torulopsis*) which often dominate the microflora of aerobically-exposed silages with lower pH (13), activities of lactic acid-utilizing yeasts (e.g. *Candida*) usually cause accelerated rise in pH and a loss of stability (6). Lactic acid-degrading yeasts therefore dictate the pace of aerobic silage deterioration especially in silages with low WSC concentration (6; 7). Enterobacteria also cause silage deterioration but this occurs only when yeasts populations are overwhelmed by the enterobacteria (10).

Cereals have higher WSC and lower protein content compared to legumes. This implies that cereals have more readily available substrates for lactic acid production. Legume silages are therefore more resistant to aerobic deterioration because they have less concentration of residual WSC and lactic acid for metabolism by yeasts upon exposure of the silage to air (11).

Management strategies to reduce deterioration of silages upon exposure to air include achieving an optimal packing density earlier during silo packing, reducing air penetration and subsequently air pockets in the silage, a good silo face management during feed-out and reducing the amount of loose silage at the base of the feed-out face (12). Direct addition of chemical additives such as acetic acid or propionic acid can also inhibit yeasts proliferation and improve aerobic stability. Finally, inoculation of silage with heterolactic LAB such as *L. buchneri* (10; 13) and some selected strains of homolactic bacteria with bacteriocin-producing capabilities such as *Enterococcus faecium* (14) have been shown to improve the aerobic stability of silage. According to (14), treatment of grass silage with a bacteriocin-producing *E. faecium* EF9296, reduced populations of *Escherichia coli*, enterobacteria, staphylococci and bacilli-like bacteria within the first 7-14 days of ensiling.

Methods of assessing aerobic stability of silage

Conventionally, aerobic stability has been determined with ambient temperature as the reference temperature (3, 4). However, the suitability of using ambient temperature for assessing aerobic stability of silages stored in farm silos has recently been questioned by (15) given the frequent fluctuation of ambient temperature. These researchers proposed using the core temperature of the silage, 20 cm from the surface, as the reference temperature above which the silage can be classified as being unstable.

A recent technology used for measuring the aerobic stability of silages is the use of thermal cameras for image analysis of thermographs that show heat distribution on the surface of silage (Fig. 1). Infrared thermal

imaging is a technique used to convert the invisible radiation pattern of an object into visible images or thermograms that show the thermal distribution of heat over the surface of a body in real-time (16; 17). It allows temperature mapping of any particular region of interest to be obtained quickly (50-60 images per second; 18) in real-time. Acquisition of data in this manner is not possible with thermocouples or other temperature sensors commonly used to measure a rise in silage temperature at a single location (17). Single-dimensional virtual thermograms constructed with spot-measurements of temperature using thermocouples (15) are limited as compared to two-dimensional thermograms indicating the temperature profile of an entire region of the silo face (20). Temperature measurements taken from several locations within the face of a silo have been used to construct virtual thermograms over the face of the silage after aerobic exposure (15). Even though this method offers an opportunity to estimate heat distribution, it is practically impossible to directly measure the temperature distribution over the entire face of the silo. Consequently, temperature at regions of the face that has not been measured directly with thermometers is estimated through interpolation. However, the use of thermal cameras offers an opportunity to directly measure and construct a two-dimensional infrared image over the entire face of the silo in real-time (Fig. 1).

Thermal imaging has been used to monitor the quality of agricultural products such as meat, fruits and vegetables (18), wheat (20) and silage (19). It also has been used to detect spoilage in grain silos (20). The advantages of thermal imaging include: 1) quick visual appraisal of heat distribution in the silage in real-time 2) temperature measurements are not based on voltage-to-temperature relationship which are potentially non-linear (21), 3) non-contact measurement of silage temperature reduces the possibility of cross-contamination that may occur during sampling in conventional approaches used to assess aerobic stability and 4) application of the technology may also reduce costs associated with personnel and chemical reagents used for conventional assessment of aerobic stability.

