

Methodology of ensiling trials and effects of silage additives

T. Pauly¹, U. Wyss²

¹Swedish University of Agric. Sciences, Depart. of Animal Nutrition & Management, Box 7024, 75007 Uppsala, Sweden, thomas.pauly@slu.se

²Agroscope, 1725 Posieux, Switzerland, ueli.wyss@alp.admin.ch

Keywords: approval schemes, ensiling trials, methodology, silage additives

Introduction

Many farmers and agricultural entrepreneurs find it difficult to choose a suitable silage additive among the large variety of additives on the market. Often additive retailers offer farmers an early season discount, which encourages farmers to buy an entire season's supply of additive long before they know which type of challenges they will encounter and hence which type of additive they require. The only way farmers can get unbiased information about an additive is through repeated ensiling tests made by independent test institutes. Without independent testing of silage additives, it is more or less impossible for the farmer or adviser to judge objectively the efficacy of an additive and to make a sensible choice from the vast variety of products. This contribution will focus on different approval schemes for silage additives within Europe, in particular in which way they test the efficacy of silage additives for the benefit of silage-producing farmers and entrepreneurs. The focus will be on today's two active approval systems, the EU authorisation of silage additives (compulsory) and the German DLG approval scheme (voluntary).

National silage additive schemes in Europe

The first countries in Europe started already in 1979 to test silage additives. At the 11th International Silage Conference in Aberystwyth, Wales in 1996 five approval schemes for silage additives had been presented. In Table 1 characteristics of these silage additive schemes are presented.

Table 1. Characteristics of European silage additive approval schemes active in 1996.

Country	Start	Compulsory	Positive control required	Farm or lab scale	Reference
Finland	1987	Yes	Yes	Both silos	Mannerkorpi et al. 1996
France	1979	Yes	Yes	4m ³ -silo	Demarquilly and Andrieu 1996
Germany	1990	No	No	Lab	Honig and Pahlow 1993 Pahlow and Honig 1996 Staudacher et al. 1999 Honig and Thaysen 2002
Ireland	1994	No	No	Both	Fitzgerald et al. 1996
UK	1995	No	No	Both	Haigh et al. 1996 Weddell et al. 1996 Weddell et al 2002
Switzerland	1979	Yes	Yes	Lab	Wyss and Vogel 1997 Wyss 1997

Unfortunately, although this commonality exists, no uniformity in either the methodology used or the interpretation of test results was obtained. This lack of common methodology and interpretation presented a particular problem for the manufacturers and retailers of additives, who demanded a single approval scheme, which was valid in all European countries. One by one national approval

schemes were abolished and by the time EU regulation No. 1831 acquired legal force (2004), only the German DLG approval scheme was still in use and is so still today.

The EU authorisation of silage additives

At the 11th International Silage Conference in Aberystwyth, Wales, Haigh et al. (1996) presented a proposal for an EU additive approval scheme. Haigh stated that active ingredient authorisation has to be the responsibility of the EU. The prove of effectiveness of formulations had to be delegated to the respective national authorities of individual member states. It was envisaged that approval at EU level allowed an active ingredient to be used throughout the entire EU. However, individual member states could appeal against the decision, if they could present good reasons against it. In Haigh's proposal only chemical active ingredients were listed. At this time, microorganisms and enzymes had not been incorporated into the scheme.

Since 2004 all silage additives in the European Union (EU) require authorisation according to EC Regulation No. 1831/2003 (Article 10) before they can appear on the market. Silage additives are considered to be 'technological additives' if their primary effect targets the improvement of silage quality (EFSA 2012). Additives that are expected to exert their primary effect on animals, are categorised as 'zotechnical additives' and their authorisation is stipulated by other regulations and guidelines, which usually require animal trials. When active components have passed through the authorisation process, which is administrated by EFSA (European Food Safety Authority), and appear on an official whitelist, they can be marketed within the entire EU. The EU authorisation process focuses on safety (regarding handling and intake) and efficacy (regarding mainly improved fermentation or aerobic stability) of single, active components of an additive. All active components of an additive must be authorised before the additive is allowed to appear on the market. Once an active component is authorised, it can be used by any additive company thereafter. This means that the EU certification has only limited value for farmers since most additives contain more than one active component. But the main objective with the EU approval system is to make sure that only safe products are sold within the EU and not to help farmers to choose a suitable silage additive.

To prove the active component's efficacy at least three successful lab-scale trials, lasting ≥ 90 days, are required. Depending on the claimed mode of action, treated silages have to show a significant improving effect against an untreated control treatment. Guidelines resemble the German DLG approval system (see below), but are less versatile regarding which problems they might be able to alleviate. Aerobic stability is determined by monitoring silage temperature over time as applied by most research institutes, but unlike the German guidelines, stability should be determined after about 90 days of anaerobic storage and without any air stress treatment (i.e. air infusion in silos during storage). The lack of an appropriate air stress treatment during storage increases the risk that the less well-fermented silages – usually the untreated controls – will demonstrate better aerobic stability than additive-treated silages. Completely anaerobic conditions such as in lab-scale silos do not mimic farm conditions and make it difficult to demonstrate an additive effect with regard to aerobic stability.

For prove of statistical significance between treated and untreated silages, EU guidelines recommend the use of non-parametric statistical tests such as the Wilcoxon-Mann-Whitney test. This type of test has the advantage that the collected data do not have to follow normal distribution like with commonly practiced analysis of variance (ANOVA) tests. ANOVA evaluations make it sometimes difficult to explain significant differences because not normally distributed parameters have to be mathematically transformed to make them normally distributed. This means that non-parametric tests usually produce probabilities, which are often more reliable and easier to interpret for a majority of readers.

