A review of silage inoculants in the New Zealand market

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Yeast and moulds in silage

Water soluble carbohydrates (WSC) - mainly sugars and other longer chain carbohydrates that serve as soluble inoculants.

Silage pH - describes acidity of silage.

Enterobacteria and Clostridia in silage - undesired microorganisms that can cause spoilage in the absence of air.

Buffering Capacity (BC) - the ability of forages to resist to a drop in pH.

Butyric acid - undesired end product of fermentation.

Enterobacteria and Clostridia in silage - undesired microorganisms that can cause spoilage in the absence of air.

Fermentation Coefficient - calculated from DM%, BC and WSC; describes how well a forage will ensile.

Granular inoculants - products are applied in dry form i.e. powder is applied via dispenser.

Homofermentative lactic acid bacteria - convert sugar only into lactic acid (see Table 8).

Lactic acid - desired end product of fermentation to drop pH for good forage conservation.

Lactic acid bacteria species - species is a biological class (like cows vs sheep).

Lactic acid bacteria strains - within species are strains (like breeds within species cow).

Silage additive - substance added to forage either microbial, chemical, nutrient or absorbent to improve silage quality.

Silage ammonia % of Total N - describes how much of protein was degraded in silage fermentation.

Silage fermentation - the process of microorganisms converting sugars (more precisely water soluble carbohydrates - WSC) into acids in the absence of air.

Silage inoculant - contain bacteria selected to dominate the fermentation of crops in the silo.

Silage pH - describes acidity of silage.

Soluble inoculants - products are applied in a liquid form i.e. sprayed onto forage.

Water soluble carbohydrates (WSC) - mainly sugars and other longer chain carbohydrates that serve as substrate (food) for silage bacteria.

Yeast and moulds in silage - undesired microorganisms that can cause spoilage when air is present.

Summary

Silage making is becoming increasingly important in New Zealand. Improved management practices have been developed and products to improve silage quality, particularly silage inoculants, have entered the market. However, it is not easy for farmers to evaluate the effectiveness of different silage inoculants on the farm. The objective of this report is to outline the usefulness of silage inoculants and provide guidelines for good decision making when selecting them.

Good silage management practices can prevent costly losses of quantity and quality. Overseas research has identified that losses in silage stacks and at open silage faces can range from below 10% to 40%. In colder climates, feed conservation has been the subject of extensive research. This is because a large part of ruminant diets is based on silage produced from home grown crops in the warmer seasons, making it an important driver of farm profitability. In Western Europe alone crops grown on nearly 15 million hectares are ensiled annually and supply a large portion of animal feed.

Silage research

In Europe and the USA extensive research has been conducted to identify the important drivers of optimal feed conservation. In the 1970s, research focused on understanding the basics of silage fermentation (i.e. processes in the closed stack), while in the 1980s and 1990s a lot of research dealt with aerobic deterioration of silage (spoilage after stack opening) and the use of silage inoculants.

Key findings from this research include:
- The most effective silage fermentation is performed by lactic acid bacteria converting sugars into acids, preferably lactic acid, to lower the silage pH quickly and reduce the potential effect of undesired microorganisms that compete with lactic acid bacteria for the substrate sugar. Silage microbiologists identified types and activity of silage microorganisms and the dynamics at different phases in the ensiling process.
- Forages differ in their ability to facilitate an efficient silage fermentation depending on their composition i.e. sugar content, buffering capacity (resistance to drop in pH) and dry matter. Benchmarks for crop fermentability were developed. Management advice was developed on how to produce a good crop and undertake good harvest and silage management to favour the lactic acid bacteria dominated fermentation.
- Keeping air out of silage stacks and reducing air exposure at silage feeding determines aerobic stability i.e. the stability of silage against deterioration when exposed to air.

Development of silage inoculants

A better understanding of silage microbiology lead to the development of silage inoculant products to add to forages to improve silage fermentation.

- Specifically selected strains of lactic acid bacteria that are able to convert plant sugar more efficiently and are more competitive than the existing microflora on the plants formed the basis of homofermentative silage inoculants in the 1980s. Some strains in research trials demonstrated the ability to produce a more favourable acid profile with more lactic acid, a faster pH drop, less protein degradation and reduced feed losses. Some of those that improved fermentation also improved the feed value of silage and could demonstrate improved performance in animal trials i.e. more milk and/or meat.
- In the 1990s strains of Lactobacillus buchneri, a heterofermentative species of lactic acid bacteria were found to produce a modest amount of acetic acid out of lactic acid in the first weeks of fermentation. Accumulation of acetic acid in silages treated with L. buchneri has been suggested as the primary reason for improvements in aerobic stability in silages i.e. less heating when exposed to air at the time of feeding.

Benefits of silage inoculants and how to evaluate them

In the 1980s a large number of silage inoculant products entered the market, creating a demand for reliable information on how to evaluate their efficacy. In Europe, different approval schemes were established. The UKASTA scheme in the UK and the German DLG approval scheme independently performed efficacy testing and published results. The manufacturer had to declare active ingredients and prove effects based on scientific trials for different categories. For silage inoculants these effects are mainly improved fermentation, improved animal performance and improved aerobic stability:
- Improved fermentation with homofermentative inoculants has been demonstrated in Europe and the US in a large number of studies. However, 40% of US studies found inoculants had no effect and in German studies, they were not effective in 39% of difficult; 12% of medium; and 7% of easily-fermentable crops.
- A large number of trials resulted in increased aerobic stability when heterofermentative L. buchneri treated silage was compared with untreated silage. However, the German DLG approval scheme reported that
between 22-48% trials in grass silage and between 56-74% trials with maize or earlage silage did not achieve an improvement of two days improved aerobic stability.

