Challenges and solutions for forage conservation for small and large enterprises

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Abstract. Forage conservation, particularly silage making, is one of the major technologies used as an interface between forage production and animal production, and the advanced technology of biological, chemical, and enzymatic additives for making silage has contributed significantly to the development of livestock production. However, the increasing demand for meat and dairy products, severe environmental deterioration induced by livestock production, and the critical risks to human health associated with mycotoxin contamination of forage crops remain to be addressed. Here we review the extant literature regarding treatment with various silage additives in relation to new paddy field forage production systems, mycotoxin contamination of forage crops, and methane and ammonia production from livestock farming.

Keywords: Paste ammonia, conservation, methane, mycotoxins, rice whole crop, silage additive.

Introduction

The consumption of meat and dairy products continues to increase worldwide, with estimates of the increased demand ranging from 229 and 580 million tons of meat and dairy products in the years 1999/2001 to 465 and 1043 million tons in the year 2050, respectively (UN Food and Agriculture Organization 2006). Particularly in Asian countries, which are home to over half of the world's population and where paddy fields occupy over 11% of the world’s total cultivated area (Maclean *et al.* 2002), the demand for livestock products is increasing. Forage and livestock production systems are thus focused on establishing the most effective use of paddy fields, especially surplus fields. For example, rice paddy fields occupy 54% of the total cultivated land area in Japan, and 46% of the paddy field is not planted due to reduced rice consumption and less suitability of other crops that are associated with humid soil conditions. This reduced use of land for rice farming is very serious in view of not only the economics of rice farming but also that of land management, including the control of water, and thus the establishment of forage rice crops is increasingly important, especially in Asian countries.

According to the UN Food and Agriculture Organization (FAO 2006) and the Intergovernmental Panel on Climate Change (2007), worldwide the livestock industry accounted for 18% of the greenhouse gas emissions equivalent to carbon dioxide, and 23% of the methane (CH_4) emission from anthropogenic activities and sources. Severe environmental deterioration induced by nitrogen imbalances from livestock farming have created ammonia pollution on the ground and in the atmosphere, acid rain, and nitrate contamination of underground water. The control of nitrogen (N) pollution and CH_4 emissions produced by livestock farming is another economic concern, since only approx. 50% of total N ingested by ruminants is retained, and the enteric CH_4 emission, a byproduct of mainly fibrous forage digestion by rumen micro-organisms, reached 3%–12% of the loss of dietary gross energy (GE), as calculated based on a value of 55 J/g CH_4. The simultaneous increase of the use and/or efficiency of dietary N and carbohydrate sources is another important goal toward directly reducing their environmental impact and enhancing sustainable development.

In addition, there are serious and valid concerns about the safety of forage. Among the many hygienic challenges involved in forage and livestock production, the existence of mycotoxins is a major problem that can damage the health of not only animals but also humans. Since these toxic compounds often contaminate feedstuff including forage crop silages and many different forage plant species (Scudamore and Livesey 1998; Miller 2008), the difficulty of controlling the generation of mycotoxins at pre- and post-harvest in livestock farming remains.

Since forage conservation, particularly silage making, is the main interface between forage production and animal production, further advances in the technology involving biological, chemical, and enzymatic additives are needed to evaluate how these additives can contribute to improvements in forage and livestock farming, especially in light of the newly developed forage production systems in Asia, the safety of meat and dairy products, and the environmental impact of livestock farming. In this paper, we review the treatment of various silage additives in relation to the new forage production system for paddy fields, mycotoxin contamination of forages, and methane and ammonia production from livestock farming.