The German DLG additive approval scheme

The German approval system for silage additives was introduced in 1990 by DLG (German Agricultural Society in Frankfurt). DLG is a non-governmental agricultural organisation that has a long history in quality approval of agricultural commodities such as concentrates, plastic films, disinfectants for stables and milking parlours, teat dips, fuels and lubricants, fertilisers, food and wines and other agricultural goods. Quality approved goods receive a 'DLG Quality Mark', which is usually printed on

the package of the approved product and signals to the user that this product had passed through a series of tests and complies with the minimum quality criteria set up by DLG. These tests must be carried out at independent research institutes and in accordance to detailed DLG guidelines (DLG 2018; Thaysen et al. 2007). The DLG committee for silage additives, consisting of 10 independent and 2 DLG-employed scientists, recommends then, based on the delivered trial dossiers, to approve or not approve the 'DLG Quality Mark' for the tested additive. The certification process is kept confidential and only approved products appear on an open DLG website (www.dlg.org/siliermittel). Several of the listed additives are identical and are sold under different names by different retailers. All identical products that wish to carry the DLG Quality Mark of the original product must apply for it and are checked by DLG if they really are identical. Once each year all products on the DLG list of approved additives are sampled and analysed to check that composition and recommended application rates of each additive comply with values from the time of approval.

If an additive company or retailer considers an application for a DLG Quality Mark, the first step would be to choose, which of the different 'action categories' (AC) would be suitable for the additive. Fermentability coefficients (FC) define how easy or difficult forages are expected to ensile. FC values are calculated from DM, sugar (WSC) and buffering capacity (BC) values of the respective forage (Weissbach, 1975; Weissbach 1996). Table 2 lists the available AC within the DLG approval system.

The DLG committee requires a dossier describing at least 5 successful lab-scale ensiling trials for AC1 (fermentation quality), AC2 (aerobic stability) and AC5a (*Clostridium* reproduction) and at least 3 feeding trials for AC4 (animal performance). For AC 6 (methane yield) at least 3 or 5 lab-scale trials are required depending on if the DLG Quality Mark is intended for a single substrate (3 trials) or for several different substrates (5 trials). In addition, the applicant is encouraged not to withhold unsuccessful trials. Unsuccessful trials often come to the DLG committee's attention anyway because its members are part of an informal silage science network in Northern Europe. Other trial reports not complying with DLG guidelines are appreciated as additional information.

AC1 tests (improved fermentation quality) are carried out with lab-scale silos (approx. 1.5 L volume, at least 3-fold replication) comparing untreated controls with additive-treated silages. Silos are stored anaerobically at 25°C for at least 90 days before silo contents are sampled and analysed for DM (corrected for volatiles lost during drying), pH, ammonia-N, organic acids and alcohols. Weight losses (% of initial DM) of silo contents during storage are determined by frequent weighing of silos.

AC2 tests (improved aerobic stability) require an air infusion 28 and 42 days after sealing. The air infusion is achieved by removing plugs from two holes (\varnothing 6 mm) on the lid and bottom of each silo for the duration of 24 h. This will stimulate yeast growth and make most control silages aerobically instable – a vital prerequisite to test the claimed effect of the additive. Exactly 7 days after the last air infusion (i.e. on day 49), silos are sampled and analysed. Aerobic stability is determined by transferring silo contents aseptically to insulated vessels (approx. 1-2 litres). Electronic temperature sensors inserted into the centre of each vessel, monitor individual silage temperatures for a period of at least 7 days at 20°C ambient temperature. A temperature increase in a silage sample is interpreted as increased activity of aerobic microorganisms (commonly yeasts or acetic acid bacteria), which consume mainly sugars (WSC) and lactate for their growth. Aerobic instability is defined as the time for the silage sample to reach 3°C above ambient temperature. Other analyses such as pH, weight losses and yeast counts at start and end of the stability test are used as supporting information.

The AC3 (reduced effluent formation) test will not be described here since no DLG Quality Marks were ever awarded in this category.

Table 2. Action categories within the DLG approval system. FC values indicating ensilability of herbage: $FC = DM, \% + (8 \times WSC, \% DM / BC, g \text{ lactate}/100 g DM)$.

Action category 1	Improved fermentation processes
Field of application	
a	Difficult to ensile forages Fermentability coefficient (FC) < 35 Roughage forages with an insufficient content of water-soluble carbohydrates and/or dry matter (DM)
b	Moderately difficult to easy to ensile forages in the lower DM range FC ≥ 35 ; DM < 35% e.g. grasses, forage legumes, silage maize, whole cereal plants, millet, Sudan grass
c	Moderately difficult to easy to ensile forages in the upper DM range FC ≥ 35 ; DM ≥ 35 to $\leq 50\%$ e.g. grasses, forage legumes, silage maize, whole cereal plants, millet, Sudan grass
d	Each with a sufficient content of water-soluble carbohydrates Grain silage e.g. corn cob mix, earlage, moist cereal grains
e	Special types of forages Forages requiring ensiling agents to develop specific actions e.g. beets, pulps, pressed pulp, stillage, brewers grains or forages for which an ensiling agent is specifically designed
Action category 2	Improved aerobic stability
Forage/substrate type	<ul style="list-style-type: none"> • Grasses or forage legumes, preferably wilted • Silage maize and maize cob products • Whole cereal plants • Cereal crops (cereals, maize) and forage legumes • Root crops • By-products of the food and fermentation industries Depending on the test reports submitted with the application, the use of the DLG Quality Mark may be limited to specific forages/substrate types
Action category 3	Reduced effluent production
Field of application	Forage with low dry matter contents
Action category 4	Secondary effect
a	Ensiling agents also capable of improving the feed intake value of treated silage
b	Ensiling agents also capable of improving the digestibility of treated silage
c Meat	Ensiling agents also capable of improving the beef production value of treated silage
c Dairy	Ensiling agents also capable of improving the milk production value of treated silage
Action category 5	Additional effects
a	Prevention of <i>Clostridium</i> endospore reproduction
b	Specific effects defined by the applicant
Action category 6	Improved methane yield value of silage by:
a	Reducing fermentation losses
b	Preventing secondary heating
c	Specific effects defined by the applicant