- Silage inoculants have also demonstrated benefits in animal performance. However, a US study found only about half of all tested products can claim such effects based on scientific data.

- The use of silage inoculants can be very profitable depending on their specific effects, but these must be proven. Combination effects e.g. improved silage quality as well as improved animal performance will bring the highest returns. The silage inoculant cost and value of milk or meat have an important impact on profitability. Using additives to prevent feed from deteriorating e.g. significantly reducing heating after opening is economical depending on the magnitude of improvement.

- New Zealand research on silage inoculants is limited. One peer reviewed study showed variable efficacy of silage inoculants on fermentation parameters in pasture silage; and improved aerobic stability in maize silage.

- An overview of silage inoculant products in the New Zealand market in the 2015/16 season shows 17 products were advertised by seven companies. Of these, 16 contain different species of lactic acid bacteria and the remaining one claims a technology without live bacteria.

- Although there is scientific evidence that silage inoculants can improve silage quality, improve animal performance and keep silage cool, they do not always work. The type of bacteria present, the presence of live organisms at harvest, application techniques and the type of forage they are added to will determine product efficacy.

**Look for the following when selecting silage inoculant products**

**Research**

An effective silage inoculant will have independent, statistically analysed, and published data supporting its use.

**Type of bacteria and targeted effects**

Some bacteria strains have shown their effect in improving silage fermentation and sometimes animal performance. If improved aerobic stability is required, look for a product with *L. buchneri*.

**Application rate**

The industry standard for application rates is a minimum of 100,000 colony forming units/gram (cfu/g) forage. A good product will have their guaranteed bacteria application rate printed on the label and the company can explain how they ensure viable bacteria are in the product at the time of use.

**Service**

The product should be supplied by a company with competent service that will support customers to make the right decisions selecting a product and provide help when required.

**Price**

Price alone should not be the driving factor for choosing an inoculant. Remember: research and ensuring good product quality are most important.

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**Introduction**

Silage making is a common practice in most countries that feed ruminants, especially in countries with hard winters, to preserve surplus feed grown in the warmer season or as a strategy to allow year round feeding of high quality forages.

Significant areas in Europe and North America are harvested for silage, a big part being grass silage, maize silage and lucerne (alfalfa) in North America. Animal production in these countries relies heavily on the production of sufficient amounts of good silage.

Wilkins (2005, p.114) notes that techniques to achieve good silage quality were not available in Western Europe until the late 1960s. Critical factors that needed to be satisfied before rapid adoption took place were (i) the ready availability of sheets of polythene, which provided rapid and effective sealing of silage; (ii) progress in mechanisation, with development of forage harvesters capable of harvesting rapidly, chopping efficiently and initiating good conditions for air exclusion and rapid fermentation through better compaction and release of plant juices; and (iii) knowledge of fermentation control and the ability to direct fermentation through either wilting or the use of easily-applied acid additives. Once this technical package was available, the production of silage in the United Kingdom increased by at least 20 percent per year for a number of years.

Wilkins and Wilkinson (2015, p.28) summarized the themes at Silage Conferences for the last 45 years and concluded that 1970-78 were dominated by themes of new technology or better compaction; 1982-86 by plant juices; and 1991-95 by new additives, cheaper and faster harvesters and better silo bags. Since 2000, the dominant topics have been the use of new technologies to improve aerobic stability.

It is recognised that feeding supplements profitably requires good management. The use of silage inoculants is considered part of best practice and as such, their use has increased. However, it is not easy for farmers to evaluate effects on farm. The objective of this report is therefore to outline the usefulness of silage inoculants and provide some guidelines for good decision making when selecting them.

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**Table 1.** Estimated areas of crops harvested for silage in Europe and North America in 1994 (Wilkinson et al. 2003, p.15).

<table>
<thead>
<tr>
<th>Area</th>
<th>Perennial grasses</th>
<th>Maize</th>
<th>Legumes</th>
<th>Whole crop cereals</th>
<th>Other crops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. Europe</td>
<td>9,646</td>
<td>3,890</td>
<td>182</td>
<td>324</td>
<td>641</td>
</tr>
<tr>
<td>E. Europe</td>
<td>1,332</td>
<td>1,288</td>
<td>193</td>
<td>208</td>
<td>1,577</td>
</tr>
<tr>
<td>Russia</td>
<td>9,736</td>
<td>7,500</td>
<td>375</td>
<td>--</td>
<td>2,251</td>
</tr>
<tr>
<td>Total</td>
<td>20,714</td>
<td>12,678</td>
<td>--</td>
<td>532</td>
<td>4,469</td>
</tr>
<tr>
<td>North America</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>2,268</td>
<td>750</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>166</td>
<td>486</td>
<td>304</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2,434</td>
<td>--</td>
<td>500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Percent of farms in different farm systems (DairyNZ, 2016).

<table>
<thead>
<tr>
<th>Year</th>
<th>Low input1</th>
<th>Farm system</th>
<th>Medium input2</th>
<th>High input3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006-07</td>
<td>59%</td>
<td>Farm system</td>
<td>28%</td>
<td>13%</td>
</tr>
<tr>
<td>2014-15</td>
<td>33%</td>
<td>Farm system</td>
<td>38%</td>
<td>30%</td>
</tr>
</tbody>
</table>

1 Farm system 1 and 2: 4% or less imported feed.
2 Farm system 3: 12% imported feed.
3 Farm system 4 and 5: 24% or more imported feed.
The silage making process and drivers for losses and silage quality

Silage production is the main practice used to store feed produced on farm. It is less dependent on the weather than hay making and thus better adapted to harvesting the crop at the optimum stage of maturity for high nutritional value. Especially for perennial grasses, high digestibility is limited to a few days (Pahlow et al. 2003, p.31).