Even though thermal cameras are not able to intercept all the infrared radiation emitted at the silage face as some of it is absorbed by moisture in the atmosphere (16), the technology still remains as one of the fastest non-contact techniques of measuring aerobic stability of silage in real time (Fig. 1).
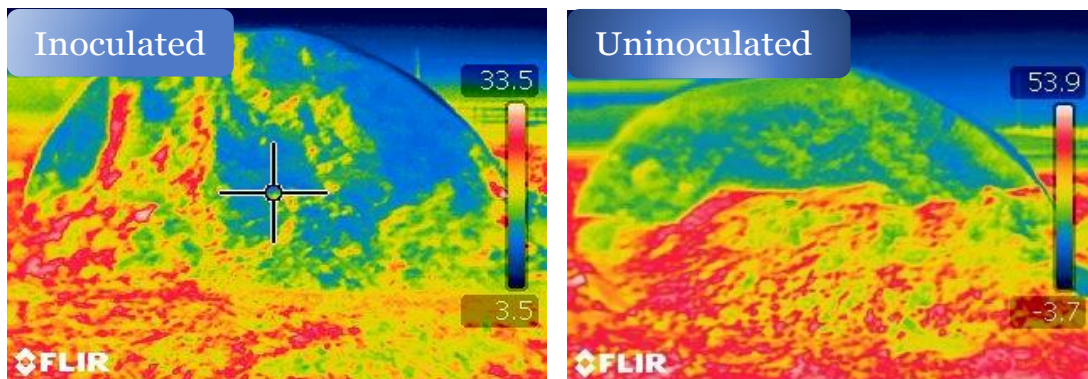

Figure 1: Real-time thermographs of silages inoculated or uninoculated with *Lactobacillus buchneri*. Thermographs were generated with an infrared camera (FLIR T865 Thermal Imaging Camera; Teledyne FLIR LLC, Oregon USA). In an increasing order of heat intensity, yellow, orange and red indicate deteriorating silage on the face of the silo due to heat and infrared radiation whereas cooler areas have black, dark blue and purple colours (Photo credit: Addah Weseh).

Factors affecting aerobic stability of silage
Forage type

The dynamics of natural silage fermentation is dependent on the interactions among the characteristics of the forage being ensiled, microbial population and the ensiling environment (22; 23). The chemical characteristics of the forage that influence its aerobic stability include DM, WSC and protein concentrations, and the epiphytic microbial composition. Microbial factors include the relative populations of desirable lactic acid bacteria and the undesirable yeasts and moulds, enterobacteria and clostridia.

Dry matter and chemical composition of the forage

Plant maturity is linearly related to DM content and the optimum DM for harvesting the crop is often a trade-off between biomass yield and nutrient yield (22; 24). The recommended optimum DM for ensiling most forages ranges from 35% to 45% (24). Forage maturity also affects the end-products of silage fermentation. Lactic acid concentration increased and acetic acid, ethanol and ammonia concentrations decreased in alfalfa silage as the DM content of the forage increased from 31% at the late-bud stage to 44% at 50% bloom stage (25). The composition of the silage affects the onset and level of deterioration of the silage. Higher DM in forage delays the onset of aerobic fermentation because it impedes the achievement of higher packing density during silo filling. This reduces bacterial growth and leads to lower population of LAB (26). The population and growth rate of LAB is reduced, lactic acid concentrations are lower and pH does not decline as rapidly in silages with higher DM (53-54%) as it does with those with optimum DM (30−32%) (27). The rise in temperature of silage is greater when the DM of the forage ensiled is higher (300–500 g DM kg^{-1}). This is because water has a higher specific heat capacity than forage material. Hence the temperature of forages with less moisture will rise faster than those with more moisture (23). Even though moisture by itself is important, it is the efficiency to which water can participate in biochemical process of silage deterioration (water activity) rather than moisture content *per se*, that indicates how much moisture is really critical for onset and duration of microbial silage deterioration (28).