AC4 tests (improved animal performance value) require feeding trials with growing or lactating cattle depending on if the AC4 application concerns improved DM intake (AC4a), improved forage digestibility (AC4b), improved beef production value (AC4c_{MEAT}) or improved milk production value (AC4c_{MILK}). Understandably these studies are considerably costlier than lab-scale ensiling trials. This might be the reason why no new applications were handed in during the last decade. Another complication might be a possible conflict with EU regulations, which require that feed additives improving animal performance, are authorised according to guidelines for 'zootechnical additives'. This is why the DLG approval system emphasises that all AC4 claims are secondary effects in contrast to primary effects in categories AC1 (improved fermentation) and AC2 (improved aerobic stability). If companies would be willing to conduct such trials, they have to prioritize compulsory EU legislation over the voluntary DLG approval system.

The AC5a test (reduced clostridial spore reproduction) should be conducted with wet forages analogous to the AC1a test. However, the forage should be inoculated with a sufficiently high amount of clostridial endospores ($\geq 10^3$ cfu/g silage) and the test requires the quantification of spores at the start and end of the storage period (≥ 90 days). Increased spore counts, butyrate and ammonia-N levels are taken as an indication of increased clostridial activity in silages. As to the question of suitable spore strains for the inoculation of forages, Pauly et al. (2008) tested 10 different *Clostridium* spore cocktails in 4 different forages with respect to their ability to produce clostridial fermentation in silage. Each cocktail contained between 1 to 3 different strains. This study confirmed that our previously selected *Cl. tyrobutyricum* strain (strain 213) produced reliably clostridial activity compared to other inoculated silages and was found to be a suitable challenge organism for ensiling trials that focus on the inhibition of clostridial activity.

AC6 tests (improved methane yield value) determine the effect of a silage additive on the methane yield from ensiled crops by comparing each substrate to untreated controls in two procedure tests.

These tests are (Fig. 1):

- Procedure test 1: 90 days fermentation, no air stress (analogous to AC1);
- Procedure test 2: 49 days fermentation, with air stress and aerobic stability test (analogous to AC2).

The ensiling tests and associated test methods are analogous to those in AC1 or AC2.

The specific methane yield is determined:

- in the fresh material;
- in procedure test 1 immediately after removal from the silo, i.e. after 90 days fermentation without air stress;
- in procedure test 2 after 49 days fermentation with air stress after aerobic stability has been tested (ASTA test).

Silage must be removed from the aerobic stability test after ten days at the latest, or three days after control silages have heated up. The control silage is classified as heated up, if two of the three sample replicates have heated up ($>23^\circ\text{C}$).

However, the weight losses during the fermentation period plus the losses during the aerobic stability test (both in g DM) must be taken into account in any event when calculating the overall effects.

This test was developed by Nussbaum (Nussbaum and Staudacher 2012) and Thaysen (Thaysen and Ohl 2015). In 2015, the first product received the DLG Quality Mark in this category.

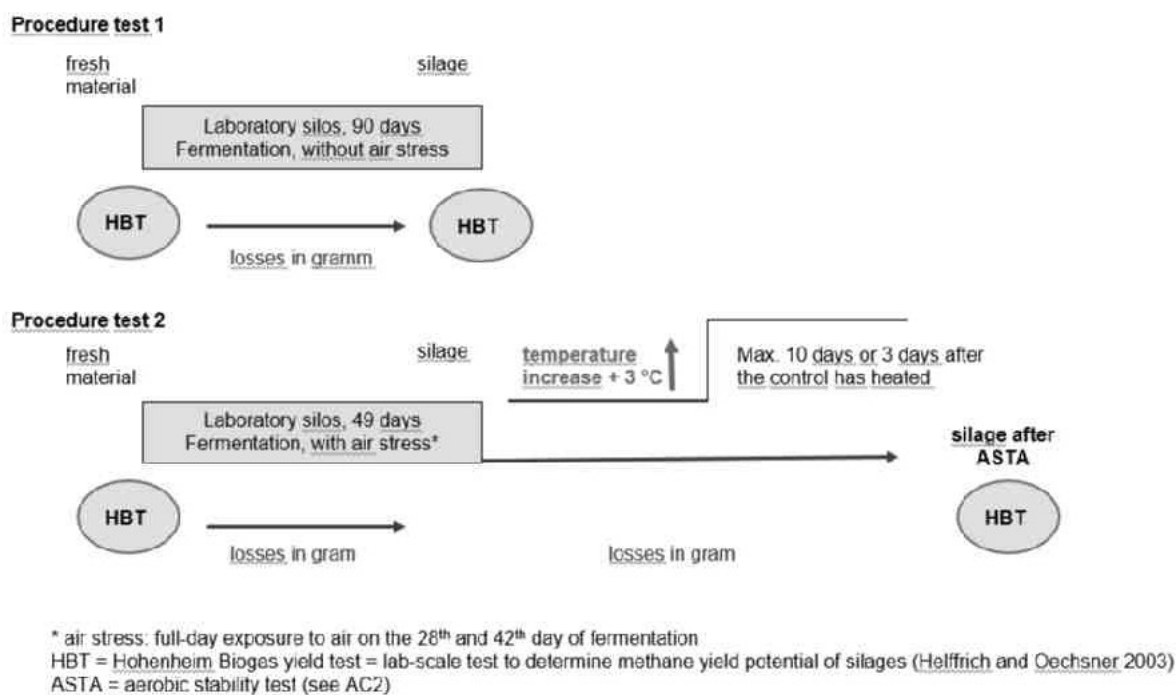


Figure 1. Test scheme for changes in methane yield values of silages associated with the use of ensiling agents for DLG Quality Mark purposes.