Basics of silage fermentation

Silage fermentation is the process of microorganisms converting sugars (more precisely water soluble carbohydrates (WSC)) into acids – preferably lactic acid because it is the most efficient acid to lower the pH quickly and to reduce the potential effect of undesired microorganism that compete with lactic acid bacteria for the substrate WSC. Undesirable fermentations from microorganisms such as enterobacteria and clostridia can dominate if the pH does not drop rapidly. Clostridia are a particular risk factor in grass and lucerne silages at lower dry matter and cause significant quality losses.

The effect of crop composition on silage fermentation

Crops vary in their nutrient composition at harvest and also in their suitability for fermentation. Dry matter (DM), water soluble carbohydrates (WSC - mainly sugars), and buffering capacity (BC) have a major effect on silage fermentation and on silage feeding value. These factors have been integrated into a single expression, the Fermentability Coefficient (FC) calculated by Pahlow & Weissbach (1999): FC = DM% + 8 * sugar/ buffering capacity.

Three categories were defined which describe how suitable crops are for a good silage fermentation process (see Table 3).

Examples of difficult fermentable crops (Group A) are lucerne or clover at low DM%, due to the high buffering capacity of legumes which counteracts a fast drop in pH. Maize and cereals however, are examples of easily fermentable crops, because they provide high levels of WSC and a low BC, i.e. enough substrate to allow high levels of lactic acid production and little "resistance" from the buffering of the crop; see more detail in Table 4.

Choosing crops and managing them well to improve fermentability is a key component for good silage management. Wilting crops efficiently without losing sugars is critical for forages like grasses, clover and lucerne, because they are cut at a low dry matter level and wilting leads to an increase in dry matter which improves their ability to ferment well during ensiling.

Factors affecting silage losses

Wilkins (2005, p.118) claimed that preventing air from entering the silage is a key management factor, as the continued ingress of air was shown by Honig (1969) to result in DM losses at the rate of 2% per month in storage. These losses would represent a higher proportion of DM in small rather than large silos and would also increase with increased ambient temperature. In addition to the gaseous losses, oxidation will result in the remaining forage being broken down to form a structure-less and inedible mass. In less extreme conditions, air ingress will result in the growth of surface layers of moulds. (Wilkinson, 1995 p.67-81). A final stage of aerobic loss occurs when silos are opened for feeding. Air penetration will stimulate activity of yeasts and bacteria and result in the metabolism of WSC, fermentation acids and even structural carbohydrates. The extent of heating in the surface zone of the silo gives an indication of the magnitude of loss, which in extreme conditions can reach 30 percent over a 10-day period (Honig & Woolford, 1980). Generally, losses from this source are increased with (i) slow feed-out rate, (ii) heavily wilted silages; and (iii) silages with low contents of acetic and butyric acids. Silages made from temperate forage legumes are much less prone to aerobic deterioration than those made from grasses (Pahlow et al 2002), but the reason for this high stability is not known. Effluent losses may reach 10 percent with very wet crops, and are determined principally by the DM content of the ensiled forage and by silage density, as influenced by silo depth. While effluent represents a loss of nutrients, a greater concern is the risk of water pollution. The high biological oxygen demand (BOD) of silage effluent is notorious. In many countries, there are regulations to prevent watercourse pollution by silage effluent, and these losses would represent a higher proportion of DM.

The silage energy losses and causative factors (MacDonald et al 1991 p.238).

<table>
<thead>
<tr>
<th>Cause of Loss</th>
<th>Classification</th>
<th>Approximate loss (%)</th>
<th>Causative factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual Respiration</td>
<td>Unavoidable</td>
<td>1-2</td>
<td>Plant enzymes</td>
</tr>
<tr>
<td>Fermentation</td>
<td>Unavoidable</td>
<td>2-4</td>
<td>Microorganism</td>
</tr>
<tr>
<td>Effluent or</td>
<td>Mutually unavoidable</td>
<td>5-7 or</td>
<td>DM content</td>
</tr>
<tr>
<td>field losses by wilting</td>
<td></td>
<td>2-5</td>
<td>Weather, technique, management, crop</td>
</tr>
<tr>
<td>Secondary fermentation</td>
<td>Avoidable</td>
<td>0-5</td>
<td>Crop suitability, environment in silo</td>
</tr>
<tr>
<td>Aerobic deterioration during storage</td>
<td>Avoidable</td>
<td>0-10</td>
<td>Filling time, density, silo, sealing, crop</td>
</tr>
<tr>
<td>Aerobic deterioration after unloading</td>
<td>Avoidable</td>
<td>0-15</td>
<td>As above, DM content, silage unloading technique, season</td>
</tr>
</tbody>
</table>

Total 7-40

Table 3. Fermentability of forages.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fermentability Coefficient</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>FC &lt;35</td>
<td>Difficult fermentable forages, such as forages with a low content of fermentable substrate and/or low DM</td>
</tr>
<tr>
<td>B</td>
<td>FC &gt;35 and at &lt; 35% DM</td>
<td>Medium to easily fermentable forages in the lower DM range</td>
</tr>
<tr>
<td>C</td>
<td>FC &gt;35 and DM 35-50%</td>
<td>Medium to easily fermentable forages in the higher DM</td>
</tr>
</tbody>
</table>

Table 4. Suitability for ensiling of forage crops (DLG Verlag 2006).