Silage production fields in Asian countries

When forage rice is to be fed to cattle, the entire rice plant including panicles, leaves and stems is generally harvested at the yellow ripening stage and conditioned in whole crop silage (WCS). The introduction of round balers with an attached crawler-type driving system successfully improved harvesting operations in wet soil conditions, which had not been achieved for forage production before, and it has contributed to the promotion of rice WCS production and use in Japan (Urakawa and Yoshimura 2003). The development of attachable short-length cutting equipment has resulted in the further improvement of fermentation quality (Kawamoto *et al.* 2007; Urakawa 2009) and has enabled a new conservation system for ensiling total mixed ration (TMR) (Shito *et al.* 2006). The livestock production system in Japan is moving toward regional cooperative farming, where TMR contractor network with rice crop farmers to supply regional self-sufficient forages.

However at the present, the rice WCS system has several problems involving yield, nutrition and feeding management, and fermentation quality. Although a new forage-type cultivar achieved an almost twofold yield (24.1 dry matter [DM] t/ha and 12.7 total digestible nutrients [TDN] t/ha) compared to common grain-type cultivars (Kato 2009), the rice-winter crop rotation system was implemented in order to increase the annual forage yield (Iijima *et al.* 2005; Kamoshita *et al.* 2007). The new cultivar “Tachisuzuka” is characterized by a high straw yield and high sugar content, with a much lower grain:straw ratio (0.08–0.14) and higher sugar content (11.5% DM) compared to the ratio 0.69 and 1.7% DM of the common cultivar “Kusanohoshi” (Matsushita et al. 2011). A higher sugar content in a cultivar is advantageous for achieving preferential fermentation profiles similar to those of forage corn and others, by reducing the influence of soil contamination (undesirable microorganisms) and lower ensiling density of round bales, which is commonly observed with rice WCS production. The lower ratio of grain:straw in the cultivar Tachisuzuka contributes to the reduction of considerable energy losses and difficulty in the feeding management of rice WCS. Shinde (2010) reported that the DM percentage of undigested rice grains was 23% of the amount of the grain ingested as harvested at the dough-ripe stage and 43% of that with advancing maturation to the yellow-ripe stage.

A number of chemical and biological additives have been proposed to improve the microbial profiles, fermentation quality, aerobic stability, and energy loss of forage crops including rice WCS, alfalfa (*Medicago sativa* L.), orchardgrass (*Dactylis glomerata* L.), and other grasses and legumes. Treatment with combinations of homofermentative and heterofermentative bacterial inoculants has been extensively demonstrated to alter the effectiveness of microbial inoculation, since they have shown no interactions and exhibited different beneficial effects on the resulting silage (Hu *et al.* 2009). The shifting of fermentation toward lactic acid with homofermentative lactic acid bacteria improved the fermentation profiles, and that toward acetic acid with heterofermentative lactic acid bacteria improved the aerobic stability and thereby both the performance of the silage and the acceptance of the silage by animals.

The potential of using commercialized lactic acid bacteria inoculants such as *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Pediococcus pentosaceus*, *Enterococcus faecium*, and *Lactobacillus buchneri* has also been described in the literature (Adesogan *et al.* 2003; Hu *et al.* 2009; Cao *et al.* 2011). A commercial lactic acid bacteria inoculant – a strain of *L. plantarum* developed for rice whole crops from a national research project – is also used for ensiling rice forage crops, and strain-dependent effect inoculation of *L. plantarum* on the fermentation quality of rice WCS were observed (Tohno *et al.* 2012).

