Silage inoculants in normal and drought-stressed forages

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According to the National Oceanic and Atmospheric Administration (NOAA) June 2021 was the hottest on record (the previous record in 2016). British Columbia set a new maximum temperature record for all Canada, with hundreds dying as result of the heat, which settled over a region ill-equipped to handle it. The West and Great Plains were extremely dry with South Dakota recording its driest June on record. Heat records and associated droughts are shortening their intervals, and it is not a matter of “IF” but “WHEN” producers in the US will have to withstand extreme conditions that challenge their bottom line. This article will concentrate on understanding plant short- and long-term adaptation to thermal and water stress, their impacts on nutritive value, and how to enhance their conservation as silage by using inoculants to improve preservation and ultimately animal performance.

Understanding metabolism in water-stressed plants

As any farmer can tell, drier than normal conditions affect plant growth and productivity. In biological terms what is really affected is the plant’s ability to carry on photosynthesis. Plants have evolved strategies that allow them to cope to a certain extent with these extremes. To increase their chance of surviving dry conditions plants have two major approaches:

1. reduce use of resources (downregulate photosynthesis/growth) and
2. reduce water losses to the environment by closing their pores (stomata).

The yellowing of the leaves for example is the first sign of a shift in the normal photosynthesis, and their crimpling (reduced turgor) is indication they are losing the battle to water losses. This is an oversimplification of a highly complex mechanism. To reduce growth by downgrading photosynthesis, the plant needs to “process” less atmospheric carbon dioxide. This is accomplished by the closure of its pores which not only reduces photosynthesis but also allows the plant to retain water. This closure is stimulated by changes in ion and water transport systems, which results in the stimulation of abscisic acid (the plants’ “stress hormone”) and a chain of events that ultimately leads to this stomata closure. Abscisic acid is also responsible of other plant processes, including seed and bud dormancy, inhibit germination, promote leaf detachment, and control organelle size. It is especially important for plants in the response to environmental stresses, including drought, soil salinity, cold tolerance, freezing tolerance, heat stress and heavy metal ion tolerance.

Of the water uptake by plants approximately only one percent is retained for metabolic processes, the rest is used to move minerals from the roots to the stem and during evaporative cooling (Buxton and Fales. 1994). The closure of the plant pores during drought saves the plant water but also shuts-off evaporative cooling which, when combined with environmental heat, results in higher foliage temperatures which further impair plant metabolism. A slowing down of the enzymatic processes allows the plant to save energy and nutrients to be used for regrowth once soil moisture improves. Apparently, photosynthesis is less affected by drought than respiration rate and growth which increases non-fibrous carbohydrates in the plant (Buxton and Fales. 1994). This mechanism helps the plant recover and facilitate regrowth once water availability improves. The restriction in plant stem development (fibrous carbohydrates), the presence of proportionally more leaves and the reduction of starch deposition in the kernels result in plants with higher fiber digestibility and crude protein content.

Soluble sugars originate through photosynthesis and are highly sensitive to environmental stressors, with drought and extreme temperatures among the most relevant. During drought growth is limited because of reduced photosynthesis and a reduction in overall metabolism. Sugars play an integral function regulating the genes associated with promoting or inhibiting growth depending on the environment. They are thus not only important as metabolic resources and the simple units from which structural fiber is built but they also act signaling processes associated with plant growth. In a way these sugars function like the hormones in animals regulating signals that control expression of genes involved in plant metabolism. For example, if sugars are in short supply, they signal the need for increased photosynthesis and mobilization of reserves to be used elsewhere by the plant. When in high concentrations they promote plant growth and carbohydrate storage. Research has shown that regulation of sugar activation and repression mechanisms take place at the transcription level (mRNA).

When plants are deprived of these sugars either because of environmental stressors and/or or defoliation, physiological/biological changes need to happen to allow for plant respiration and metabolism to continue. Almost 80% of the CO2 assimilated during photosynthesis goes towards the synthesis of sucrose, which is then transported to "sink" plant organelles. Research has shown that different soluble sugars play different roles in the metabolism of stressed plants. Sucrose and glucose are substrates for cellular respiration or maintain cell homeostasis; fructose on the other hand, appears to be associated with secondary metabolite synthesis, among them acting as substrate for lignin and phenolic compounds synthesis.