<table>
<thead>
<tr>
<th>Crop</th>
<th>DM%</th>
<th>Sugar %DM</th>
<th>Buffering capacity</th>
<th>Fermentation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryegrass - fresh</td>
<td>20</td>
<td>17.3</td>
<td>52</td>
<td>47</td>
</tr>
<tr>
<td>Ryegrass - wilted</td>
<td>35</td>
<td>17.3</td>
<td>52</td>
<td>62</td>
</tr>
<tr>
<td>Other grasses - fresh</td>
<td>20</td>
<td>9.2</td>
<td>55</td>
<td>33</td>
</tr>
<tr>
<td>Other grasses - wilted</td>
<td>35</td>
<td>9.2</td>
<td>55</td>
<td>48</td>
</tr>
<tr>
<td>Red clover - fresh</td>
<td>20</td>
<td>11.5</td>
<td>69</td>
<td>33</td>
</tr>
<tr>
<td>Red clover - wilted</td>
<td>35</td>
<td>11.5</td>
<td>69</td>
<td>48</td>
</tr>
<tr>
<td>Lucerne - fresh</td>
<td>20</td>
<td>6.5</td>
<td>74</td>
<td>27</td>
</tr>
<tr>
<td>Lucerne - fresh</td>
<td>35</td>
<td>6.5</td>
<td>74</td>
<td>42</td>
</tr>
<tr>
<td>Maize silage – dough maturity</td>
<td>30</td>
<td>11.0</td>
<td>32</td>
<td>58</td>
</tr>
<tr>
<td>Whole plant cereal silage (barley)</td>
<td>43</td>
<td>6.3</td>
<td>41</td>
<td>55</td>
</tr>
<tr>
<td>Whole plant cereal silage (wheat)</td>
<td>42</td>
<td>5.5</td>
<td>32</td>
<td>56</td>
</tr>
</tbody>
</table>

Table 5. Silage energy losses and causative factors (MacDonald et al 1991 p.238).
Mode of action of silage inoculants

Dominating the fermentation process

At ensiling, crops contain a variety of naturally occurring microorganisms that have an influence on silage quality. Aerobic bacteria dominate prior to ensiling; however lactic acid bacteria generally dominate once anaerobic conditions are established i.e. when silage is well sealed and all oxygen in plant material has been used up in the initial first few hours.

Table 6. Typical populations of bacterial and fungal groups on plants prior to ensiling (Pahlow et al. 2003).

<table>
<thead>
<tr>
<th>Group</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic bacteria</td>
<td>&gt;10,000,000</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>10 - 1,000,000</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>1,000 - 1,000,000</td>
</tr>
<tr>
<td>Yeasts</td>
<td>1,000 - 100,000</td>
</tr>
<tr>
<td>Molds</td>
<td>1,000 - 10,000</td>
</tr>
<tr>
<td>Clostridia (endospores)</td>
<td>100 - 1,000</td>
</tr>
<tr>
<td>Bacilli (endospores)</td>
<td>100 - 1,000</td>
</tr>
<tr>
<td>Acetic acid bacteria</td>
<td>100 - 1,000</td>
</tr>
<tr>
<td>Propionic acid bacteria</td>
<td>10 - 100</td>
</tr>
</tbody>
</table>

During ensiling, lactic acid bacteria dominate and produce lactic acid to generate an anaerobic environment. Other microorganisms including yeasts, fungi, and enterobacteria are present and can cause spoilage if not controlled. Generally lactic acid is the preferred end product of fermentation in silage because it lowers pH more efficiently than other acids. Fast initial acidification is the key to controlling the growth of competing enterobacteria and clostridia. These competitors will continue to grow until an inhibitory low pH has been achieved. The pH at which these microorganisms are inhibited also depends on the dry matter content of the crop (Pahlow et al., 2003).

Inhibiting the growth of detrimental microorganisms helps to reduce proteolysis (degradation of protein) and other plant enzyme activity; and ultimately ensures fermentation with less losses and a good quality silage produced.

There are different kinds of lactic acid bacteria. Homolactic lactic acid bacteria ferment 6-carbon sugars like glucose and fructose (important part of WSC in crops) to lactic acid. They are more efficient producers of lactic acid and generate no CO2 i.e. the pH drops quickly and silage losses are reduced. Therefore, most silage inoculants contain strains of homolactic lactic acid bacteria.

In general, lactic acid bacteria can be divided into two types: homofermentative and heterofermentative.

Homofermentative (glucose) Lactic acid 100 99
Heterofermentative (glucose) Lactic acid, ethanol, CO₂ 76 98
Homofermentative (fructose) Lactic acid, acetic acid, mannitol, CO₂ 95 99
Yeast (glucose) Ethanol, CO₂ 51 99
Clostridia (glucose and lactate) Butyric acid, CO₂ 49 82

Table 7. Predominant fermentation pathways in silage and theoretical recovery of dry matter and energy (Kung et al., 2003).

<table>
<thead>
<tr>
<th>Type of fermentation</th>
<th>End products</th>
<th>DM recovery %</th>
<th>Energy recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homofermentative</td>
<td>Lactic acid</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Heterofermentative</td>
<td>Lactic acid, ethanol, CO₂</td>
<td>76</td>
<td>98</td>
</tr>
<tr>
<td>Heterofermentative</td>
<td>Lactic acid, acetic acid, mannitol, CO₂</td>
<td>95</td>
<td>99</td>
</tr>
<tr>
<td>Yeast</td>
<td>Ethanol, CO₂</td>
<td>51</td>
<td>99</td>
</tr>
<tr>
<td>Clostridia (glucose and lactate)</td>
<td>Butyric acid, CO₂</td>
<td>49</td>
<td>82</td>
</tr>
</tbody>
</table>

Improving silage aerobic stability

Even in well fermented and well managed silos, there is still opportunity for large losses of nutrients due to aerobic spoilage during storage and feed out. Aerobic spoilage starts with heating. It is initiated by acid tolerant aerobic microorganisms present in silage as a result of air infiltration. Yeasts are the most important microorganisms, oxidising residual sugars and lactic acid, causing heating and pH rise which favours growth of other organisms e.g. moulds (Pahlow et al. 2003 p.76). Hornig et al (1998) presented losses of net energy of between 16 and 38% resulting from silage with a slow feed-out rate of 1 m per week, air penetration 1-2 m into the silage and heating occurring in 1 day.