Epiphytic populations of spoilage organisms and environmental factors

The population of epiphytic microflora varies among forages. Scientists (25) have observed higher numbers of epiphytic organisms on corn compared to alfalfa forage. The numbers of Streptococci, Enterobacteriaceae, yeasts and moulds, lactic acid-utilizing yeasts, and carbohydrate-fermenting clostridia were higher on corn than on alfalfa. Even among corn hybrids, distinct differences in epiphytic populations and fermentation characteristics have been observed (25). Spoilage organisms such as yeasts and moulds increase rapidly upon exposure to air. During feed out, the population of spoilage organisms on the face of the silo exceeds those of lactic acid bacteria. The growth rate of spoilage organisms during feed-out rather than the population of lactic acid bacteria determines whether the silage will deteriorate rapidly or not.

Environmental factors such as variations in daily temperature, precipitation and humidity prior to harvesting may also affect epiphytic populations on the forage (29). Environmental factors affecting aerobic stability of silages in cold and hot regions have been discussed extensively (1). Aerobic deterioration of silage in the tropics is further enhanced by environmental conditions. Higher environmental temperatures in the tropics give aerobic yeasts (1) and spoilage bacilli (2) competitive advantage over lactic acids bacteria. This results in silages with lower aerobic stability compared to those ensiled in temperate regions. This constraint is one of the

main reasons why silage production in warmer regions of the tropics is low.

Type of silo

Factors such as silo design, packing density, as well as temperature, radiation, wind and precipitation at the time of aerobic exposure may all affect aerobic stability of silage. In most experiments, silages are stored in laboratory-scale silos. The main advantages of laboratory silos over large farm silos have been noted (30). They include easy replication of treatments, uniformity of conditions such as density in the silos, and the use of small amounts of forage for ensiling. However, results from mini-silo experiments should always be field-tested using commercial silos before being applied at the farm-scale as results from laboratory silos cannot always be extrapolated to farm-scale silos (31; 23). According to (33), laboratory silos may be useful for assessing the trajectory of fermentation and aerobic stability but are not suitable for the assessment of the effects of factors such as silage feed-out rate and face management on aerobic stability (12).

Silage Inoculants

Whether an inoculant will be effective at improving aerobic stability of silage or not will depend on the following:
1. The LAB population delivered by the inoculant. This should be at least 10% greater, and have a faster growth rate than the epiphytic population. Inoculation rates that are even 1% less than the epiphytic population may have minimal or no effect on aerobic stability.
2. The forage being ensiled. It should have sufficient substrates (e.g. WSC) for fermentation (34; 32) into acids that inhibit proliferation of spoilage organisms.
3. Crop-inoculant synergy; some strains of LAB are crop specific, for example, LAB originally isolated from corn may

not perform as well when applied to alfalfa.
4. The LAB should be resistant to phage (34).
5. The type of inoculant (1 G, 2 G or 3 G) and the efficiency at which WSC is fermented to fermentation acids, and the ratio of acetic: lactic acid produced. Silages with higher acetic: lactic are likely to have a longer shelf life upon exposure to air because of the stronger inhibitory effects of acetic acid.

First-generation (1 G) silage inoculants

The production of sufficient lactic acid to cause a rapid decline in pH during ensiling was the original criteria for the selection and development of suitable 1 G silage inoculants (5; 30). Most early silage inoculants were however inefficient and a rapid reduction in pH was often not observed (31). Following many years of selection, the inclusion of efficient LAB species and strains in most 1 G silage inoculants has resulted in a consistent rapid pH decline and improvements in DM retention across a range of forages (Table 1). The criteria for selection of LAB to be included in a silage inoculant was first proposed by (5) to include:
1. They must be able to grow vigorously and overwhelm the dominant epiphytic population.
2. They must possess a homolactic fermentation pathway to produce sufficient lactic acid that will inhibit the growth of other microorganism while at the same time being tolerant to low pH.
3. They must be able to ferment a wide range of sugars (e.g. hexose, sucrose, fructans and pentoses) but should not metabolize organic acids.
4. They should be capable of enduring and growing at temperatures up to $50^{o}C$.
5. They should be able to grow on material of lower moisture content such as wilted forage.

