Progress in Silage Research in Relation to Animal Production and Food Safety

N. Nishino
Okayama University, Japan

The 21st International Grassland Congress / 8th International Rangeland Congress took place in Hohhot, China from June 29 through July 5, 2008.

Proceedings edited by Organizing Committee of 2008 IGC/IRC Conference

Published by Guangdong People's Publishing House

Progress in silage research in relation to animal production and food safety

N . Nishino
Graduate School of Natural Science and Technology , Okayama University , Okayama 700-8530 , Japan , E-mail : j1oufeed @cc .okayama-u .ac .jp

Key points :

1 .The role of ensiling is expanding from preservation of moist crops to bioprocessing , to reduce contamination by undesirable microbes and chemicals .
2 .Silage can be a good vehicle to increase the population of probiotic microorganisms fed to animals . Research on probiosis is increasing , but criteria for screening and the probiotic functions to be implemented have not yet been clearly defined .
3 .Microorganism community analysis will help us understand competition and synergy among silage microorganisms . Such an approach could accelerate the development of novel inoculants with specific functional properties .

Introduction

Consumers are increasingly concerned about the hygiene and safety of animal foods , because certain diseases in livestock can also threaten human life and health . Toxins , pathogens and contaminants have been studied previously , but these concerns have recently encouraged a greater research emphasis on food safety aspects . Because by-products of animal origin are now prohibited in principle from cattle feed , dairy cows need good protein supplements as alternatives to fish meal . At the same time , researchers are showing increasing interest in protecting and restoring the environment because consumers value more highly foods produced by methods that generate less pollutants and in a sustainable way . Furthermore , because the sharp rise in demand for biofuels has raised the price of concentrate feeds , we need to use forage more effectively than ever , while also caring for the environment and avoiding both animal and human health problems .

Globally , forage is more often preserved as hay rather than silage , although silage makes a major contribution to ruminant feeding in temperate and cold regions (Wilkins *et al* ., 1999) . The increasing use of big bale machinery has meant that hay making can easily be switched to silage making when periods of dry weather are shorter than predicted . This change has increased the production of low-moisture silage with a long length of cut , restricted fermentation and a pH greater than 5 .0 . Inefficient N utilization in silage-based diets may have been ameliorated as a result of the shift from high-moisture to low-moisture silage , but the risk of contamination with enterobacteria , listeria , fungi and other undesirable microorganisms may also have increased . In contrast , in large-scale farming silage may now be more often made with a bunker silo using finely chopped forage compacted without appropriate wilting (Nonaka & Furukawa , 2006) . These two contrasting systems may become more common due to high efficiency of labor and investment ; family-based farmers would prefer large bale preparation while contractors would prefer bunker silo preparation . Forage yields will probably increase in high latitude regions if predicted climate changes occur . Ensiling will sustain its current importance under the predicted unstable weather conditions .

This paper describes recent advancements in silage research . Ensiling is now expected to reduce the risk of pathogens , toxins and contaminants , and improve the health and performance of silage-fed animals . Researchers have shown that although some undesirable microbes and chemicals can be eliminated by fermentation in a silo , others cannot , so that the risk of transmission to animals remains high . Attention has also been directed to examining the probiotic effects of bacterial inoculants because there is evidence that adding specific lactic acid bacteria (LAB) increases dry matter (DM) and cell wall digestibility (Weinberg & Muck , 1996) . The scientific basis of this hypothesis remains inadequate . However silage could serve as a medium to increase the population of probiotic microorganisms before being fed to animals . Such research is therefore worth accelerating , while criteria on the function and properties of probiotics need to be defined .

Ensiling effects on undesirable microbes and chemicals

The principle of ensiling is based on the acidification of moist forage , mainly by LAB , under anaerobic conditions . Undissociated acid is primarily important for suppressing undesirable microorganisms , while dissociated molecules may also have minor effects . Undissociated acid can passively diffuse into microbial cells through the membrane and split into anions and protons inside the cell according to the internal pH , leading to acidification of the cytoplasm , disruption of the proton-motive force , and inhibition of substrate transport , energy-yielding processes and macromolecular synthesis (Ostling & Lindgren , 1993) . Although pH value does not indicate the inhibitory activity of silage , this simple measurement can sometimes work because the normal quantity of undissociated acid could greatly exceed the minimum inhibitory concentration (MIC) for undesirable microorganisms . Big bale silage has made the removal of oxygen from a silo more difficult , which in turn could increase the risk of contamination by undesirable microorganisms . A number of pathogens and chemicals that may or may not be affected by ensiling are described .