Fermentation factors such as carbohydrate assimilateion, pH range, temperature allowing growth, and growth regulation of undesirable microorganisms are important, and the diversity of epiphytic lactic acid bacteria (fermented juice of epiphytic lactic acid bacteria; FJLB) was observed to affect the anaerobic fermentation of forage crops including rice whole crop, alfalfa, Italian ryegrass (*Lolium multiflorum* L.), orchardgrass, and some tropical grasses (Cao *et al.* 2002; Yahaya *et al.* 2004; Hiraoka *et al.* 2006). A simple screening of effective microflora by using FJLB, which can be obtained by prefermenting fresh rice leaf and stem fractions for 2–3 days at about 30°C in water containing 1–2% sugars (w/v) and a few drops of acetic acid (vinegar) satisfactorily comprised over 2.0×10^8 colony-forming units per milliliter (cfu/ml) of the compositional percentage of 55% *L. lactis subsp. lactis*, 17% *L. fermentum*, 16% *L. plantarum*, and 11% *Weissella confuse* (Hiraoka *et al.* 2006). The diverse members of *L. lactis subsp. lactis*, *L. fermentum*, *L. plantarum*, and *W. confuse* respond to various environmental and material conditions. Further research using FJLB preparations of mixed and balanced inoculants of homofermentative and heterofermentative lactic acid bacteria is of interest, and is expected to contribute to preferential fermentation quality without reducing the advantages of ease of use and cost-effectiveness.

Antifungal and anti-yeast activities of chemical additives strongly improved the stability of silage when it was exposed to air (Kung *et al.* 2004). Propionic acid-based additives and commercial preservatives (additives containing a mixture of ammonium formate, propionate, ethyl benzoate, and benzoate) did not affect fermentation at low rates but altered the aerobic stability of forage crops including corn, barley, wheat grains and TMR, and, in some circumstances when treated with microbial inoculants, the silage was preferentially fermented

(Adesogan *et al.* 2003; Kung *et al.* 2004; Tyrolová and Výborná 2011). Chemical additives, in combination with enzymatic treatment, certainly improved fermentation quality in the silage (Chuncheng *et al.* 2008; Sun *et al.* 2009), while chemical treatment including urea and sodium bicarbonate alone promoted the *Enterococcus* dominant microbial flora and induced preferential fermentation in whole crop wheat silage (Bal and Bal 2012).

The amino acid fermentation byproduct (AFB), which is the mother-liquid residues of amino acid-producing bacteria and is normally used as an N supplement for ruminants, has been recognized as a very useful silage additive with beneficial effects on the fermentation, aerobic stability, and digestibility of silages of Italian ryegrass, rice whole crop, barley whole crop (*Hordeum vulgare* L.), and corn (*Zea mays*)(Yimiti *et al.* 2006a; 2006b, Okiyama *et al.* 2008, Yamamoto *et al.* 2008, Yimamu *et al.* 2008). The addition of AFB at the ensiling of rice whole crop increased the digestibility of rice grains of lactating dairy cattle, which was advantageous for the feeding management because the byproduct possess the ability to assist the microbes to access morphological and anatomical tissues of forage plants. The *in situ* rumen degradability of DM and NDF of AFB-treated silages was also increased by averages of 7% and 6.5% compared to untreated silages and in some circumstances the milk production was increased after the treatment of rice WCS (Fang *et al.* 2006; Okiyama *et al.* 2008).

AFB additives consistently enhanced the growth of *L. lactis* subsp. lactis, *P. acidilactici*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, and *L. plantarum*, to levels higher than the growth of *Clostridium butyricum* and the growth of yeast isolated from Italian ryegrass silage (Yimiti *et al.* 2006). Aerobic stability after the silo was opened was also in evidence based on lowered lactic acid consumption and lowered pH rising in AFB-added corn silage, although the colony numbers of yeast and fungi were increased, with no significant difference between the untreated and treated silages (Yimamu *et al.* 2008). AFB consistently contains higher concentrations of ammonia N, free amino acids, reducing sugars and carbohydrates and various minerals, showing that concentrations of total N, potassium, calcium, magnesium, and total cations were related to the improvement of both lactic acid fermentation and rumen degradability (Yamamoto *et al.* 2008). Significant improvements in NDF degradability might therefore be expected with the commonly used agricultural byproducts.