The number of DLG-approved silage additives and number of brand owners with at least one DLG-approved silage additive in their portfolio is depicted in Fig.2 below. The number of DLG-approved silage additives was rather constant and varied between 60 – 72 per year during the last years.

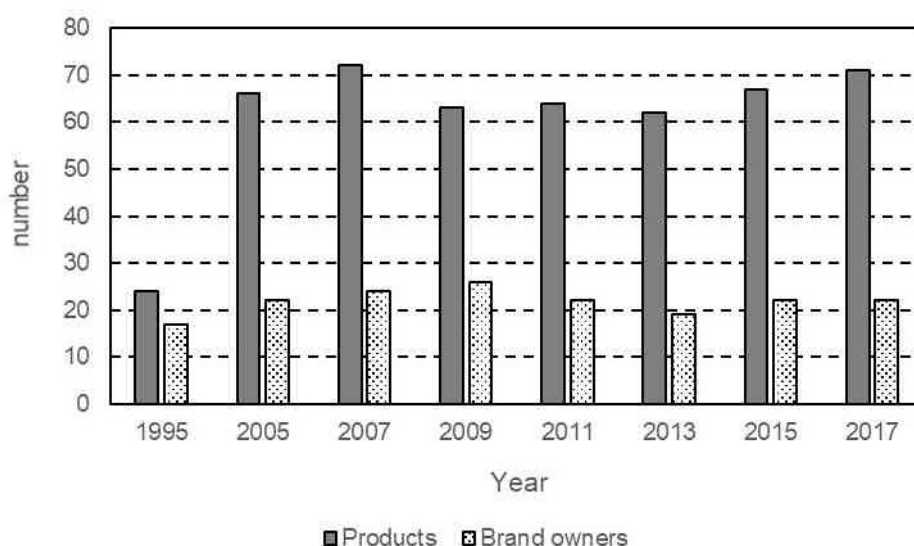


Figure 2. Products and brand owners with a DLG Quality Mark.

Osmotolerance

All biological ensiling agents are additionally tested for osmotolerance during the annual quality test. Lactic acid bacteria with a low osmotolerance do not perform well in high DM silages such as in AC1c. Where the detected osmotolerance levels in products certified for action category 1c are below 30% of

the declared microbial counts over three consecutive years, the respective manufacturer is advised accordingly.

The osmotolerance-test or “Rostock Fermentation Test” (RFT) is an *in vitro* test using forage juice in test tubes. By adding a certain amount of potassium chloride (KCl) it is possible to increase osmolality and simulate higher dry matter levels in the test tubes. The test analyses the activity of natural occurring and supplemented lactic acid bacteria together with the contents of fermentable carbohydrates in the forage (Richter et al. 2010). The pH decrease after 3 days fermentation in tubes with and without KCl will give a good indication about how osmotolerant the additive is. The basic principle of the test is the adaption of the fermentation media to the conditions of a three days fermented silage using the osmolality, a parameter that includes the total concentration of soluble ingredients with osmotic behaviour.

One application of the RFT is the check-up of different silage additives from one year to the next for the DLG. A great advantage of this test is the good standardisation of the test conditions and the fastness compared to ensiling trials. With the help of a cluster analysis it is possible to identify additives that work not very well (Cluster IV).

Comparison of the German DLG and the French INRA schemes

The big difference between the German DLG and the French IRA schemes was the size of the silos and the wilting degree of the forage (Pflaum et al. 1997). In France the test silos had a capacity of 4 m³, which were close to practical conditions and the forage was cut and ensiled without any wilting. In Germany, laboratory silos with a volume of 1.5 litres were used and the forage was wilted to different DM contents. Furthermore, the French approval scheme was compulsory for the authorisation for a product and included an obligatory determination of silage intake and digestibility with sheep for chemical additives. In Germany, the DLG scheme is on a voluntary basis and the applicant chooses among various AC tests according to the additive’s specific mode of action.

In 1994 and 1995 comparative ensiling trials with the same forage and the same wilting degree were carried out in Theix, France, for a direct comparison between the DLG and INRA schemes. In 1995, Switzerland joined in on the comparison. The Swiss approval system is similar to the DLG method.

In Figure 3 fermentation acids of the first trial of 1995 are presented. All silages treated with the inoculant contained in comparison to the untreated silages more lactic acid and less acetic and butyric acid. Also the second trial, where besides a negative control without additive, a chemical additive and an inoculant were tested in 1 L laboratory silos (Germany) and 4 m³ silos (France), showed similar results (Fig. 4). The differences between the fermentation acids between the three countries can partly be explained by the different storage temperatures (inside or outside).

In general, the aim of the silage additive testing system was fulfilled with both methods.