One strategy to increase the efficacy of 1 G inoculants was co-culturing of more than one species or strain of the same species, in a single inoculant (32). Co-culturing of LAB in a single inoculant takes advantage of the fact that different LAB grows at different rates and have different requirements for nutrients, temperature and anaerobiosis. Mixed-species inoculants have improved feed intake and growth performance of feedlot cattle as compared to single-species inoculants (33).

Improvements in aerobic stability of silages inoculated with 1 G silage inoculants have generally been poorer compared to improvements in fermentation characteristics (Table 1). Efficient fermentation patterns result in higher concentrations of lactic acid and residual WSC in the silage. These substrates are then metabolized by aerobic organisms. It is difficult to accurately ascertain the real causative agents of silage deterioration. For example, yeasts population of 10^6 CFU g^{-1} which is greater than 10^5 CFU g^{-1}, proposed as threshold for silage deterioration (6), failed to initiate spoilage of a corn silage-based total-mixed ration. It thus suggests that other species of bacteria and unknown microorganisms other than yeasts may be responsible for aerobic deterioration of silage (34).

Review of experiences, challenges, and opportunities of forage conservation in sub-Saharan Africa has shown that many research institutions lack adequate laboratory facilities for assessing fermentation characteristics and aerobic stability of silage (36). In Nigeria, 1 G inoculants containing *Lactobacillus plantarum* have been used to improve fermentation characteristics of guinea grass but aerobic stability of the silage was poorer resulting in lower preference by sheep (37).

Second-generation (2 G) silage inoculants

Second-generation silage inoculants were developed to improve DM retention during fermentation and aerobic losses during feed-out. The growth of silage spoilage organisms such as fungi (yeasts and moulds) and bacteria (enterobacteria and bacilli) is inhibited by the end-products (organic acids) of silage fermentation. The inhibitory effects of organic acids on yeasts and moulds increase with increasing chain length of the acid (41). Of the major volatile fatty acids produced during silage fermentation, butyric acid, a four-carbon compound, has the greatest inhibitory effect on silage spoilage microorganisms, compared to lactic, acetic or propionic acid. However, increased production of butyric acid is a reflection of the fermentation of sugars and lactic acid, and of the breakdown of proteins and amino acids by clostridia, and is therefore a hallmark of poor-quality silage (5; 6; Table 1). Consequently, only the production of acetic and propionic acids is desirable for improving aerobic stability of silages. Inhibitory effect of an organic acids on spoilage organisms is caused by diffusion of undissociated acids across their cellular membrane into the cytoplasm, where dissociation increases intracellular acidity, denaturing essential proteins and potentially causing cell death (42; 43).

Two major species of bacteria used to formulate 2 G inoculants include *L. Buchneri* and *Propionibacteria*. *Lactobacillus buchneri* converts lactic acid into mainly acetic acid whereas *Propionibacteria* converts WSC into propionic acid, during ensiling. Both acetic and propionic acids are more anti-mycotic than lactic acid. Studies have shown that propionic acid inhibited the growth of acid-tolerant yeasts more than acetic acid, with a combination of these acids being the most effective against silage deterioration (44). However, the concentration of propionic acid as compared to acetic acid is often too low to appreciably prevent aerobic deterioration of silage.

Table 1. Effects of first -generation silage inoculants on fermentation and aerobic stability of silages

Lactic acid bacteria	Crop	Terminal pH	Aerobic stability	DM recovery	Reference
1. *L. casei,*	Corn	Higher	No effect	No effect	(35)
2. *E. faecium*[1]	Grass	Lower	Increased	Improved	(14)
3. *L. plantarum* and *E. faecium*	Alfalfa	No effect	Reduced	Not determined	(33)
4. *L. plantarum* and *E. faecium*	Barley	No effect	Reduced	Not improved	(9)
5. *L. plantarum* and *E. faecium*	Wheat	Lower	Reduced	Not improved	(38)
6. *L. plantarum* and *E. faecium*	Corn	No effect	Reduced	No effect	(34)
7. *L. plantarum* and *P. Pentosaceus*	Perennial ryegrass	Lower	No effect	Improved	(39)
8. *L. plantarum, P. acidilactici* and *E. Faecium*	Barley	Lower	Increased	Not improved	(40)
9. *L. plantarum, L. bulgaricus* and *L. Acidophilus*	Barley	Lower	Reduced	Improved	(41)

[1]Bacteriocin-producing strain EF9296.