Hygienic measures in forage production and conservation

Aflatoxin (AFT), fumonisin (FUM), trichothecin mycotoxins (TRCs) such as deoxynivalenol (DON), nivalenol (NIV), and T-2 toxin (T2) and zeararenon (ZEN), the secondary metabolites produced by fungi, are the major mycotoxins detected in forage crops and silage including corn, sorghum (*Sorghum bicolor* Moench), barley, wheat (*Triticum aestivum* L.), rice plants, and food-processed byproducts including bran, gluten, and corn distillers' dried grains with solubles (Scudamore and Livesey 1998; González *et al.* 2008; Miller 2008). Approximately 2–6.2% of the AFT B_1 ingested was carried over to milk (Fink-Gremmels 2008), and some was metabolized in the rumen and detected as AFT M_1 in the milk; this toxicity is almost the same as that of AFT B_1 (Trucksess *et al.* 1983; Allcroft *et al.* 1968). DON was completely metabolized in the rumen and detected as the de-epoxides in the milk (Miyazaki 2007; Fink-Gremmels 2008). The ZEN was converted into α-zearalenol in the rumen (Kiessling *et al.* 1984), and the ZEN and metabolite in the milk was only detected at low concentrations of 0.06-0.08%.

Several studies have described the occurrence of liver cancer and other toxic mutations in domestic animals and humans due to AFT (Park and Stoloff 1989), liver and renal toxicities in domestic animals and humans and equine leukoencephalopathy due to FUM (Voss *et al.* 2007), alimentary and immune-toxic injuries due to TRCs (Miyazaki 2007), and inactivation of female hormones due to ZEN (Donga *et al.* 2010). Thus the concentrations in feed ar required to be <10 μg/kg for AFT, <100 mg/kg for FUM (outside Japan), <5 mg/ka for DON (in Japan, EU, USA *etc.*) and <100 mg/kg for T2.

Toxic compounds are widely spread throughout Northern, Southeast, and Southern Asia, Oceania, Northern, Central, and Southern Europe, and the Mediterranean littoral (Binder *et al.* 2007). AFT and related compounds (AFTB_1, B_2, G_1, G_2) produced by *Aspergillus flavas* and *A. parasiticus* are detected particularly in the tropical zones, whereas FUM and related compounds (FUM B_1, B_2, B_3) produced mainly by *Fusarium fujikuroi* and *F. proliferatum* are detected especially in corn in temperate zones. T2 produced by *Fusarium* fungi is present primarily in Northern Europe and rarely in the Oceania and Asia. ZEN, produced by *Fusarium* fungi such as *F. graminearum*, is spread widely from the cold to the tropical zones.

Plant breeding has been conducted to obtain new resistant varieties and to deepen the understanding of mechanisms of toxin generation related to climate conditions and plant–pathogen interactions (Oerke *et al.* 2010; Woloshuk and Shim 2013). However, the control of mycotoxins is a complex undertaking in light of the generation behavior of each mycotoxin; for example, as reported for FUM, a higher toxic concentration was observed in plants showing slight or no symptoms of *Fusarium* head blight, while the opposite was also observed (Oerke *et al.* 2010). Another difficulty is that very little information has been obtained about AFT generation behavior during the pre- and post-harvesting periods and the interactions of AFT generation and environmental conditions. At present, it is thus practical to harvest properly at the growing stage before starting the generation of mycotoxins, to ensile forage immediately after harvest, and to maintain the hygienic conditions of silos and animal housing (Magan 2007).

Using as an example FUM in corn, the yellow-ripe stage is suitable for harvesting, since FUM mostly begins to increase in some of the kernels and ears at the yellow-ripe stage (Warfield and Gilchrist 1999; Ariño *et al.* 2009; Uegaki *et al.* 2012a). Uegaki *et al.* (2012b) reported that there were no changes in the FUM concentration after and during the ensiling of contaminated forage crops. Commercial adsorbent might be also practically useful as a method for protecting animals against mycotoxicosis; a mixed adsorbent of zeolites and hydrated sodium calcium aluminosilicates was found to be near-perfect for preventing aflatoxicosis, but no adsorbent possesses complete effectiveness against all of the various types of mycotoxins (Alexander *et al.* 2001). The microbial and/or relative enzymatic degradation of mycotoxins collected by the *Nocardioides* sp. in wheat fields was capable of degrading DON to 3-epi-deoxynivalenol and its intermediates after prolonged incubation (Ikunaga *et al.* 2011), and microbial and enzymatic technology is expected to eventually provide detoxifying systems for toxic fungi in field soil and their secondary metabolite products during ensiling.