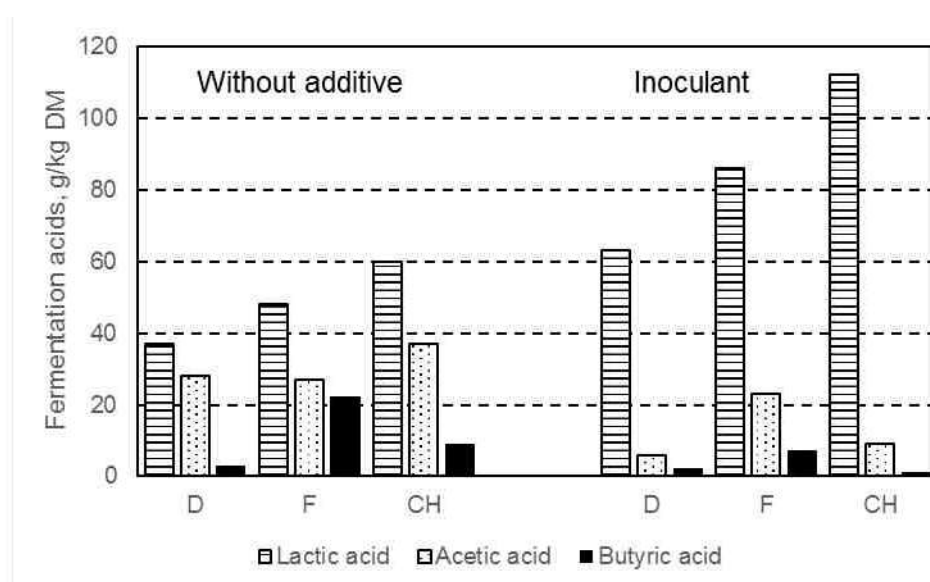


Figure 3. Results of trial 1 in 1995 – ryegrass, second cut, 25% DM, 84 g crude protein/kg DM and 120 g WSC/kg DM (D: Germany; F: France; CH: Switzerland).

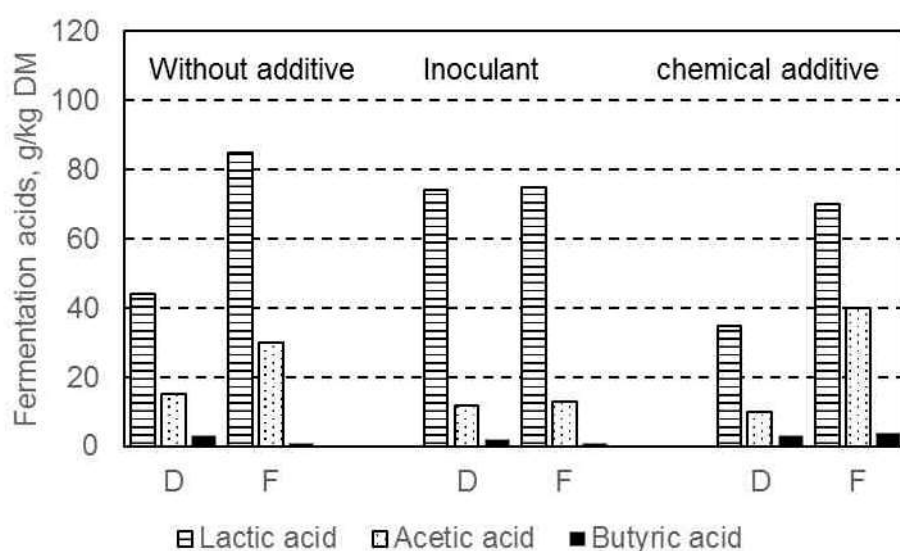


Figure 4. Results of trial 2 in 1995 – ryegrass, second cut, 25% DM, 80 g crude protein/kg DM and 122 g WSC/kg DM (D: Germany; F: France).

Testing silage additives in round bales

Experience from many round bale experiments indicated that some additives, which have proved their efficacy in bunker silos, were often failing in round bales. Two important differences to bunker silage are a) bale silage is recommended to be wilted to 45 – 55% DM and b) bale silage is usually unchopped. We believe that the key issue is how the additive is distributed within the herbage. During baling the additive is sprayed on top of the windrow just when it is fed into the pick-up unit of the baler. A reasonable assumption is that any blending of additive and forage in a baler is rather inefficient given that the forage usually is unchopped. Efforts to apply an additive to moist hay (Charlick et al. 1980; Holden & Sneath 1980) demonstrate the problem of distributing an additive evenly in unchopped forage.

A test scheme for the approval of silage additives for big bales was already presented at the 15th International Silage Conference in Madison (Pauly and Rubenschuh 2009). In 2010 and 2011 trials were carried out in Germany, Sweden and Switzerland with the main goal to compare laboratory silos (1.5 Liter) with round bales (Wyss et al. 2012). In 2010 round bale trials with identical protocols were conducted in Germany, Sweden and Switzerland to compare the effect of two additives against an untreated control. The inoculant contained the strains *L. plantarum*, *L. rhamnosus*, *P. pentosaceus*, *L. buchneri* and *L. brevis* and was applied at a rate of 1 g per tonne, respectively 100.000 cfu/g FM. The product was diluted with water and the application rate was 4 litres / tonne FM. The chemical product contained hexamine, sodium nitrite, sodium benzoate and sodium propionate and was applied undiluted at a rate of 4 litres / tonne FM. The applied dosage of the inoculant amounted 118, 148 and 108% and for the chemical product 131, 138 and 103% of the targeted doses in Germany, Sweden and Switzerland, respectively. This showed us that the application of silage additives in round bales, especially sticking to the target rate, was not easy and required skill and experience. In 2011, the study was repeated in Germany and Switzerland but with a slightly modified protocol. The DM contents were 37 and 41% in Germany and Switzerland, respectively. This time the applied rate for the inoculant amounted to 67 and 113%, respectively. In addition a part of the laboratory silos and round bales were exposed to an air stress treatment. In laboratory silos two 6 mm holes were opened for 24 h (stress 1) one week before silos were sampled. In bales four holes (diameter 20 mm) were made and closed again (taped) after 24 h (stress 2). For another air stress variant 20 holes were made with a nail (diameter 2 mm) and holes were not sealed until bales were sampled seven days later.