When *Propionibacteria* was applied to either corn or sorghum forage prior to ensiling, the bacteria could not be detected in either silage and there was no measurable increase in propionic acid or improvement in aerobic stability (45). Other studies (46) have also shown that the addition of *Propionibacteria* inoculant had no significant influence on fermentation or aerobic stability of corn silage. Inoculation of sorghum, wheat or corn silages with *Propionibacteria* increased acetic and propionic acid concentrations and decreased yeast and mould populations resulting in improved aerobic stability (45). However, in other studies aerobic stability of pearl millet and corn were marginal and no significant production of propionic acid was observed with the addition of *Propionibacteria* (47). The latter researchers attributed the results of their findings to the acidic environment in in the silo which affected the survival of *Propionibacteria*.

Acetic acid, especially in its undissociated form, prevents the deterioration of silage by reducing the growth of yeasts during ensiling and aerobic exposure (34; 39). Whereas WSC is the main substrate for production of lactic and acetic acids through the heterolactic pathway of fermentation, *L. Buchneri* metabolizes lactic acid into acetic acid. The degradation of lactic acid into acetic acid by *L. buchneri* therefore occurs only during the latter stages of fermentation when pH is lower (pH = 4.3−3.8) and temperature is between 15°C and

37°C (3; 48). One major characteristic of all 2 G inoculants is therefore their ability to produce higher concentrations of acetic or propionic acids that inhibit the growth of spoilage organisms.

Aerobic stability of silages treated with inoculants containing *L. Buchneri* has been improved across a wide range of forages including; corn (13; 34; 35), barley (49), sorghum and wheat (50), alfalfa (51), and total mixed ration containing silage (35; 49; 52). However, there are studies in which 2 G inoculants failed to improve the aerobic stability of silages. The acetic acid concentration of corn (53) and bi-crop of peas and wheat (11) silages inoculated with *L. Buchneri* increased compared to the control but this did not result in a consistent improvement in aerobic stability. In other studies, acetic acid concentration did not differ between silages treated with or without *L. buchneri* but yeasts numbers were reduced and aerobic stability was improved (54; 55). The lack of improvements in aerobic stability of silages treated with *L. buchneri* can therefore be attributed to: 1) higher acetic acid concentration in the untreated silage due to heterofermentation by epiphytic microbial population 2) the type of forage being ensiled; legume silages being more resistant to spoilage because their low residual WSC and lactic acid concentrations that serve as substrates for spoilage organisms and 3) aerobic deterioration of silage may be caused by microorganisms

other than yeasts that are known to be susceptible to acetic acid. 4) co-culturing of homolactic LAB with *L. Buchneri* may reduce the activity of *L. buchneri*.

The irony of yeasts improving the aerobic stability of silage has been hypothesized. Treatment of sorghum and wheat silages with a combination *L. buchneri, L. plantarum* and yeasts (Y-56; isolated from local wheat and *Hansenulasub pelliculosa*) showed that neither the addition of yeasts alone nor with LAB at ensiling reduced the aerobic stability of silage. This observation was attributed to increased acetic acid concentration in the silage produced by the yeasts (50).

Use of microbial agents for enhancing silage fermentation and mitigating aerobic losses during feed-out is not common in West or East Africa but in South Africa, heterolactic 2 G inoculants have been used to improve the aerobic stability of maize silage (56).