The plant microbiota

When studying plant biology and their environment we usually divide it in two areas, the rhizosphere and the phyllosphere. The rhizosphere is the region of soil in the vicinity of the plant roots. The plant roots and their exudates interact with soil microorganisms in what has been also termed the "root microbiome". There is an increased interest in dealing with soil health and all its implications for sustainable farming. The phyllosphere on the other hand, has not been as extensively studied, and it represents the ecosystem on the aerial portion of the plant inhabited by countless microorganisms. Among these are bacteria which live non-parasitically on the plant surface on leaves, flowers, buds, and seeds. Different bacteria prefer different plants and plant organs depending on the bacteria's colonization system which is controlled by the plant. Bacteria which live on leaves and stems are called phyllobacteria, and those on the root system are referred to as rhizobacteria. Standing forages contain an abundance of epiphytic microorganisms such as bacteria, yeasts, and molds. One of the most extensively studied genus because of their importance in forage conservation are Lactobacilli and the purely homofermentative Pediococcus (placed within the family lactobacillaceae).

When forage is ensiled, the epiphytic lactic acid bacteria convert water soluble carbohydrates mostly into lactic acid, decreasing the pH to the point at which undesirable microbes (yeasts and molds) are inhibited. During exposure to air in the storage and feeding phase, aerobic spoilage of silage is a major cause of low nutritive value, bringing about the proliferation of undesirable microorganisms. Susceptibility to spoilage is a critical problem when ensiling forages under warm and dry conditions, and additives that protect the silage upon exposure to air are helpful. One of the key factors for the successful application of microbial additives in silages is the harmony between the ensiled forage plant and the microorganisms used.

Lactobacillus are homofermentative (metabolizing hexoses by glycolysis to lactate as major end product) or heterofermentative, where hexoses are metabolized to lactate, CO_2 and acetate or ethanol as major end products. The Lactobacillaceae are the only family of lactic acid bacteria that includes both homo and heterofermentative organisms. Pediococcus a member of this family of bacteria, are often used in silage inoculants and are commonly added as beneficial microbes since they are considered probiotics. Pediococcus pentosaceus is a promising strain that improves the taste and nutrition of preserved feedstuffs. It possesses bacteriocins or bacteriocin-like substances that play effective antibacterial roles in the microbial ecosystem as probiotics due to their anti-inflammation, antioxidant, and detoxification, abilities.

Effects of warm, dry conditions on epiphytic bacteria.

Some microorganisms withstand relatively dry conditions because they have a physiological capacity to sense and respond to drought. Dry conditions cause losses of water from the cell, which increases their osmolality. These changes produce protein alterations in the cell, and affect metabolic pathways related to DNA replication, transcription and translation, integrity of the membranes, critical metabolic processes essential for cell viability. A reduction in water activity increases oxygen concentration and oxidative stress.

At extreme dryness (loss below 0.1 g of water per gram of dry weight), the acidic groups, side polar chains and peptide bonds of proteins are no longer saturated, and they lose functionality, which ultimately leads to cellular death (Manzanera. 2020). Bacterial cells have protective mechanisms such as the accumulation of K^+, Mn^{+2} and Fe^{+2}, to counteract increases in the osmotic pressure of the extracellular medium. When the cell cannot achieve iso-osmolality, the cell will try to incorporate external osmolytes ('osmoprotectans"), that protect the cell against both an increase in salts and desiccation. Those compounds that protect the bacteria against desiccation are called xeroprotectants and are sometimes able to also act as osmoprotectants. Microorganisms that cannot incorporate or synthesize the appropriate levels of these molecules perish during the drying processes. Under these conditions trying to rely exclusively on native epiphytic bacteria to obtain a good silage fermentation would clearly be a mistake that would lead to extensive organic matter losses, and deterioration of the ensiled mass.

The use of additives to enhance silage preservation.

Farmers have been using different additives to enhance the conservation of stored forages for a long time. In fact, forage preservation by fermentation was used in ancient Egypt as depicted in a mural from the second millennium BC. Advancing to the early 20th century, A. I. Virtanen, a Finnish researcher, developed a system by which silage preservation was attained by adding acids to enhance conservation. Since then, multiple other additives have been developed to achieve a desired or inhibit an undesirable fermentation, inhibit aerobic deterioration, and add nutrients and absorbents. The appearance in the market of additives such as organic acids, enzymes, and bacteria or their combinations, has greatly helped improve silage quality.

