L.buchneri :The rest of the story

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Lactobacillus buchneri bacteria were originally identified by researchers from buckets of corn silage that stayed surprisingly cool after being exposed to air for several days. These bacteria were isolated and grown in pure cultures to be used as an inoculant. These inoculants are promoted for preventing forage heating and spoilage after exposure to air. Before deciding to use a *L. buchneri* product, you need to hear the rest of the story.

Chapter 1: **Forage stability after being exposed to air is related to forage pH and concentrations of acetic acid levels.** In a study to determine what compounds keep forages cool, it was shown that forage stability increases exponentially with acetic acid concentration (Danner, 2003). The higher the acetic acid concentration, the longer the forage stayed cool. What most people do not discuss is how acetic acid actually keep forges stable. The food industry has known for sometime that acetic acid inhibits molds and yeast, which produce heat. However, the second part of the story is that the ability of acetic acid to inhibit molds and yeasts depends on pH. A more acidic environment (i.e., lower pH) results in more acetic acid activity for increasing forage stability (Danner, 2003). For acetic acid, the pH needs to be below 4.79 (i.e., pKa) to get 50% effectiveness for keeping forage cool. For a good description of how acetic acid actually works to inhibit yeasts see Thomas (2002). With this understanding, acetic acid should be corrected for pH level and discussed in terms of "effective" acetic acid. Figure 1 shows examples of forage fermentation expressed as effective acetic acid.

Producer take home message: because a low pH (< 4.79) is necessary to make acetic acid highly effective in inhibiting molds and yeasts, a *L buchneri* bacterial product would not be appropriate for hay crop silage.

***L. buchneri* bacteria:**

- **produce acetic acid,**
- **produce 1,2-propanediol ,**
- **grow slowly (45-60 days),**
- **do not tolerate low pH,**
- **are not appropriate for wet (< 33% DM) corn silage.**

Acetic acid:

- **increases forage aerobic stability,**
- **is pH dependent for yeast inhibition,**
- **has a pKa of 4.79,**
- **should be expressed as effective acetic acid,**
- **results in increased fermentation losses.**

At least 10 pounds of corn meal are required to replace the energy lost in producing 2% DM acetic acid.

Forage	pH	Acetic acid %DM	Effective Acetic acid % DM	% Effective
Corn Silage	3.9	3.7	3.25	88%
Corn Silage	4.1	3.7	3.07	83%
Haylage	5.0	3.7	1.41	38%
Haylage	4.5	2.1	1.39	66%

Figure 1. Example forages expressed as "Effective Acetic Acid".

Chapter 2: ***L. buchneri* bacteria must grow to produce acetic acid.** *L. buchneri* bacteria are sensitive to low pH and are slow growing. The industry standard for lactic acid producing inoculants is 100,000 CFU/g of forage. Work at the University of Delaware (Ranjit and Kung, 2000) showed that this level of *L. buchneri* bacteria was not enough to impact acetic acid or aerobic stability of corn silage. Most commercial products containing *L. buchneri* bacteria are formulated using at least 400,000 CFU/g in order to out compete native bacteria. This is one reason why *L. buchneri* based inoculants are typically more expensive per treated ton of forage compared to traditional lactic acid producing inoculants. Researchers (Grazia,1984) have found that *L. buchneri* bacteria do not grow below a pH of 4.1. In a personal communication, Dr. Muck (USDA-ARS, 2011) indicated that the optimal pH for growth of *L. buchneri* bacteria is above 5. Consequently, if there is a rapid pH drop, *L. buchneri* bacteria will be inhibited. In a 2007 presentation, Muck pointed out that the slow growth of *L. buchneri* bacteria requires 45-60 days of storage to achieve much effect for improving aerobic stability. This slow growth was confirmed by other researchers (Schmidt, 2009). Since acetic acid does not begin to accumulate from the *L. buchneri* bacteria until 45-60 days, a *L. buchneri* bacteria product is not appropriate for forages that will be fed shortly (< 60 days) after ensiling.