Both chemical and biological methods have been used to improve aerobic stability and are primarily based on limiting the growth of lactate assimilating yeasts in silage, as these organisms are usually the primary initiators of aerobic spoilage.

The initial commercialisation of Lactobacillus buchneri, a heterofermentative lactic acid bacterium, to improve aerobic stability, was controversial because it went against the dogma that only homofermentative acid bacteria were suitable as silage inoculants. To date, however, specific strains of L. buchneri appear to be the most effective at improving aerobic stability (Kung & Muck, 2015).

Oude Elferink et al. (2001) reported that L. buchneri possesses an anaerobic pathway that degrades lactic acid to acetic acid, 1,2-propanediol and ethanol. Accumulation of acetic acid in silages treated with L. buchneri has been suggested as the primary reason for improvements in aerobic stability in silages (Kung et al., 2003). Muck (2010) mentions the slower speed at which L. buchneri bacteria grow compared to homofermentative silage inoculants. After the initial fermentation is completed by homofermentative lactic acid bacteria, L. buchneri slowly convert lactic acid to acetic acid.

Improving animal performance

European silage additive approval schemes recognise improved animal performance (Weddel et al 2002, Staudacher et al 1999). It is assumed that the typical mode of action for improved animal performance (more milk, more weight gain) is based on improved silage fermentation; with reduced losses, improved acid profile and reduced protein degradation having a positive effect on silage feed value and animal intake which then results in higher energy intake and better animal performance (LDO Verlag 2006 p.178).

On the other side, Kung & Muck (2015 p.75) state: “For more than three decades, animal trials have revealed that some lactic acid bacterial silage inoculants have improved milk production, daily gain and/or feed efficiency. Among these experiments are instances where the inoculant did not affect silage fermentation compared to untreated silage even though animal productivity was increased by inoculation. Such cases have been frequent enough to suggest that the inoculated silage is having some effect on the rumen microbial community”. Muck p.51 (2010): “We have evidence that homofermentative inoculants can change rumen fermentation in a way that may explain their effects on gain and milk production. The results also suggest that animal effects may be more strain specific than effects in the silo”.

New developments of silage inoculants

Muck (2010) reports on ‘combination inoculants’ that combine homofermentative strains with L. buchneri. These products are available in the market. They seek to gain the best of both types of inoculants – an initial control of fermentation with better silage quality and improved stability.


Figure 1. Critical pH values for anaerobically stable silages as influenced by dry matter % (Weissbach, 1996).
Benefits of silage inoculants and how to evaluate them

Silage inoculants are the most common silage additives in most countries. Weddel et al. (2002) reported that were 25 inoculants (64%) from a total of 39 silage additive products were approved for the UK additive approval scheme from 1993 to 2001. Muck (2010) writes: “Inoculants are the most common silage additives in the United States. These products contain lactic acid bacteria to supplement the lactic acid bacteria naturally on the crop and help ensure a consistent fermentation in the silo. The standard type of silage inoculant that has been marketed for several decades contains one or more homolactes of lactic acid bacteria. Recently, a heterolactes species, *L. buchneri* has begun to be marketed alone or in combination with homolactes species. The entry of the *L. buchneri* products has made it more difficult to know what, if any, inoculant should be used in making silage”.

Approval schemes for silage inoculants

With an increasing number of silage additives appearing in European markets in the 1990s it became apparent that the industry would benefit from independent recommendations on their potential benefits. Silage is of huge importance in Western Europe; Table 1 shows that crops grown on nearly 15 million hectares are ensiled annually and therefore they supply a large portion of animal feed. Because of this, silage losses and quality have an important impact on farm profitability.

Silage additive quality testing schemes were introduced in the United Kingdom in 1993 (Weddel et al. 2002) and 1991 in Germany (Staudacher et al. 1990). These were voluntary schemes with the main objective to prove product efficacy. Weddel (2001) reports that “Numerous studies on various product types had been reported in the scientific literature and it was apparent that there were major differences in effectiveness between products, particularly amongst the inoculants”.

Testing schemes and experimental facilities were developed to test product effects. Approvals for demonstrated effects are given for different categories: fermentation quality, aerobic stability, effluent run-off and animal performance.

The UK and German schemes are similar. They require statistically significant research results from trials with a scientific protocol which have been reviewed by experts, for an approval in each category. Both schemes also maintain regular independent product quality monitoring.

Since 2003, in the EU, all feed additives, including all silage additives, have required approval before being allowed on the market. The main focus of this legislation is food safety. The EU approval requires submission of an extensive technical dossier covering quality, safety and efficacy.

The following results refer to the United Kingdom and German approval schemes because of their focus on extensive efficacy testing.

Inoculant effects on fermentation quality

The German approval scheme recognises that different crops present different challenges to achieving good silage quality. As shown in Table 3, crops are different in their ability to ferment, so the fermentation coefficient (Table 4) was used to create different groups (ranging from difficult to easily fermentable crops) and silage inoculants were tested for each of these groups.