Some of the more common lactobacilli used as inoculants are Lactobacillus plantarum, L. acidophilus, Pediococcus acidilactici, P. pentosaceus, and Enterococcus faecium. The combination of more than one species allows for potential synergistic actions. Enterococci for example, have the fastest growth followed by Pediococci, and then Lactobacilli. Similarly, their tolerance to different DM conditions and range of optimal temperatures and pH for growth, allows them to complement each other. To be able to exert a significant effect in the fermentation however, these bacteria must be present at enough concentration. A commonly recommended inoculation rate has been to supply 100,000 cfu per g of wet forage; there seems to be no cost-effective response to doubling or tripling this amount.

Other additives of interest are enzymes that digest fiber and starch. These may be single enzymes, or their combinations intended to degrade more than one type of carbohydrate. The enzymes degrade plant cell walls allowing the bacteria to access and degrade the cell contents faster and reduce the pH of the silage. The most popular have been cellulases, hemi-cellulases, and xylanases, which increase the concentration of lactic acid in relation to acetic, thus improving the efficiency of fermentation. In addition, the rapid pH reduction inhibits proteolytic enzymes, and thus ammonia release which would negatively affect palatability and intake.

Cattle performance

Holtshausen et al. (2011) evaluated the effects of on endoglucanase and xylanase on the digestibility of selected forages and the performance of dairy cows. For alfalfa hay, DM, NDF, and ADF disappearance was greater compared with no enzyme. Barley silage NDF and ADF and alfalfa silage NDF disappearance tended to be greater for the highest enzyme dosage compared with no enzyme addition. The enzyme product improved fat corrected milk production efficiency for early-lactation dairy cows by 11.3%, but its effect depended upon the dosage.

Eun and Beauchemin (2017) evaluated four enzyme products that varied in enzymatic activities added to alfalfa hay and corn silage. The enzymes were endoglucanase, exoglucanase, and xylanase, and were tested at different dosages. The activities of endoglucanase and exoglucanase were positively correlated with improvement in NDF degradability of corn silage, whereas only the endoglucanase tended to be correlated with improvement in NDF degradability of alfalfa hay. Combining effective polysaccharidases further improved fiber degradation of both forages, with greater improvements for corn silage. Combination treatments generally resulted in additive effects with increases in fiber degradation equal to the sum of improvements of individual enzymes. Improved fiber degradation in corn silage was associated with decreased acetate/propionate which suggests greater conservation of the silage organic matter.

Positive results have also been reported when Lactobacilli or cellulolytic enzymes were added to the TMR. Refat et al. (2018) pretreated dairy cow TMRs with xylanase and cellulase on a barley silage–based diet. The addition of enzymes linearly increased in vitro DM digestibility. Milk fat yield, fat-corrected milk, and energy-corrected milk responded quadratically to the incremental enzymes. Milk protein percent improved linearly in response to the dosage with feed efficiency showing a quadratic effect.

Romero et al. (2016) evaluated the performance of cows fed a TMR which included 10% bermudagrass. The use of xylanase at 1 mL/kg of TMR dry matter increased DM intakes (23.5 vs. 22.6 kg/d), organic matter (21.9 vs. 20.9 kg/d), and crude protein (3.9 vs. 3.7 kg/d). It increased milk yield (kg/d) during week 3 (41.2 vs. 39.8 kg/d), 6 (41.9 vs. 40.1 kg/d), and 7 (42.1 vs. 40.4 kg/d). Application of xylanase increased DM intake and production, implying that it could be used to improve performance of dairy cows fed diets with up to 10% bermudagrass.

Vasconcelos et al. (2008) conducted two extensive experiments, on the effects of feeding live cultures of Lactobacillus acidophilus plus Propionibacterium freudenreichii on performance and carcass characteristics of feedlot cattle. Four treatments were evaluated, which included a control diet (lactose carrier only) or diets containing 1 x 10(9) cfu/(steer x d) of P. freudenreichii (strain NP 24) with 1 x 10(7) (L), 1 x 10(8) (M), or 1 x 10(9) (H) cfu of L. acidophilus strain NP 51/(steer x d). Gain efficiency on a live BW basis was improved (P = 0.02) by NP 51 treatments compared with the control, with G:F responding quadratically to NP 51 dose for the overall feeding period (P = 0.05). These data indicate that live cultures of L. acidophilus strain NP 51 plus P. freudenreichii strain NP 24 increased feed efficiency of feedlot cattle fed steam-flaked corn-based diets by approximately 2%, the effects depending on the dose of Lactobacillus.