In general, the silages from the laboratory silos and round bales had a good fermentation quality. The fermentation was more intensive and the pH was lower in small scale laboratory silos in comparison to round bale silages. The acid profiles of the silages from Sweden and Switzerland (Fig. 5 and 6) show similar responses to the additive treatments and bales versus lab silos. As expected acid formation was larger in the wetter Swiss than drier German forages. The more intensive fermentation in the laboratory silos can be partly explained by the different length of cut of forages. The results of the aerobic stability tests are presented in fig. 7 and 8. In Germany, the aerobic stability in the treated bales was improved in only 2 of 5 cases. Here the low dose rate (67%) can explain this result. In Switzerland, the inoculant did improve the aerobic stability of all laboratory and round bale silages. The experiments indicated that silage additives can be tested in round bales when treated and untreated forages have the same DM content and when silage additives have been applied evenly and at the targeted dose. Furthermore, it is possible to expose round bales to an air stress treatment and thereby create more suitable conditions (i.e. aerobically instable controls) for the testing of silage additives. Aerobic stability of bales is usually not an issue for most farmers since bales are consumed within a day or two. However, an increasing number of horse owners taking care of only few animals require a long aerobic stability when they buy silage or haylage from farmers.

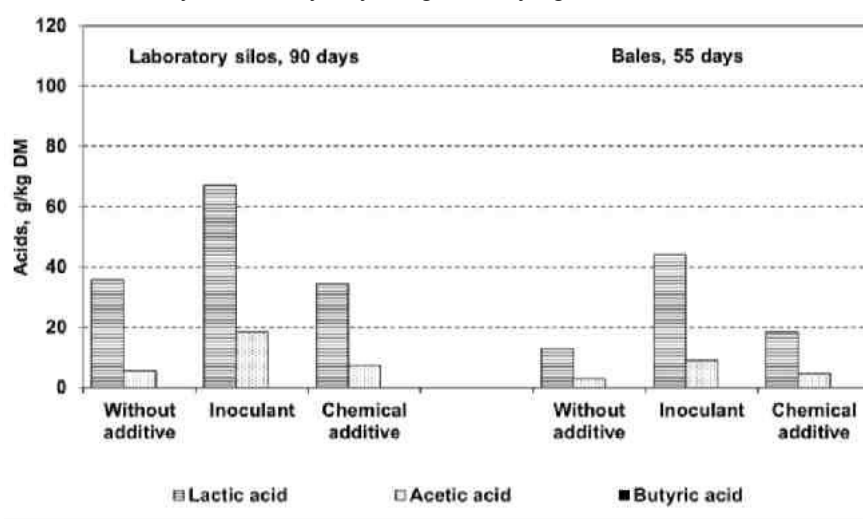


Figure 5. Fermentation acids of treated and untreated silages from Sweden (Herbage: DM 50.2%, crude protein 116 g/kg DM, NDF 495 g/kg DM, WSC 153 g/kg DM)

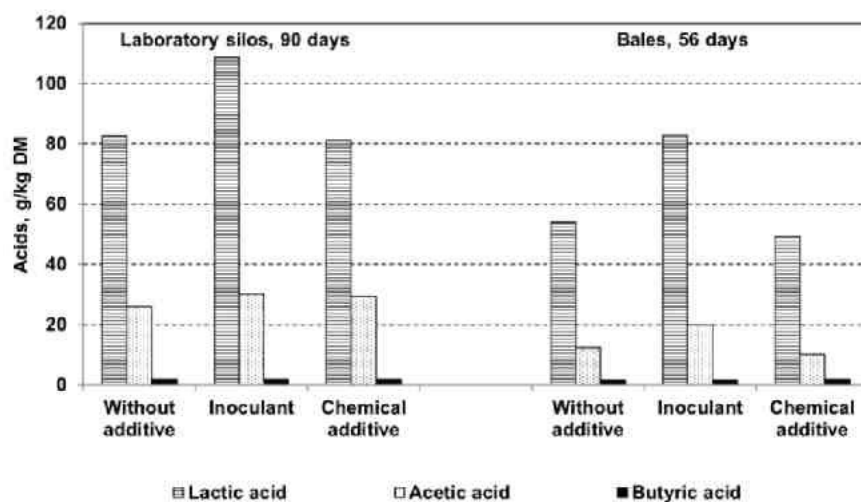


Figure 6. Fermentation acids of treated and untreated silages from Switzerland (Herbage: DM 38.0%, crude protein 195 g/kg DM, crude fibre 214 g/kg DM, WSC 145 g/kg DM)

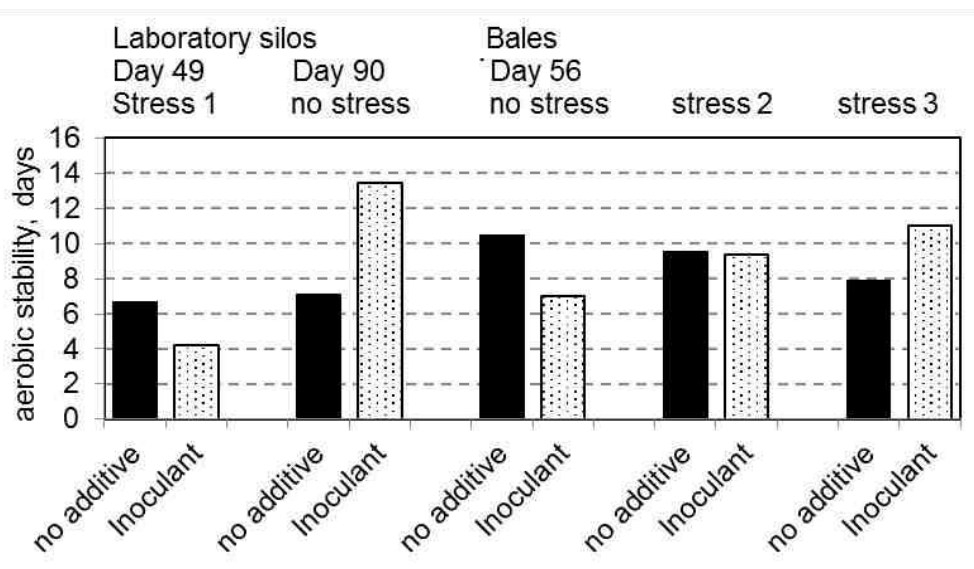


Figure 7. Aerobic stability of the silages from Germany made from the same herbage (Herbage: DM 37.1%, crude protein 128 g/kg DM, crude fibre 290 g/kg DM, WSC 98 g/kg DM). ‘Stress’ indicates different air stress treatments applied during storage.