For each group at least five independent trials have to be conducted (DLG 2000) with results showing a positive effect on silage quality in order to receive approval for the respective group.

Silage quality is compared between inoculated and untreated silage using laboratory scale silos (1-20 litres) with 3 replicates per treatment. The decline of pH after a few days, fermentation acid profile, protein degradation (NH3-N as % of total N) and losses (determined as weight loss) after 90 days are measured.

Results of 15 years testing homofermentive silage inoculants submitted for approval showed, on average, a positive effect on silage quality (Table 9), with only 39% of difficult-, 12% of medium- and 7% of easily-fermentable crops showing no overall significant improvement of silage quality when expressed as a silage quality index. The conclusion is, that proven silage inoculants have, on average, a positive effect in medium-fermentable and easily-fermentable crops.

Weddel (2001) presented results from 2000 farm samples treated with different silage additives and concluded that although there were no statistical differences (as is common with data of this kind) there were clear indications that silage additive treatments had consistently improved the quality of fermentation.

Muck (2010) summarised results of inoculant research: “Various scientists have summarized the published literature, and a general picture emerges of what to expect from homofermentative inoculants. In one of the largest surveys, Muck and Kung (1997) reviewed over 230 trial comparisons (inoculated vs. untreated silage) published between 1990 and 1995. Homolactes inoculants lowered pH and shifted fermentation toward lactic acid and away from acetic acid in approximately 60% of the cases they summarized”.
Inoculant effects on silage aerobic stability

Well fermented silage can still deteriorate under the influence of air when silage is opened and exposed to air. As mentioned before, lactate assimilating yeasts can metabolise lactic acid which elevates silage pH and therefore creates a favourable environment for other detrimental microorganisms e.g. moulds to grow. In addition, silage starts to heat which indicates energy and dry matter losses.

Measuring inoculant effects on aerobic stability is difficult, because factors like air influence prior to opening of sites, compaction density of silage, feed-out rate, ambient temperature and others are variable and influence the measurement. Honig (1986) presented a method to measure aerobic stability in a standardised way. He identified that measuring heat development in a laboratory model was highly correlated with losses in aerobic stability. During fermentation in laboratory silos, silage is removed from the laboratory silos and placed into an insulated container that allows a controlled airflow through the silage. Continuous recording of silage temperature in a temperature controlled room is performed over 7 days. Aerobic stability (h) is defined as the duration of the silage staying cool after exposure to air. Aerobic losses (%DM) are calculated based on the accumulated temperature over time, greater than the ambient temperature, using a formula developed by Horlig.

Again, results of 15 years testing different products revealed that there was on average a positive effect of heterofermentative lactic acid bacteria (L. buchneri) on aerobic stability. However, not all results were positive: 22% of medium fermentable and 48% of easily fermentable grass silage in trials didn’t increase in aerobic stability by >2 days and in maize silage and earlage 56% (medium fermentable) and 74% (easily fermentable) didn’t achieve this target.

Table 9. Effect of silage inoculants on silage fermentation in grass with different fermentability (DLG Verlag 2006 p.172).

<table>
<thead>
<tr>
<th>Fermentability</th>
<th>Difficult</th>
<th>Medium</th>
<th>Easy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr of trials</td>
<td>101</td>
<td>216</td>
<td>103</td>
</tr>
<tr>
<td>Crop DM%</td>
<td>19</td>
<td>25</td>
<td>41</td>
</tr>
<tr>
<td>FC#</td>
<td>29</td>
<td>48</td>
<td>57</td>
</tr>
<tr>
<td>pH</td>
<td>5.10a</td>
<td>4.60b</td>
<td>4.60a</td>
</tr>
<tr>
<td>Acetic acid %DM</td>
<td>3.10</td>
<td>2.80</td>
<td>2.50a</td>
</tr>
<tr>
<td>Butyric acid %DM</td>
<td>3.90a</td>
<td>3.63b</td>
<td>2.20a</td>
</tr>
<tr>
<td>Losses %</td>
<td>12.0a</td>
<td>8.8b</td>
<td>10.0a</td>
</tr>
<tr>
<td>Untreated</td>
<td>Inoculant</td>
<td>Untreated</td>
<td>Inoculant</td>
</tr>
<tr>
<td>DM%</td>
<td>24.40</td>
<td>24.60</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>4.41</td>
<td>4.18</td>
<td></td>
</tr>
<tr>
<td>Ammonia-N (%Total N)</td>
<td>8.50</td>
<td>6.60</td>
<td></td>
</tr>
<tr>
<td>Lactic acid %DM</td>
<td>2.08</td>
<td>2.64</td>
<td></td>
</tr>
<tr>
<td>ME MJ/kgDM</td>
<td>10.20</td>
<td>10.70</td>
<td></td>
</tr>
</tbody>
</table>

The method standardises factors that influence aerobic stability. During fermentation in laboratory silos, silage is exposed to air for 24 hours after 4 and 6 weeks to create a challenge typically causing untreated silage to heat. After 7 weeks of fermentation, 100g dry material of silage is removed from the laboratory silos and placed into an insulated container that allows a controlled airflow through the silage. Continuous recording of silage temperature in a temperature controlled room is performed over 7 days. Aerobic stability (h) is defined as the duration of the silage staying cool after exposure to air. Aerobic losses (%DM) are calculated based on the accumulated temperature over time, greater than the ambient temperature, using a formula developed by Horlig.