When to use inoculants?

Some farmers would not even think to put up silage without them, others never use them! Research results are also hit and miss; some show excellent results, some not so much. Oftentimes we realize we should have used them once we open the silage at feed-out. Similarly, we only value the money spent on insurance the moment we claim it, otherwise it seems like an unnecessary expense. There are crops that under a good growth year, and excellent management, do very well without inoculants. Other crops seem to be great candidates for their use regardless of growing conditions. In recent times there has been a trend to use cover crops both to enhance soil health, and as an alternative source of forage during times of shortage. An extensive review of those silages yielded very interesting results and insight on their fermentation. The dataset came from between 500 to 10,000 samples (depending on the crop) analyzed across fifteen seasons by the Dairy One Laboratory of New York. Bear in mind these figures are averages of high and low values, and to predict their potential for preservation and/or livestock performance it is always advisable to analyze samples.

Table 1. Carbohydrate fractions of small grain silages

	Barley	Millet	Oats	Rye	Triticale	Wheat
% Acid Detergent Fiber; ADF	35.0	40.2	38.4	37.5	38.1	36.9
% Neutral Detergent Fiber; NDF	54.4	61.3	58.6	57.7	58.5	56.6
% Ethanol sol. carbs (sugars)	5.5	4.3	5.1	6.7	5.1	6.3
% Starch	9.2	3.1	3.5	1.7	2.3	6.3
% TDN	62.5	54.6	60.5	61.8	60.8	60.8
NDFD 30hr, % of NDF	60.0	53.6	59.9	65.2	63.9	60.9

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Of the carbohydrate fractions, Millet showed the highest NDF at 61.3% (wheat straw has 73%), with all the other silages in the mid to high fifties. Millet also showed the highest ADF (40.2%) with triticale and oats close behind, the rest of the silages had similar values that went from the mid- to upper thirties. Analyses of the fiber fractions clearly showed millet at a disadvantage from a nutritional standpoint compared to the rest. All silages had hemicellulose content (NDF-ADF) in the twenties. It is important however not to just look at the concentration of NDF but also its digestibility. Its digestibility at 30 hours showed rye silage as having its highest value at almost 65%, followed by triticale at 64% and wheat at 61%. This is interesting since triticale is precisely a hybrid of wheat and rye. The NDF digestibility of the rest was at 60% except for millet at 53.6%.

Well-fermented silages show very low values of ethanol-soluble carbohydrates (simple sugars) since they are used up during fermentation. Corn silage for example has on average approximately 2.1 percent. For cover crop silages the values were between 5 and almost 7 percent, except for millet at 4.3 percent. If we compared them to corn silage it is clear there is still room for utilization of this fraction to enhance silage fermentation. One could argue that the digestibility of the plant cell walls in these plant species could difficult their utilization during the ensiling process. It is likely then that any steps taken to improve the accessibility to plant cell contents (e.g., chopping, inoculants) could result in improved fermentation in the silo.

Hill et al. (2013) compared spring and late summer planted millet both with and without a microbial inoculant to corn silage (CS) fed to growing beef cattle. In Experiment 1 separate plantings of pearl millet were harvested at the soft dough stage in either July or September. Wilting the spring-planted first crop millet (FCM) prior to ensiling or direct cutting and adding an inoculant to the summer-planted second crop millet (SCM), did not improve silage fermentation. The pH was higher, and lactic acid much lower for millet silages compared with corn silage as follows: for FCM, 5.74 and 3.22, for SCM, 4.12 and 3.34, and for CS, 3.66 and 8.69. Dry matter intake by growing heifers (initial weight 272 kg) was 2.95 kg/d for both FCM and SCM silages, and higher for CS at 6.08 kg/d for HCS. Average daily gains (ADG) were 0.15 and 0.20 kg/d, respectively, for FCM and SCM silages, and ADG was higher with CS at 0.95 kg/d. These results confirm our findings in that millet silage is not an adequate forage to obtain acceptable daily gains when fed to growing heifers.