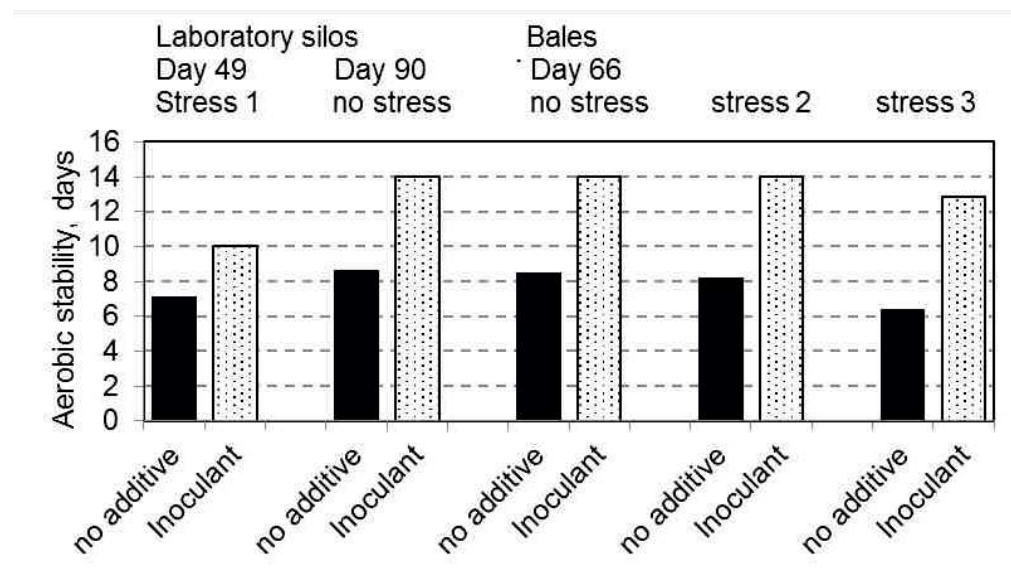


Figure 8. Aerobic stability of the silages from Switzerland made from the same herbage (Herbage: DM 40.6%, crude protein 147 g/kg DM, crude fibre 231 g/kg DM, WSC 167 g/kg DM). 'Stress' indicates different air stress treatments applied during storage.

Future perspectives

Guidelines for the test of silage additives should not be static but should be updated regularly to meet new arising challenges. The DLG Commission for Silage Additives investigates currently the possibility to introduce new test protocols for: a) silage additives, which show a positive response after a shorter storage time (AC2), b) silage additives that reduce the extent of protein degradation during ensilage or c) TMR additives, which extend the aerobic stability of total mixed rations (TMR).

Summary

In the period between 1979 and 1995 five national silage additive approval schemes appeared in Europe (Table 1). Today only two approval schemes are still in use, the EU authorization of additive components (compulsory) and the German DLG approval scheme of complete additives (voluntary). The EU authorization focuses on safety and environmental properties. Efficacy is primarily tested with regard to fermentation quality and aerobic stability (without air stress). Since EU authorization is compulsory for all additives and most additives are composed of more than one single active component, it offers no immediate help to advisors or farmers to help selecting a suitable additive.

The DLG approval scheme has a more consumer-oriented approach and can test complete additives under a rather large variety of conditions, so called 'action categories' or ACs. There are 6 different ACs and most ACs have several subgroups (Table 2). That offers a rather wide variety of conditions under which an additive can be tested. Approved additives get the privilege to carry a 'DLG Quality Mark' with the approved ACs on their package, which signals to the consumers the specific qualities the additive has demonstrated in a series of impartial tests. Additives which fail a test are not made public. After 2005 between 60 and 72 approved additives (Figure 2) are listed each year on an open DLG website (in German: <http://www.dlg.org/siliermittel.html>). This web list represents the only source of impartial information about silage additives in the German speaking regions of Europe and is used extensively by many advisors and farmers.

Comparative trials from 1995 between the German and the French approval schemes were described as well as trials from 2010 to evaluate a DLG test protocol for testing additives in round bales. Round bales present a challenge because additives are usually sprayed on top of the windrow with very limited blending of additive and forage (which remains unchopped!). Many additives do not perform well when not properly distributed in the forage. Other challenges were controlling the application rate

in each bale and minimizing DM variation between the first and the last bale in the experiment. Even experiments which introduce an air stress in bales to create bales with short aerobic stability were presented. An air stress in bales might be important for horse owners, who require a longer period of aerobic stability. With aerobically instable bales it is possible to test additives, which are able to extend the time from bale opening until bales start to heat and mould.