In the US a meta-analysis of 43 experiments (Kleinschmit & Kung, 2006) provides an overview of the effects of L. buchneri on silage quality: these inoculants on average reduce lactic acid and increase the amount of acetic acid, with the effect that pH is increased in treated silage compared to untreated silage, because lactic acid reduces pH more effectively. Inoculated silage had reduced yeast counts and increased aerobic stability. In maize silage, those products which were applied at 100,000 cfu/g of crop and above had a larger effect on fermentation and aerobic stability. This analysis also indicated that silage DM losses increase by 1% with the use of L. buchneri products due to the less efficient pathway (see Table 8). There is no reference about the number of trials with positive effects in this meta-analysis.

Conclusion: a large number of trials resulted in increased aerobic stability when silage treated with heterofermentative L. buchneri were compared with untreated silage. The German DLG approval scheme reported between 22-48% of trials with grass silage and between 56-74% trials with maize or earlage silage did not achieve an improvement of two days improved aerobic stability.

Inoculant effects on animal performance

Although silage inoculants were initially used to improve silage quality, their effects on animal performance are also important because they have a significant impact on profitability. Both the UKASTA and DLG approval schemes evaluate animal performance improvements from the use of silage additives. They require statistically significant increases in animal performance from trials performed with a scientific protocol to prove claims.

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The expected improvement for different approvals is listed in Table 12. Although these differences are economically meaningful, they are not very large when it comes to demonstrating a statistical difference. The average standard deviation in milk yield and weight gain is often larger than these differences i.e. it needs careful selection of similar animal groups for the different treatments to perform a good trial.

<table>
<thead>
<tr>
<th>UKASTA1</th>
<th>DLG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestibility (%)</td>
<td>2.5</td>
</tr>
<tr>
<td>Energy MJ NEL/kgDM</td>
<td>0.1 – 0.3</td>
</tr>
<tr>
<td>Feed intake %</td>
<td>8</td>
</tr>
<tr>
<td>Feed efficiency %/kg DM</td>
<td>5% milk; 10% gain</td>
</tr>
<tr>
<td>Live weight gain g/day</td>
<td>90</td>
</tr>
<tr>
<td>Milk yield kg/day</td>
<td>1</td>
</tr>
</tbody>
</table>

1 UK Agric. Supply Trade Assoc. 2 German Agric. Society.

The German DLG approval scheme listed a total of 51 lactic acid bacteria products in June 2016 (DLG 2016), with 23 products receiving approval for improved animal performance (more milk or more live weight gain).

Kung (1998) summarised results of animal response to silage inoculants in the US. About half of all studies showed a positive effect on animal performance.

Conclusions: silage inoculant have demonstrated animal performance benefits. However, only about half of all tested products can claim such an effect based on scientific data.

### Profitability of silage inoculants

The expert group of the German DLG approval scheme included a chapter about “the profitability of silage additives” in their technical manual about silage additives (Raue, 2006). It was stated upfront that the profitability of using silage additives can only be determined based on individual farm conditions. However, the authors also intended to give some general statements.

A modelling scenario was performed with the following assumptions: reduced energy losses in stack of 3%, increased feed energy density of 0.25 MJME/kgDM, increased intake of 0.5 kgDM/cow and day, increased milk yield of 1.1 kg/cow and day and the following cost: 1 UK Agric. Supply Trade Assoc. 2 German Agric. Society.

New Zealand research on silage inoculants

Although results about silage inoculants were published in New Zealand in the 1970s e.g. the use of formic acid and formaldehyde in ryegrass clover (Binnie & Barry, 1976), Rogers et al (1979) and formic acid in lucerne (Lancaster et al, 1977), the author is aware of only one New Zealand paper published in a peer reviewed Journal in recent years. Kienzmann et al (2011) investigated effects of different silage inoculants on silage fermentation in pasture silage and on aerobic stability in maize silage compared to untreated silage. Results show variable efficacy of silage inoculants on fermentation parameters in pasture silage. Only one product showed a significant difference (P<0.01) to untreated silage across six trials in pH decline (see Table 14) and an improved acid profile (lactic/acetic acid ratio) and protein degradation (NH3-N) (see Table 15). Possible reasons for the different performance of products are discussed below.

Silage inoculants claiming improved aerobic stability in maize silage were tested in eight different trials. Standard measurements of silage fermentation acid and propanediol were taken and the Honig air stress method (Honig, 1986) was used to measure aerobic stability. One product showed elevated lactic acid and propanediol and increased aerobic stability, as is expected with a L. buchneri product (see “improving silage aerobic stability”), with the other products not differing from untreated silage.

Some products contain the same species (e.g. L. plantarum); but many contain different strains of the species (e.g. cow is a species and breed is like a strain; B. plantarum (Table 17).

The main silage inoculant products in the New Zealand market

Inoculants are widely advertised in New Zealand. An overview of products and recommendations for their use lists a total of 18 products sold by 7 companies (Table 17).
**Table 17. Overview of silage inoculant products in NZ market** (alphabetical order distributor) as advertised in October 2016. **2x** indicates there are two separate strains of the species present.**

<table>
<thead>
<tr>
<th>Company</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BioStart</strong></td>
<td><strong>Silage King</strong></td>
</tr>
<tr>
<td>PPP Probiotics</td>
<td><strong>Bio Power Gold</strong></td>
</tr>
<tr>
<td><strong>Donaghys</strong></td>
<td><strong>Ecosyl 100</strong></td>
</tr>
<tr>
<td><strong>Grevilla-Ag</strong></td>
<td><strong>Si-Lac</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>BioSil</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>Maize</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>Hay Sil</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>Si-All 4x4</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>Si-All 74</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>Si-All 74</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>Si-All 74</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>Si-All 74</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>1HF0</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>1I2Z</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>1C23</strong></td>
</tr>
<tr>
<td><strong>Nutritech</strong></td>
<td><strong>1CF7</strong></td>
</tr>
</tbody>
</table>