Enterohemorrhagic Escherichia coli

Escherichia coli O157 :H7 causes diarrhea , hemorrhagic colitis and hemolytic uremic syndrome in humans . Ruminants are regarded as natural reservoirs of *E . coli* O157 :H7 , and may excrete large numbers of the organism in their feces over long periods . Because this pathogenic organism can persist in feces for up to 3 months , application of cattle manure to pasture may pose a potential risk of infection for animals (Wilkinson , 1999) . The prevalence of *E . coli* O157 :H7 has been shown to be as high as 28% in feces sampled at slaughter houses , indicating that the organism is widely distributed in farmed cattle (Gansheroff & O Brien , 2000) . Ensiling can serve as a means to prevent its entry into the food chain because normal lactate-type silage may contain 10 times more undissociated acid than the MIC for enterobacteria (Ostling & Lindgren , 1993) . However , *E . coli* O157 :H7 may be more tolerant of acid conditions than the usual enteric bacteria , so that concerns about its growth and survival during ensiling have not been allayed . In well-fermented silage the numbers of *E . coli* O157 :H7 decreased from 10^{4-5} cfu/g to below detectable levels within 20 d and addition of homofermentative LAB could help to ensure elimination (Bach *et al* . , 2002) . When the rate of acidification was slow , the population of pathogenic *E . coli* increased at the start of ensiling and remained high at 10^7 cfu/g at 20 d (Fenlon & Wilson , 2000) . Because no marked difference was shown in survival between the usual and enterohemorrhagic *E . coli* , transmission of *E . coli* O157 :H7 could have been avoided by proper ensiling with a combination of low pH , undissociated acid and anaerobic environment .

Mycobacterium avium subsp . *Paratuberculosis*

Paratuberculosis (Johne's disease) is a serious bacterial disease of ruminants that is spreading widely and causing considerable economic losses . The etiologic agent is *Mycobacterium avium* subsp . *paratuberculosis* (MAP) . Infected cows may show symptoms of chronic granulomatous enteritis , with clinical signs of persistent diarrhea and progressive weight loss . Early diagnosis has been difficult because of the long incubation time ; several months are required for a culture test and infected animals show no clinical signs for more than a year (Hasonova & Pavlik , 2006) . Asymptomatic animals may thus shed MAP with feces for a long period and the agent may survive in bovine feces , ponds and soil . Hay and silage could therefore be a source of MAP transmission when harvested from contaminated pastures , although MAP can be eliminated from feces through proper manure management . Katayama *et al* . (2000 , 2004) investigated the effects of fermentation and ammonia treatment to decontaminate MAP . In high-moisture silage the inoculated agent was undetectable even with a high pH of 4 .8 , but its survival was revealed to be high without ensiling or fermentation products . Survival rate increased as the moisture level and fermentation products decreased , suggesting that undissociated acid in water may primarily account for the efficacy of decontamination . Ammonia treatment was shown to exclude MAP from contaminated hay when applied at 3% , while about half of the agent remained detectable when ammonia was applied at 1% . These results indicate that ensiling diminishes the opportunity for MAP transmission and when clinical disease is found a high level of ammonia treatment effectively disinfects and decontaminates hay and silage .

Table 1 *Effect of ensiling and ammonia treatment on survival of* Mycobacterium avium *subsp .* paratuberculosis *in alfalfa silage (Katayama et al . , 2000)*

	DM (%)	Survival (%)	pH	Lactic acid	Acetic acid
Silage	90	100	5 .6	nd	nd
	70	87	5 .4	nd	0 .1
	55	13	4 .8	2 .4	0 .3
	40	0	4 .4	2 .5	0 .6
	25	0	4 .5	1 .2	0 .6
NH_3 1%	70	47	6 .0	nd	nd
3%	70	0	7 .0	nd	0 .1

nd ; not detected .