Third-generation (3 G) fibrolytic silage inoculants

Over time, emphasis on development of inoculants shifted from merely improving fermentation to reducing DM loss and improving forage fibre digestibility (57; 58). In addition to improving aerobic stability of silages, 3 G silage inoculants have a capability of hydrolysing fibre during ensiling and thereby improving ruminal fibre digestion when fed to ruminants. Third-generation silage inoculants contain a new generation of *L.buchneri* that produces an enzyme called ferulic acid esterase, capable of hydrolyzing esters that link arabinoxylans to ferulic acid. Ferulic acid may serve as an etherification site for lignin formation (59). Ferulic acid is one of the most abundant hydroxycinnamic acids present in cell walls of cereal crops and its presence inhibits fibre digestion in ruminants (60). The dual benefit of improving aerobic stability and fibre digestibility is the mainstay of 3 G silage inoculants (19; 57; 61).

More studies are required to further validate research results on the effect of 3 G inoculants on ruminal fibre digestion. Literature on 3 G silage inoculants is scarce. Persistence of 3 G LAB throughout the ensiling duration is a challenge especially at lower pH. A major species of most 3 G inoculants is *Lactobacillus buchneri*. This heterolactic LAB is however denatured when pH declines below 3.5 and their population is not high enough to induce degradation of lactic acid into acetic acid (3). Diffusion of undissociated acetic acid across the cellular membrane into the cytoplasm of *L. buchneri* can cause increased intracellular acidity thereby denaturing essential cellular proteins leading to microbial cell death (42).

Effects of forage chop length on aerobic stability

Chopping forage to a theoretical length greater than 1.9 cm prior to ensiling impedes packing and increases porosity in the silo. Porosity in the silo increases the susceptibility of the silage to aerobic deterioration. Chopping forage to a theoretical length greater than 1.5 cm resulted in silages with lower WSC concentration, and higher pH and DM losses compared to those chopped at 7.5 cm or 15 cm (62). In other studies, DM losses were lower for medium chop lengths (1.3 and 2.5 cm) compared to lower (0.63 cm) and higher (3.8 cm) chop lengths (63). Data from (12) suggest that higher silage packing density was associated with improvements in aerobic stability at the feed-out face of bunker silos. This is because loosely packed silages enhanced porosity of the silage thereby exposing silage microorganisms to oxygen and increasing the rate of deterioration and heating of the silage (23). In most mini silo experiments, aerobic stability is often assessed by placing loose silage samples in a container and exposing it to air hence the effect of chop length in most mini silo experiments is expected to be negligible.

Effect of aerobic deterioration of silage on the feed value chain and animal growth performance

The loss of DM during aerobic silage deterioration represents a loss of potentially digestible nutrients. Yeasts that metabolize WSC and lactic acid are the first microbial agents of silage deterioration. They convert WSC and lactic acid into CO_2, water and ethanol, resulting in higher pH and evolution of heat (5). The higher pH and depletion of WSC and lactic acid stimulates the growth of acetic acid bacteria. The activities of acetic acid bacteria further deplete the silage of soluble substrates leaving a residue of more complex sugars, which are then further utilized by bacilli and moulds (8; 6). Pathogenic bacteria such as *Listeria* may also multiply following this succession (6). The depletion of potentially digestible nutrients decreases the nutritive value of silage by as much as 16% compared to its value at time of opening of the silo (64). Exposing wheat silages to air for 7 days increased silage temperature and CO_2 concentration, and reduced DM and NDF digestibility of the silage by 14% and 7%, respectively (65).