### Factors influencing silage inoculant effectiveness

Many years of inoculant research overseas, and also recently in New Zealand, shows variable results with the use of silage inoculants. Well-designed studies have demonstrated that inoculants can improve fermentation, aerobic stability of silage and improve animal performance. However, a number of studies showed no effect of inoculants in any of these categories. There are several possible reasons for silage inoculants not to work:

#### Active ingredients

In countries with approval schemes (UKASTA and DUL), there are controls of active ingredients and their levels in products. Products with unknown product composition and unproven ingredients are not allowed to be listed in these approval schemes. Weddel (2001) describes the experience of the introduction of the UKASTA approval scheme: "...in the early 1990s, prior to the introduction of the Scheme, up to 40% of the products being marketed in the UK were unable to show scientific proof that they actually worked. In contrast, the products which remained within the scheme were extremely successful in providing scientific evidence of efficacy.

In New Zealand, some products fail to provide information about active ingredients. For example one product claims: "All other inoculants rely on live bacteria whereas our inoculants don’t." Our inoculants boost lactic acid producing bacteria which naturally occur on the grass or crop with Signal Molecule Technology”. Unless the technology has been proven scientifically it can’t be considered an effective ingredient.

Even apparently proven inoculant species e.g. Lactobacillus plantarum are not all the same Contreas-Goves & Muck (2006) developed a Q&A on silage inoculants and answered the following question: Are there performance differences between specific strains of an organism? A. "Yes, for ex. not all Lactobacillus plantarum strains grow at the same speed. Some L. plantarum strains may grow better on alfalfa, others better on corn. Some strains may grow better under drier conditions or higher temperatures than others, etc.”

There seem to be differences between strains regarding their effectiveness on animal performance as well. Muck (2010) p.51: "We have evidence that homologous fermentative inoculants can change rumen fermentation in a way that may explain their effects on gain and milk production. The results also suggest that animal effects may be more strain specific than effects in the silo”.

### How to select a silage inoculant

Kung (2014) and Muck (2010) published some guidelines on how to select a silage inoculant. The following list gives the main points:

1. Research, research, research: An effective silage inoculant will have independent, statistically analyzed, and published data supporting its use. Some companies may claim other products’ research results because their lactic acid bacteria species have the same name. Many bacteria have the same species name (not strain), but they don’t necessarily have the same effects.

2. Factors influencing silage inoculant effectiveness: There are a number of reasons why a product might not change fermentation. First, a product might be a dead or mishandled or misapplied. A significant number of studies never measured the number of lactic acid bacteria in the product they used to make sure that the product contained the number of bacteria claimed on the package.

3. Silage inoculants are live organisms and production quality control and proper product storage handling are fundamental steps in supplying a product that inoculates the guaranteed number on the label to the forage harvested.

4. Product form and application: Some products are delivered dry e.g. as a granular product, and as a water soluble powder that is applied in a liquid form. Muck (2010) p.55 states: “Lactic acid bacteria must be mixed thoroughly with the crop for best results. These bacteria cannot move by themselves. Spraying the silo at the chopper affords several opportunities for the inoculant to be mixed with the crop. Finally, in drier silages (> 40% DM), the inoculants applied wet can get started faster than the dry products that must be moistened by the crop to be activated. Considering all of these issues, products applied wet generally have an edge over dry products. On the day of use two potential issues may affect viability of soluble products: chlorine and temperature. If water is chlorinated it needs to be less than 1ppm or purchase an inoculant that has compounds which react with the chlorine so it doesn’t kill the lactic acid bacteria. Inoculant viability is best below 40 °C. Some companies have done temperature tolerance research specifically for their products.”

5. Forage type: Forages are different and conditions may be too challenging. As shown in Table 3 and 4, fermentability varies widely between different crops. Silage inoculant bacteria need sugar to convert into lactic acid. If it is missing e.g. in a pasture crop that had lower sugar levels from the start and was exposed to several days of rain, then silage inoculant efficacy will be limited. Conversely, a maize crop may already show a rapid pH drop without any silage inoculant applied and an improvement in pH drop might not be possible.
2. Type of bacteria and targeted effects: some bacteria strains, mainly homofermenters, have shown their effect in improving fermentation and sometimes animal performance. If improved aerobic stability is required, one should look for a product with L. buchneri which is a heterofermenter.

3. Application rate: the industry standard for application rates is a minimum of 100,000 cfu/litre of forage. A good product will print the guaranteed bacteria application rate on their label, and the company can outline how the product will print the guaranteed bacteria application rates. Determine your needs based on research, research, and then pick a research-shelf life of silages. Determine your needs based on the crop, silo, or challenges, and then pick a research-inoculant to help.

4. Service: although it is not directly linked to product quality, competent service will support customers to make the right decisions selecting a product and provide help when required.

5. Price: price alone should not be the driving factor for choosing an inoculant. Remember: research, service, application rate, type of bacteria and targeted effects: some bacteria strains, mainly homofermenters, have shown their effect in improving fermentation and sometimes animal performance. If improved aerobic stability is required, one should look for a product with L. buchneri which is a heterofermenter.

Conclusions from Kung (2014): Silage inoculants should not be used in place of good management. However, there are a variety of silage inoculants that are extremely helpful in improving the fermentation and shelf life of silages. Determine your needs based on the crop, silo, or challenges, and then pick a research-proven inoculant to help.

References


DLG (German Agric. Society) (2000) DLG guidelines for the testing of silage additives for compliance with DLG quality label requirements.