There are two common products made by *L. buchneri* bacterial (Oude Elferink, 2001). The first product is acetic acid. Since most untreated forage will normally have 2-2.5% acetic acid, forage treated with *L. buchneri* bacteria should produce over 2.5% acetic acid to demonstrate effectiveness. The second product is 1,2-propanediol (aka., propylene glycol). This product is only produced by *L. buchneri* bacteria. Forage samples with an effective *L. buchneri* fermentation should have 1,2-propanediol concentrations greater than 0.4% DM. See Figure 2 for an example of an effective *L. buchneri* fermentation. It is important to use a forage testing laboratory that tests for 1,2-propanediol to accurately assess the effectiveness of *L. buchneri* added to produce acetic acid during fermentation.

CORN SILAGE	Value	Unit
Dry Matter	33.8	%
pH	3.93	
Titratable Acidity	8.72	meq/100gm
Lactic acid	4.0	
Acetic acid	3.70	% DM
Propionic acid	0.39	% DM
Iso-butyric acid	< 0.01	% DM
Butyric acid	< 0.01	% DM
Total VFA	8.09	% DM
Lactic acid/VFA	49.44	%
1,2 Propendiol	0.403	% DM
Ammonia, CPE*	1.2	% DM

Figure 2. Example of corn silage with an effective *L. buchneri* fermentation. Note the high (> 2.5% DM) acetic acid concentration and presence of 1,2 propanediol. There will be approximately 1% DM loss during this conversion of lactic acid to acetic acid and 1,2 propanediol.

Figure 3 contains an example of corn silage sample with poor *L. buchneri* fermentation. This is an example of the pH dropping too fast and the *L. buchneri* bacteria not producing acetic acid. **Producer take home message: treating forage with an *L. buchneri* product should result in > 2.5% DM acetic acid and > 0.4 %DM 1,2 propanediol. But, these products costs energy to produce.**

Qualitative		
pH	3.58	
Total VFA	8.83	% DM
Lactic acid	6.70	% DM
--Lactic/TVFA		
Acetic acid	2.15	% DM

Figure 3. Corn silage with an ineffective *L buchneri* fermentation. Note the low acetic acid concentration (< 2.5%) which suggests the added *L buchneri* bacteria did not survive. The low pH and low acetic acid suggest energy and dry matter conservation during fermentation.

Chapter 3: **Acetic acid production results in dry matter loss.** As discussed above, *L. buchneri* bacteria convert lactic acid to acetic acid and 1,2 propanediol. In particular, *L. buchneri* bacteria convert 2 lactic acid molecules into 1 molecule of acetic acid, 1 molecule of 1,2 propanediol and trace amounts of ethanol (Oude Elferink, 2001). All of these end products result in energy loss in the forage. This energy loss is practically important given recent escalating grain prices.

Lactic acid in forage has a ME value of 1.69 Mcal/lb (Knapp, 2012). If you start with 6% DM lactic acid (42 pounds; 70 MCal) before the *L. buchneri* bacteria begin to convert it to lactic acid, and you end with 3% lactic acid and 2% acetic acid when the *L. buchneri* bacteria are done, you will need 10 pounds of corn meal per ton of forage to make up the lost energy. The reason for this loss is that *L. buchneri* bacteria use lactic acid as a food source to make acetic acid. In this process, a 3 carbon molecule (i.e., lactic acid) is converted to a 2 carbon molecule (i.e., acetic acid) and a 1 carbon molecule (i.e., carbon dioxide) is released. It can be shown that there is a 2:1 relationship between acetic acid concentration and fermentation-based dry matter loss. This does not take into account the energy and dry matter loss associated with 1,2 propanediol and ethanol. **Producer take home message: there is a 1% DM loss to make 2% acetic acid and 10 pounds of corn meal per ton is needed to replace the energy.**