Mycotoxins

Interest in animal mycotoxicosis is increasing , although fungal metabolites have been proven to cause only a few cases of livestock disease . Teratogenic , carcinogenic and immune-suppressing effects are known with mycotoxins ; thus , a reduction in feed intake , body weight gain , disease tolerance and reproductive capacity might be ascribed to consumption of mycotoxin-contaminanted diets (Scudamore & Livesey , 1998 ; Binder *et al* . , 2007) . Because the principle of ensiling , i .e . , acidification and anaerobiosis , does not suit most molds , exclusion of air is crucial to reduce the contamination by storage fungi such as *Aspergillus* spp . and *Penicillium* spp . (Wilkinson , 1999) . Mycotoxins produced by field fungi such as *Fusarium* spp . , however , appear difficult to remove once growing crops are contaminated . Deoxynivalenol (DON) is considered the most common mycotoxin in silage . The extent of contamination can vary according to region , weather , climate and harvest year . In a survey of mycotoxin contamination in northern Japan , DON was often found in corn silage , mostly at less than 4 mg/kg

(Hiraoka & Deguchi, 2005). Other mycotoxins were not detected in corn silage except for trace levels of zearalenone in a few silage samples. Grass silage did not contain any detectable amount of mycotoxins suggesting that high-energy forage corn is much more susceptible to infection by field fungi. The concentration of DON in forage corn naturally contaminated did not decrease during ensiling (Wilkinson, 1999; Hiraoka & Deguchi, 2005); however, silage fermentation was shown to detoxify a number of mycotoxins (Karlovsky, 1999). This implies that microbes capable of detoxification could be found in the natural environment and thus, research may need to be expanded to isolate mycotoxin-detoxifying microbes. Niderkorn *et al*. (2006) examined the binding of *Fusarium* mycotoxins by LAB; the removal was up to 55% for DON, 82% for fumonisin B1 and 100% for fumonisin B2, and the binding ability differed considerably among LAB strains. The mode of action was assumed to be binding and not biodegradation.

Dioxins

Polychlorinated dibenzo-*p*-dioxins, dibenzofurans and coplanar polychlorinated biphenyls (collectively called dioxins here) are extraordinarily toxic compounds. About 90% of the total human intake in Japan was reported to originate from foods and people fear that dioxins may be concentrated biologically when animals are fed polluted feeds. Dioxins have been shown to contaminate forages by exposure to the atmosphere and not by absorption via the roots from the soil. Although early-maturing corn appeared to have lower concentrations of dioxins than late-maturing corn, the level of contamination per unit area may be limited and no correlation was found between dioxin concentration and period of forage growth (Uegaki *et al*., 2005). Frequent harvesting or a short growing period would not reduce dioxin contamination and unfortunately ensiling did not decrease dioxin concentration. Uegaki *et al*. (2003) examined dioxins in silages made on a laboratory-scale and on a practical-scale and found that ensiling affected neither the concentration nor the isomer profiles. Although a number of aerobic microorganisms have been shown to be able to decompose dioxins, their use in ensiling is difficult. Decreasing air pollution may be a better way of managing contamination of silage by dioxins.

Tools for improving animal production and food safety

Bacterial inoculants

The primary purposes of inoculation, i.e., enhancing the decrease in pH from the time of initial ensiling and reducing energy loss and extensive proteolysis, have almost been achieved. Most of the commercial preparations now contain several strains of homofermentative LAB such as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Enterococcus fecium* and *Pediococcus* spp. Many recipes for LAB mixtures are used according to target forages; recommended preparation may different among grass, corn and whole-crop cereal silages. *Lactobacillus buchneri*, a heterofermentative LAB, is considered an exclusive species which can inhibit aerobic spoilage after silo opening. This was first commercialized as a single-strain inoculant, but is now used in mixtures with homofermentative LAB, probably due to worries about intensive acetic acid fermentation.

Improvement by inoculants is seen much more clearly in fermentation patterns than in animal performance. On average about 30% of bacterial inoculations improved performance in silage-fed animals, while others showed improvements only in storability or sometimes had no effect at all (Weinberg & Muck, 1996). Some of the positive results coincided with increases in DM and cell wall digestibility and in some cases animal performance improved even without advantageous effects on fermentation patterns. Weinberg & Muck (1996) speculated on the presence of probiotic effects and since then research on probiotic inoculants appears to have increased. If an animal consumes 30 kg silage containing 10^{7-8} cfu/g of LAB, a probiotic strain can be delivered to the rumen at 10^{6-7} cfu/ml initially (rumen volume is assumed to be 150 l). These probiotics face competition from other rumen microorganisms and some would flow into the animal's lower gut.