References

- Charlick, R.H., Holden, M.R, Klinner, W.E & Shepperson, G (1980) The use of preservatives in haymaking. *Journal of Agricultural Engineering & Research* 25, 87-97.
- Demarquilly, C. & Andrieu J.P. (1996) Legislation for use of silage additives in France. *Proceedings of the XI International Silage Conference, Aberystwyth, Wales*, 138-139.
- DLG (2018) DLG Testing Guidelines for the award and use of the DLG Quality Mark for ensiling agents", prepared under the auspices of the DLG commission for ensiling agents, at <http://www.dlg.org/siliermittel.html>.
- EFSA (2012) Guidance for the preparation of dossiers for technological additives. EFSA Panel on Additives and Products or Substances used in Animal Food. *EFSA Journal* 2012; 10 (1): 2528, 1-23.
- Fitzgerald, L., O'Kiely, P., Fitzgerald, S. & Murphy, J.J. (1996) Silage Additive Scheme – Republic of Ireland (1996) *Proceedings of the XI International Silage Conference, Aberystwyth, Wales*, 150-151.
- Haigh, P.M., O'Kiely, P., Pahlow, G. & Viuf, B.T. (1996) European Union proposed silage additive scheme. *Proceedings of the XI International Silage Conference, Aberystwyth, Wales*, 140-141.
- Haigh, P.M., Weddel, J.R & Agnew, R. (1996) The United Kingdom forage additive approval scheme. *Proceedings of the XI International Silage Conference, Aberystwyth, Wales*, 142-143.
- Helffrich, D. & Oechsner H. (2003) Hohenheimer Biogasertagstest. *Landtechnik* 3, 148-149.
- Holden, MR & Sneath, RW (1980) Application of preservative chemicals to hay at baling. EGF Occasional Symposium No.11, *Proceedings of a Conference on Forage Conservation in the 80's* (ed. C. Thomas), British Grassland Society, Brighton, UK, 248-251.
- Honig, H. & Pahlow, G. (1993) The DLG-evaluation scheme for silage additives. *Proceedings of the XI International Silage Conference, Aberystwyth, Wales* 148-149.
- Honig, H. & Thaysen, J. (2002) 10 years testing of silage additives by DLG – a comprehensive data evaluation. *Proceedings of the XIIIth International Silage Conference, Auchincruive, Scotland*, 232-233.
- Mannerkorpi, P., Jaakkola, S., Toivonen, V., Heikkilä, T., Huhtanen, P. & Rankanen, R. (1996) The Finnish scheme for official approval of silage additives. *Proceedings of the XI International Silage Conference, Aberystwyth, Wales* 148-149.
- Nussbaum, H. & Staudacher, W. (2012) Yield of methane gas – a new DLG-test scheme for silage additives. *Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland*, 450-451.
- Pahlow, G. & Honig, H. (1996) The German silage additive approval scheme. *Proceedings of the XI International Silage Conference, Aberystwyth, Wales*, 146-147.
- Pauly, T.M. & Rubenschuh, U. (2009) A test scheme for the approval of silage additives for big bales. *Proceedings of the XV International Silage Conference, Madison, USA*, 291-292.
- Pauly, T.M., de Paula Sousa, D., Spörndly, R. & Christiansson, A. 2008. Inoculation of experimental silages with different *Clostridium* spores. In: *Proceedings of the 22th General Meeting of the European Grassland Federation (EGF) 'Biodiversity and Animal Feed'*, Uppsala, Sweden. *Grassland Science in Europe*, vol. 13, p. 678-680.

- Pflaum, J., Gartner, L., Demarquilly, C., Andrieu, J.P., Honig, H., Staudacher, W. & Wyss, U. (1997) Silage Additive Testing. Comparison of the German DLG and the French INRA Schemes. *Das wirtschaftseigene Futter*, 42 (3), 217-248.
- Richter, W., Spieker, H. Schuster, M. & Baranowski, A. (2010) Rostocker Fermentationstest – Nachprüfung biologischer Siliermittel mit DLG-Gütezeichen. *Schriftenreihe der Bayerischen Landesanstalt für Landwirtschaft* 11, 1-65.
- Staudacher, W., Pahlow, G. & Honig H. (...) Certification of silage additives in Germany by DLG. *Proceedings of the XII International Silage Conference, Uppsala, Sweden*, 239-241.
- Thaysen, J., Honig, H., Kalzendorf, C., Spiekers, H. & Staudacher, W. (2007) Silage Additives: Aspects of feed legislation, efficacy of DLG-approved products and recommendations for application. *Übersichten Tierernährung* 35, 55-91.
- Thaysen, J. & Ohl, S. (2015) Effects of silage additives in whole-crop maize and grain silages to enhance methane yield. *Proceedings of the XVII International Silage Conference, Piracicaba, Brazil*, 86-87.
- Weddell, J.R., Haigh, P.M. & Steen R.W.J. (1996) The UK Forage additives Approval Scheme – Development and Guidelines. *Proceedings of the XI International Silage Conference, Aberystwyth, Wales* 144-145.
- Weddell, J.R., Agnew R. & Cottrill B. (2002) The UK Forage Additives Approval Scheme – Developments and Product Approvals. *Proceedings of the XIIIth International Silage Conference, Auchincruive, Scotland*, 230-231.
- Weissbach, F. (1975) Methoden und Tabellen zur Schätzung der Vergärbarkeit. *Akademie der Landwirtschaftswissenschaften der DDR. Agra Markkleeberg*.
- Weissbach, F. (1996) New developments of crop conservation. *Proc. of the 11th International Silage Conference* (eds. Jones *et al.*, IGER), Aberystwyth, Wales. 11-25.
- Wyss, U. (1997) Siliermittel zur Verbesserung der aeroben Stabilität. *Agrarforschung* 4 (7), 273-276.
- Wyss, U. & Vogel, R. (1997) Ergebnisse der Siliermittelprüfung 1996. *Agrarforschung* 4 (3), 121-124.
- Wyss, U., Thaysen, J., Pauly, T. & Rubenschuh, U. (2012) Testing inoculant and chemical additives in round bales in comparison to laboratory silos. *Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland*, 294-295.