Environmental load related to forage production and utilization

Fecal and urinary N excretion per unit of dietary protein ingestion is influenced by low dietary protein quality alone, and in combination with the feeding of low-quality forage. Similarly, the enteric CH_4 emission per unit of organic matter (OM) and dry matter intake (DMI) is extensively influenced by the higher composition and lower digestibility of the fibrous components of feeds. Ominski *et al.* (2006) reported that when four formulations of chopped alfalfa-grass silage ranging in NDF content from 46.4 to 60.8% (DM basis) were fed to steers during the overwintering period, the enteric losses were lower at 27.1 L/kg DMI with a silage of 60.8% NDF than at that of 23.7 L/kg DMI with a silage of 46.4% NDF.

A similar trend was observed when CH_4 emissions were considered as a percent of GE. Therefore, treatment with chemical, biological, and/or enzymatic additives for silage making has been considered worthwhile to reduce the influence of agronomic factors including plant species and variety, fertilization, planting density, harvest time, and climate conditions, not only to improve fermentation and animal performance but also for reducing environmentally hazardous substances as ensiled forages.

Generally, forage conservation and hay or silage making have significant effects on the compositional percentage of non-protein N. It has been reported that the concentrations of non-protein N in silage accounted for 25–85% of the total N, higher than the 10–35% in the original materials (Muck 1988). Lowering the pH to near 4.0 or 4.5 immediately after ensiling is of great importance, since the protein degradation of forage crops is activated by plant proteolytic or silage bacterial activities at pH 5.5–6.0. Therefore, chemical additives, as mentioned above, are effective to control the protein degradation, particularly with lactic acid bacterial additives. The addition of cell-wall degrading enzymes was also effective to reduce ammonia production during silage fermentation, similar to biological treatments (Nadeau *et al.* 2000; Zahiroddini *et al.* 2004; Dehghani *et al.* 2012).

Lactic acid bacterial additives including both homo- and hetero-fermentative lactic acid bacteria were observed to reduce the ammonia concentration in the silages of various forage crops by 9.4–41.1% (average of 22.7%) (Filya 2003; Kung *et al.* 2004; Hu *et al.* 2009; Chen *et al.* 2013). Since nowadays most silage bacterial additives are designed to protect the aerobic stability of the silage, their contribution to the environmental impact is more reliable and effective, although it is influenced by fermentation factors such as plant species, conservation period, pre-treatment of wilting, and year. Cao *et al.* (2002) demonstrated using alfalfa silage that the enhancement of lactic acid fermentation using FJLB significantly increased the *in vivo* DM and OM digestibility, probably as result of the acidic breakdown of pectin-rich alfalfa cell walls. The N retention was also higher at 13.9% of the total indigested N with FJLB-added silage compared to that at 2.1% with the control silage, accompanied by decreased NH_3 production during the ensiling. A similar trend was observed with higher urinary allantoin of cattle fed the FJLB-added silage, which was associated with higher bacterial growth in the rumen.

Methane is produced as a result of the microbial breakdown of forage cell walls in the rumen, reflected in the concentrations of NDF and hemicellulose. Ensiling alfalfa, compared with hay making, was assessed to decrease 32% and 28% of the enteric CH_4 production per unit of energy intake and per unit of DE, respectively, according to a mathematic and dynamic model of rumen digestion in which the fiber content was the primary definition factor (Benchaar *et al.* 2001). That decrease was also estimated to be higher compared to decreases obtained by changing the harvest time and the cutting length. However, anaerobic fermentation does not decrease the NDF and hemicellulose concentrations in all of the resulting silage of various forage crops (Nadeau *et al.* 2000; Zahiroddini *et al.* 2004; Xu *et al.* 2008).