Weinberg *et al*. (2003) examined survival of commercial LAB inoculants in an *in vitro* rumen. They found that after inoculation at 10^7 cfu/ml of LAB, a similar number remained after 72 h. The potential to increase DM and cell wall digestibility was also determined by Weinberg *et al*. (2007), while benefits were limited after 24 h of *in vitro* incubation. Cai (2006) indicated that *L. plantarum* inoculated to silage could be detected in both rumen and feces at 10^6 cfu/g in silage-fed sheep. Survival in the rumen environment does not necessarily imply probiotic potential and criteria for screening and functions to be implemented have not been well defined. Even so, the goal of LAB inoculants should be directed to stable improvement of animal performance. This view is now paramount during the development of novel inoculants for silage.

Probiotics are also being developed as substitutes for antibiotics and to prevent gastrointestinal disorders in animals. Due to concerns over the spread of antibiotic-resistant genes, antibiotics as growth promoters are completely or progressively being banned in the animal industry. Many of the bacteria that cause opportunistic infections in animals are closely related to LAB species and therefore the use of bacteriocins, antibacterial peptides synthesized ribosomally by microorganisms could have potential to avoid such infections in animals. Gollop *et al*. (2006) examined antibacterial activity of LAB inoculants and their respective silages with *Micrococcus luteus* and *Pseudomonas aeruginosa*. Most of the tested strains showed weak or strong activity against target bacteria and part of the activity was imparted to inoculated silage. However, antibacterial activity in silage expressed by LAB inoculants depended on forage types and inoculant strains. A strain of LAB was shown to confer antibacterial activity only to corn silage, while another strain could pass the activity to corn and wheat silages. Further research is warranted on what types of bacteria should be targeted for improving gut function and how the activity could be retained in various crops after ensiling.

Microorganism community analysis

Even in inoculated silages, bacteria other than inoculated species can remain predominant or maintain a significant role. Because many silage microorganisms can produce lactic acid, volatile fatty acids and alcohols, the microorganism community structure is difficult to understand through determination of fermentation products. Conventional cultivation methods might cause a biased view because no information can be obtained on unpredictable microorganisms until suitable media are used. During the past decade, bacterial identification based on molecular methods, especially those including the sequencing of genes coding for ribosomal 16S rDNA (16S rRNA genes), has become an important tool to study microflora in environmental samples (Ercolini, 2004). To realize the beneficial effects of bacterial inoculants and to find out novel mixed preparations for specific use, microflora are better understood as a whole rather than as limited groups. Survival in silage and in the gastrointestinal tract is important when prophylactic function and bacteriocin activity are to be implemented in inoculants. Because conventional plate cultures require much time and labor, methods such as 16S rDNA clone library, PCR-RFLP and PCR-DGGE may soon be used to evaluate the dynamics of microbial populations. Hiraoka *et al*. (2006a, b) used these three methods when examining the microflora associated with whole crop rice ensiling. The materials were found to be contaminated with many organisms of probably soil origin, while after sealing only a few LAB species were shown to predominate fermentation. Survival of added *L. plantarum* was confirmed by these methods, but other bacteria indigenous to the untreated control also survived in inoculated silage. Wang & Nishino (2008) incorporated PCR-DGGE to examine microflora that could account for the high aerobic stability of mixed by-products silage. The method was helpful in narrowing down the candidates to be tested in inoculation experiments and then *L. buchneri* was indentified as the LAB primarily responsible for establishing the stability. These approaches may also work well to indentify microflora that are involved in resistance to deterioration in legume silage and characteristic production of acetate-type silage from tropical forages.