Albeit small, heterolactic fermentation by 2 G inoculants also results in DM losses but these losses are less than the losses that occur upon aerobic exposure of silage (64). About 5% of the energy in the fresh unensiled corn and sorghum forages is lost during ensiling (64) whereas about 14% of DM is lost when wheat silages were exposed to air for 7 days (65). Nearly 0.7 moles of CO_2 is produced per every one mole of acetic acid produced (23). Also, 50% of lactic acid degraded by *L. buchneri* is converted to acetic acid during anaerobic fermentation (1 lactic acid → 0.48 acetic acid + 0.48 1, 2-propanediol + 0.04 ethanol + 0.52 CO_2; 48). Under aerobic conditions, more acetic acid is further produced. Such high concentration acetic acid and butyric acid in deteriorating silages further contributes to the concentration of organic acids that are associated with reduction in dry matter intake (DMI) of silages. Acetic acid by itself may not depress DMI, but because most poorly fermented silages also contain higher concentrations of acetic acid, depression in DMI of such silages is often erroneously attributed to acetic acid concentration (39). Higher concentration of acetic acid alone may not depress silage intake in the short term, but intake may be enhanced when the silage contains the same concentration of acetic acid in conjunction with other acids such as lactic acid (65). Some studies (66) have therefore found that lactic, propionic and butyric acids but not acetic acid, had the greatest impact on DMI of silage. The depressive effect of acetic acid on DMI for dairy cows was 54% greater than that of lactic acid (67). Dry matter digestibility of sorghum silages exposed to air for 14 days has increased from 53% for uninoculated silages to 55% and 61% for 1 G and 3 G inoculants, respectively (23).

First-generation silage inoculants consist mainly of homolactic lactobacilli that produce copious lactic acid as the end product of fermentation of hexose sugar. The lactic acid formed causes a rapid decline in silage pH that preserves the silage but also serves as a residual substrate for spoilage microorganisms upon exposure of the silage to air. Co-culturing of these homolactic strains with heterolactic strains of LAB such as *Lactobacillus buchneri* and *Propionibacteria*, that mainly produce acetic and propionic acids, respectively was used as a strategy to overcome this problem. *L. Buchneri* and *Propionibacteria* are classified as 2 G silage inoculants because they particularly produce acids that inhibit aerobic spoilage of silages but their ability to improve the digestibility and nutritional value of silage has not been consistently supported by available literature as they lack fibrolytic enzyme activity during ensiling.

As opposed to 1 G inoculants, most 2 G and 3 G inoculants are not tolerant of very low pH (below 3.5). Heterolactic 2 G and 3 G inoculants counteract the problem of lower pH by degrading lactic acid into acetic acid and

propanediol, thereby increasing the pH slightly. Real-time information about the quality of silage offered to animals is needed for appropriate decision-making by ruminant farmers. The aerobic stability of silage during feed-out cannot be obtained in real-time through laborious conventional temperature measurements with thermocouples. The use of infrared cameras to generate thermograms in real-time will enable farmers make quick management decisions.

Losses associated with silage spoilage also have large financial implications on the profit margins of farmers. Conservative estimates suggest that the use of 3 G silage inoculants may reduce aerobic losses and cost per kg live weight gain of feedlot cattle by 6%, resulting in a net return of up to $ 10.70 per head of feedlot cattle (61). The next step in microbial silage inoculants research will be the development of inoculants with 3 G characteristics capable of surviving and enhancing fibre digestion in the rumen.

Conclusion and Applications
1. Compared to temperate species, tropical forages have a photosynthetic pathway that aids the accumulation of more recalcitrant fibres and less soluble carbohydrates. The use of 3 G inoculants in the treatment of tropical forages prior to ensiling can increase the nutritional value of native forages and fresh crop residues by increasing the solubility of fibre through ferulic acid esterase during ensiling.
2. Microbial treatment of silages with inoculants will have less harmful effects on local farmers because, unlike chemical additives, they are not corrosive. However, viability of LAB in most inoculants may be a challenge as most farmers in rural areas of sub-Saharan Africa lack refrigeration facilities to store microbial inoculants given the vagaries of weather conditions in most tropical regions.
3. In Africa, there are no commercial companies engaged in the sale of silage

inoculants. Most extension agents therefore do not have the technical knowledge on handling, storage and application of silage inoculants. The model used for storage and distribution of human vaccines in rural areas of West Africa where some rural clinics use solar-powered refrigerators to store vaccines, can be adopted to maintain the viability of silage inoculants for use by farmers in rural areas.

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