Chapter 4: Formulation of *L. buchneri* products has a significant impact on their effectiveness. Originally, *L. buchneri* products contained only these bacteria. Consequently, the early fermentation phase of silage was dominated by native bacteria. Given time (approximately 45 days), *L. buchneri* bacteria grow and convert accumulated lactic acid to acetic acid. Fermentation losses associated with only *L. buchneri* bacteria can be enormous. Second generation products include traditional lactic acid producing bacteria, in addition to *L. buchneri* bacteria, in an effort to control the early phase of fermentation. The problem is that if the early phase is controlled very well (rapid pH drop) then the *L. buchneri* will not have a good environment in which to grow. It is likely that including a *L. plantarum* bacteria, which is a traditional silage inoculant, would result in an overly acidic environment unfavorable to *L. buchneri* bacteria. Furthermore, *L. plantarum* bacteria are noted for accumulating compounds such as hydrogen peroxide which inhibit most other bacteria. Another approach is to include bacteria from the genus *Pediococcus*. These bacteria have approximately the same optimal pH range as *L. buchneri.* Yet, the fact remains that there can be significant dry matter loss in the early fermentation phase if silage pH is not controlled to a low level. Within a week, corn silage should have a pH less than 4 and haylage should have a pH between 4 and 4.7 for maximal dry matter conservation in silage fermentation. **Producer take home message: a product that drops pH fast will inhibit *L. buchneri* bacteria growth.**

Chapter 5: The appropriate use of *L. buchneri* bacteria as an inoculant is when aerobic (exposed to air) heating is not controllable through management. Kung (2010) discussed management approaches to improve aerobic stability of silage. Generally, heating for forages occurs when feed cannot be consumed within 24 hours of air exposure. For example, when a corn silage pile will be move from one location to another. In general, a well packed pile (>50 pounds/ft^3) with good face management (> 8" fed/day) will not experience prolonged periods of feed heating. For short episodes, adding propionic acid to the specific heating feed is a possible solution. Commonly, adding 0.1-0.2% (wt/wt) of propionic acid to the feed that is heating (generally corn silage) will inhibit yeast. This equates to 2-4 pounds of propionic acid per ton of forage during the heating episodes. Using a current price of $1/lb for propionic acid, the cost to

control heating during feedout would be about $3/ton. This compares to energy costs of $1.50 (10 lbs *$0.15/lb for corn meal) plus the cost of the *L. buchneri* bacteria product (about ~$1.30/ton). The difference being that the propionic acid is applied only during times of heating while a *L. buchneri* bacteria product is applied to the entire crop.

Conversely, inappropriate use of *L. buchneri* bacteria is when native *L. buchneri* bacteria will naturally produce acetic acid. According to data summarized from Cumberland Valley Analytical Service, corn silage that has a dry matter content of 32% or less will typically have acetic acid content greater than 2.5%. These wet corn silages will naturally have high aerobic stability from this high acetic acid. Adding additional *L. buchneri* bacteria to wet corn silages is generally not recommended. **Producer take home message: only use a *L. buchneri* product when additional acetic acid is needed to keep forage cool. However, 3 pounds/ton of propionic acid during feedout may be a more cost effective treatment.**

Conclusion: *L. buchneri* bacteria based inoculants generally will not provide the rapid and significant pH drop during the early fermentation phase of a traditional lactic acid producing inoculant. When the pH is left in an elevated range favorable for *L. buchneri* bacteria, they will grow slowly (> 45 days) to produce acetic acid and 1,2-propanediol. The tricky part of acetic acid is that it will increase the stability of the silage but at a significant fermentation-based dry matter loss. The cost to produce an additional 2% acetic acid is 10 pounds of corn meal. Under current prices, the cost of the *L. buchneri* bacteria product plus the energy loss will approach $2.80/ton. Due to the added risk of dry matter loss associated with slow or poor fermentation, *L. buchneri* inoculants should be considered only in scenarios where heating of forages (i.e., aerobic stability) cannot be controlled through management.

An alternative scenario is to focus on minimizing fermentation losses with a lactic acid producing inoculant that rapidly drops pH (~$1.00/ton) to conserve dry matter. During times when this forage has a tendency to heat, add 2-4 pounds of propionic acid per ton of heating forage during feedout to stabilize the forage (~$3/ton). Adding this rate of propionic acid for 220 days has the same cost as the *L. buchneri* bacteria treatment. Since most farms will not need to add propionic acid for more than 90 days, this options is more cost effective. Once heating become more chronic in nature (> 220 days) the *L. buchneri* bacteria options is more cost effective.

Finally, references to dry matter loss estimates should be separated into fermentation losses and losses from aerobic stability (i.e., feed out). Some companies will combine fermentation losses with feed out losses for marketing reasons. However, they are controlled separately and should be discussed separately.

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