Conclusions

The main purpose of ensiling was formerly to maintain the forage nutritive value as high as that of the parent crop; however, the present aim is increasingly directed to adding benefits and advantages through processing with silage microorganisms. Efforts to prevent toxin formation and pathogen build-up have been made to promote good animal performance and farmer's health, but the present objectives are expanding into improvements of the quality and safety of animal products. Ensiling can inactivate several undesirable bacteria and chemicals and this beneficial action is expected to find application against potent organisms and substances. Probiotic function could be a good addition to attract silage users and consumers because people have now a better understanding of the potential benefits of food microorganisms in human and animal health. However, there is much room for dispute regarding the screening criteria for beneficial microorganisms and which of their functions are to be utilized. There will always be differences in the role of dietary microorganisms between monogastrics and ruminants and many claims of beneficial effects for human health would not be true for high-producing and short-lived livestock. Moreover, feeds and forages cannot be sterilized before inoculation, complicating the treatment effects by synergistic and anergistic activities of indigenous microorganisms. Furthermore, interactions of these microorganisms with plant compounds and rumen bacteria may be difficult to control because of diverse farming practices in relation to area, climate, productivity and managing systems. Considerable improvements are still needed to maintain the security of the food chain from forage production to the consumer.

References

Binder, E. M., Tan, L. M., Chin, L. J., Handl, J. & J. Richard (2007) Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Animal Feed Science and Technology*, 137, 265-282.

Cai, Y. (2006) Progress in silage research and analytical procedures. *Research results 18-3*, *National Institute of Livestock & Grassland Science*, pp. 87-95.

Ercolini, D. (2004) PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. *Journal of Microbiological Methods*, 56, 297-314.

Fenlon, D. R. & J. Wilson (2000) Growth of *Esherichia coli* 157 in poorly fermented laboratory silage: a possible environmental dimension in the epidemiology of *E. coli* 157. *Journal of Applied Microbiology*, 30, 118-121.

Gansheroff, L J. & A. D. O Brien (2000) Escherichia coli O157:H7 in beef cattle presented for slaughter in the U.S.: Higher prevalence rates than previously estimated. *Proceedings of the National Academy of Sciences of the USA*, 97, 2959-2961.

Gollop, N., Zakin, V. & Z. G. Weinberg (2005). Antibacterial activity of lactic acid bacteria included in inoculants for silage and in silages treated with these inoculants. *Journal of Applied Microbiology*, 98, 662-666.

Givens, D. I. & Rulquin, H. (2004) Utilization by ruminants of nitrogen compounds in silage-based diets. *Animal Feed Science and Technology*, 114, 1-18.

Hasonova, L. & I. Pavlik (2006) Economic impact of paratuberculosis in dairy cattle herds: a review. *Veterinarni Medicina*, 51, 193-211.