Regarding cell wall constituents and digestibility, the mixture of cellulase and lactic acid bacteria additives is expected to be effective (Xu *et al.* 2008; Sun *et al.* 2009; Xing *et al.* 2009). Xing *et al.* (2009) reported that treatment with cellulolytic enzymes effectively decreased the NDF and ADF concentrations and improved the *in vitro* dry matter digestibility and *in vitro* NDF digestibility. However, those authors observe that the NDF degradation by cellulolytic enzyme activity during the ensiling was primarily the result in the lactic acid bacterial consumption of the enzyme-degradable NDF fraction, the subsequently increased NDF concentration, and the decreased digestibility of enzyme-undegradable NDF residues by rumen microorganisms.

The surfactant Tween 80, registered as a food additive, was reported to increase the water and enzyme absorption and cellulose degradation of grass hay when used in a buffered enzyme solution with a low concentration of 0.005% Tween 80 (Goto *et al.* 2003a). A considerable increase in forage degradability at *in vitro* rumen digestion was also obtained by adding a surfactant at a low concentration, together with significant increases in the ratio of VFA:CH_4. The surfactant probably induces hydration and alters the surface of plant materials, in accord with the nonspecific enhancement of pure culture growth of *Streptococcus bovis*, *Prevotella ruminicola*, *Megashaera elsdinii*, *Selenomonas ruminatium*, *Butyrivibro fibrisolvens*, and *Fibrobacta succinogenes*, which are fiber-, starch-, protein-, and metabolite-degrading bacteria in the rumen (Goto *et al.* 2003b; Kim *et al.* 2005). In addition, AFB, a promising agent for silage additives as described above, was revealed to possess the ability to assist the accessibility of morphological and anatomical tissues to cellulolytic enzymes, probably due to the increased ion strength of higher concentrations of ammonium sulfate and ammonium chloride. The current research suggests the importance of the further development and use of agents that aid microbial and enzymatic additives, in cross-relation to agricultural factors such as forage species and growth and maturation stages at harvest.

Moss *et al.* (1994) studied the enteric CH_4 emissions from sheep fed straw of three types (oat, wheat, and barley), and they found that treatment with ammonia (35 g/kg DM) and sodium hydroxide (45 g/kg DM) decreased the enteric CH_4 emissions per digestible OM by 20.5% and 12.5%, respectively. According to a very common observation following alkali treatment, the application to major forage crops must be considered. Additionally, nutrition and feeding management techniques such as supplementation with ionophores and fumaric and malic acids, plus lipid-rich diets with ingredients such as whole cotton seed, canola seed, sunflower, flax, coconut oil, and copra meal, are important to reduce methane emissions produced by livestock farming (Beauchemin *et al.* 2008).

Conclusion

The main purpose of ensiling is to preserve the nutritive and feeding values of a crop until it is fed to livestock. The present goals are to contribute to the expanding of forage and livestock production areas by increasing fermentation quality and to reduce both environmental deterioration and the risks to food safety. Ensiling technology including the development of new bacterial, enzymatic and chemical additives has already improved the fermentation and aerobic stability, and it has helped improve the nutrition and feeding management of silage. Increased effectiveness and efficiency of silage additives that have contributed to decreased environmental impact and improved livestock production have also been achieved, particularly with new silage additive candidates and supporting agents, as shown by AFB (mother-liquid residues of amino acid producing bacteria) and surfactant Tween 80. However, mycotoxin contamination in forage crops and silage remains a problem that must be addressed by plant breeding, cultivation, and conservation. Due to the possible effects of ensiling technology, especially silage additives, further microbiology research is needed to develop microbial and/or enzymatic detoxification techniques to protect against food contamination and risks to animal and human health.

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