The authors followed the first with a second experiment where silage treatments included CS, direct-cut millet treated with an inoculant (MS) or inoculant plus a source of fermentable carbohydrates (0.5% ground corn; MSC) added to improve silage fermentation. These millet silages had improved fermentation compared with those in the first experiment. The three silage treatments were fed to beef steers (initial weight 272 kg) in a drylot, for 56 days. Steers fed CS had higher ADG and improved feed efficiency than steers fed millet (MS) or millet + corn grain (MSC). Respective 56-d ADG (kgs) and feed efficiency for treatments were: MS, 0.78 and 7.35; MSC, 0.78 and 7.92, and for SCS 1.22, and 5.05. Since both millet silages either with inoculant or with inoculant and corn grain showed the same weight gains is clear that the inoculant increased the energy supplied by the forage to an extent like that of the supplemental grain. Steer performance indicated that millet silages can still be used in growing cattle diets if they are supplemented with energy and/or a microbial inoculant.

Does using inoculants pay?

The decision of whether to use an inoculant in the silage depends on how good feed management is in a particular farm. Using an inoculant will make good feed management even better but will not turn bad feed management into good feed. After all, not even insurance companies take high risk clients! We also want to make sure we use an inoculant with proven results backed by sound science, and at the rates suggested by the company. Just remember what was mentioned above, there needs to be at least 100,000 CFU/g for a good inoculant to take over the fermentation. Anything less than that, and the bacteria in the inoculant will not stand a chance to overcome those already present in the ensiled mass. Remember also that the bacteria in the inoculant need substrate on which to act upon. If the forage is chopped at a point where it has used up most of its sugars (e.g. nearly mature corn), then chances are inoculants will not work. There is also the need to use inoculants that contain a combination of bacteria with proven effects on the target silage.

An inoculant may contain one or more strains of lactic acid bacteria. The most common homofermentative species is *Lactobacillus plantarum*. Other common homofermentative are various *Lactobacillus* or *Pediococcus* species and *Enterococcus faecium*. Lactobacillus plantarum, Enterococcus faecium, and Pediococcus acidilactici all act on the silage differently. Enterococcus species and Pediococcus species can tolerate higher dry matter conditions and can grow more rapidly than Lactobacillus species can.

Homofermentative inoculants can improve animal performance by 3-5% (Kung and Muck, 1997). Summarizing 14 lactation studies McAllister et al. (2017) reported that Lactobacillus plantarum improved dry matter intake by 4.8 percent and milk yield by 4.6 percent when applied to grass, corn, or alfalfa silages. The authors also reported that a single inoculant was used in five lactation studies and in four studies with beef cattle; milk yield per cow increased on average 0.76 liters per day and average daily gain by 11.9 percent.

Let's take the first example of a dairy cow which increases intake by 4.8% and production by 4.6%. If we assume the cow eats 24 pounds of silage DM per day and the cost of inoculant of $1 per ton of wet silage, then it would roughly be $0.03 per cow per day in inoculant alone (DM basis). If the cow produces 88 pounds of milk ($15 at $17/CWT) and increases production by 4.6%, it will produce 92 pounds or $15.6 returned in milk. Assuming an efficiency of 1.5, that cow will eat 2.7 pounds more feed, which coincides with the 4.6% percent increased intake reported by research. For feed at $15 per CWT, 2.7 additional pounds of DM are $0.40, plus 3 cents for the inoculant it ends at $0.43. If the increase in milk) is $0.68 gross return (4 lbs. at $17/CWT, then deducting from it $0.43 it will be $0.25/cow/day. The same calculations used for the more conservative approach of 1.67 lbs. (0.76 L) would result in $0.08 gross return. In short using inoculants applied properly to the right forages can return between 8 and 24 cents per cow per day.

Since feed costs are the highest individual component of the cost of livestock production, producers need to take every approach possible to get the most out of them. Their adequate conservation and utilization become then a must for every profitable livestock operation. Using silage inoculants to try to make up for deficient feed management practices will not pay. Their use can make good feed management even better but will not make bad management good. There is a solid and extensive body of research that suggests inoculants both as cellulolytic enzymes and/or bacteria should improve silage fermentation. Despite these evidence, research results are often variable, and so are experiences in the field. It is very likely that this inconsistency is due to the different scenarios observed during silage fermentation and crop year. Because of this reason, when conducting research work with inoculants it would be advisable to use a range of potential rates (at least low, recommended, and high), rather than just using the "standard" rate suggested by the company that commercializes the product.

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