Hiraoka, H. & K. Deguchi (2005) A report on the security of domestic roughage in Japan. *Research results 17-2, National Institute of Livestock & Grassland Science*, pp. 43-52.
Hiraoka, H., Yamamoto, Y., Urakawa, S., Yamada, H., Karita, S. & M. Goto (2006a) Effective fractions and microbiota of fermented juice of epiphytic lactic acid bacteria of rice plant (*Oryza sativa* L.). *Japanese Journal of Grassland Science*, 51, 379-384.
Hiraoka, H., Yamamoto, Y., Urakawa, S., Yamada, H., Karita, S. & M. Goto (2006b) Effect of fermented juice of epiphytic lactic acid bacteria on fermentation quality of rice whole crop silage in the undesirable harvesting condition. *Japanese Journal of Grassland Science*, 52, 89-94.
Huhtanen, P. & Shingfield, K J. (2005) Grass silage: factors affecting efficiency of N utilization in milk production. In: R. S. Park and M.D. Stronge (eds), *Silage Production and Utilization*, Wageningen Academic Publishers, Wageningen, pp. 35-50.
Johnson, L.M., Harrison, J.H., Davidson, D., Hunt, C., Mahanna, W.C. & K. Shinners (2003) Corn silage management: Effects of hybrid, maturity, chop length, and mechanical processing on rate of extent of digestion. *Journal of Dairy Science*, 86, 3271-3299.
Karlovsky, P. (1999) Biological detoxification of fungal toxins and its use in plant breeding, feed and food production. *Natural Toxins*, 7, 1-23.
Katayama, N., Tanaka, C., Fujita, T., Saito, Y., Suzuki, S. & E. Onouchi (2000) Effects of ensilage on inactivation of *Mycobacterium avium* subsp. *paratuberculosis*. *Grassland Science*, 46, 282-288.
Katayama, N., Shibata, M., Suzuki, T., Ootake, M., Kamata, S. & Y. Yokomizo (2004) Effects of ammonia treatment under various conditions on viability of *Mycobacterium avium* subsp. *paratuberculosis* inoculated in low-moisture roughage. *Grassland Science*, 50, 280-284.
Keady, T.W J. (2005) Ensiled maize and whole crop wheat forages for beef and dairy cattle: effects on animal performance. In: R.S. Park and M.D. Stronge (eds), *Silage Production and Utilization*, Wageningen Academic Publishers, Wageningen, pp. 65-82.
Kozakai, K., Nakamura, T., Kobayashi, Y., Tanigawa, T., Osaka, I., Kawamoto, S. & S. Hara (2007) Effect of mechanical processing of corn silage on in vitro ruminal fermentation, and in situ bacterial colonization and dry matter digestion. *Canadian Journal of Animal Science*, 87, 259-267.
Nonaka, K. & K. Furukawa (2006) A farm survey on the contractor-assisted silage making with bunker silos and the silage quality in Tokachi district, Hokkaido. *Japanese Journal of Grassland Science*, 52, 33-39.
Niderkorn, V., Boudra, H. & D.P. Morgavi (2006) Binding of Fusarium mycotoxins by fermentative bacteria in vitro. *Journal of Applied Microbiology*, 101, 849-856.
Oshita, T., Otsuka, H., Nishino, H., Takayama, H., Takatori, M. Igarashi, H., Nonaka, K. & T. Nakui (1999) Milk production and cost performance of high-producing dairy cattle fed on corn silage in the early lactation period. *Grassland Science*, 45, 59-66.
Ostling, C.E. & S.E. Lindgren (1993) Inhibition of enterobacteria and *Listeria* growth by lactic, acetic and formic acids. *Journal of Applied Microbiology*, 75, 18-24.
Shito, H., Takahashi, K., Shibuya, Y. & N. Yamana (2005) Fermentation quality of silage ensiled by the roll baler for chopped material. *Japanese Journal of Grassland Science*, 51, 87-92.
Shito, H., Yamana, N., Shibuya, Y. & K. Takahashi (2006) Development of the roll baler for chopped materials. *Japan Agricultural Research Quarterly*, 40, 233-237.
Scudamore, K.A. & C.T. Livesey (1998) Occurrence and significance of mycotoxins in forage crops and silage: A review. *J ournal of the Science of Food and Agriculture*, 77, 1-17.
Uegaki, R., Kurokawa, S., Yoshimura, Y., Cai, Y. & Y. Fujita (2003) The fate of dioxins in corn during the silage fermentation process. *Grassland Science*, 49, 352-354.
Uegaki, R., Seike, N. & T. Otani (2005) Influence of atmospheric exposure period on the concentrations of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and coplanar polychlorinated biphenyls (dioxins) in forage. *Grassland Science*, 51, 215-221.
Wang, F. & N. Nishino (2008) Ensiling of soybean curd residue and wet brewers grains with or without other feeds as a total mixed ration. *Journal of Dairy Science*, in press.
Weinberg, Z.G. & R.E. Muck (1996) New trends and opportunities in the development and use of inoculants for silage. *FEMS Microbiology Reviews*, 19, 53-68.
Weinberg, Z.G., Muck, R.E. & Weimer, P.J. (2003) The survival of lactic acid bacteria in rumen fluid. *Journal of Applied Microbiology*, 94, 1066-1071.
Weinberg, Z.G., Shatz, O., Chen, Y., Yosef, E., Nikbahat, M., Ben-Ghedalia, D. & J. Miron (2007) Effect of lactic acid bacteria inoculants on in vitro digestibility of wheat and corn silages. *Journal of Dairy Science*, 90, 4754-4762.
Wilkins, R.J., Syrjala-Qvist, L. & K.K. Bolsen (1999) The future role of silage in sustainable animal production. *Proceedings of 12th International Silage Conference*, Swedish University of Agricultural Sciences, pp. 23-40.
Wilkinson, J.M. (1999) Silage and animal health. *Natural Toxins*, 7, 221-232.