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Review

Effect of Silage Quality on the Health and Performance of High-Producing Dairy Cows: A review

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Silages represent the principal component of the diet of high-producing dairy cows. The objective of this review is to provide a comprehensive summary of the existing literature on the impact of silage quality on the feed intake, performance, and health of dairy cows. The review described the impact of the nutritional quality of silages and fermentation products on dairy cow performance, including the implementation of preventive practices to avoid the undesirable fermentation process. The following section focused on the prevalence of pathogens that present a risk to animals and humans. A summary was provided of the most recent findings on the occurrence of *Clostridium spp*., *Enterobacteriaceae*, *Bacillus spp.* and *Listeria monocytogenes* in silage. The final section of the review addressed the most recent findings on the prevalence of undesirable metabolites, such as mycotoxins and biogenic amines, in silage. The mycotoxin section concentrated on the adverse health effects of elevated mycotoxin concentrations in dairy cow feed rations.

Keywords: dry matter intake, silage additives, microorganisms, aerobic deterioration

1 Introduction

The quality of silages has a significant impact on dry matter intake, milk yield, and the health status of dairy cows. Dry matter intake and milk yield can be influenced by the nutritional value of silages (Khan et al., 2015) and by fermentation products of poorly made silages. Poorly made silages can pose a risk to dairy cow health (Queiroz et al., 2018). The procedures for silage storage are not standardised, and the quality of silage varies in chemical, biological, and fermentation parameters. In particular, the marginal parts of silage have a lower fermentable acid content, a lower pH for the safe preservation of forage, and a poorer nutritional value (Gallo et al., 2016). Since the mid-20th century, biochemical pathways in silage fermentation have been described, and silage additives have been proposed to improve this process. One of the future research perspectives is to improve the hygiene of ensiled feed to improve the production of safe food and animal health (Wilkinson & Rinne, 2018).

2 The influence of silage quality on the feed intake, performance and health of dairy cows

The technological processes employed during ensiling have a direct influence on the nutrient composition, quality of fermentation, digestibility, palatability, and the maximisation of intake by cattle, as well as the prevention of the development of undesirable microorganisms (Borreani et al., 2018). The chemical, microbial and organoleptic components of silage produced during fermentation may be a contributing factor to the poor performance of dairy cattle. The presence of a high acetic acid content, the development of undesirable microorganisms and the poor digestibility of the resulting silage are significant undesirable parameters that have been observed and described (Kung et al., 2018). Other parameters that have been extensively investigated are the use of silage additives in relation to the final quality of the silage, its intake and its effect on the performance of dairy cows (Muck et al., 2018).

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2.1 The effect of acetic acid on feed intake

Acetic acid is an important product in the fermentation of silages. In successful silage production without the use of silage additives, acetic acid levels are low (Grant & Ferraretto, 2018). A meta-analysis conducted by Oliveira et al. (2017) indicated that an inoculant comprising homofermentative and facultative heterofermentative lactic acid bacteria can result in a in reduction the concentration of acetic acid in silage. The typical acetic acid concentration in silages ranges from ≤ 5 to 30 g/kg dry matter. Higher concentrations are typical for the use of heterofermentative bacterial strains and for low dry matter silages (Kung et al., 2018). The utilisation of inoculants comprising heterofermentative lactic acid bacteria was found to elevate the acetic acid content of legume silage by 19.7% and grass silage by 52.9% in comparison to untreated silages (Irawan et al., 2021). In a meta-analysis conducted by Bernardi et al. (2019), the mean acetic acid concentration in silage treated with an inoculant comprising heterofermentative lactic acid bacteria strains was found to be 27 g/kg dry matter of the silage. In a study, Gerlach et al. (2021) compared the results of 33 studies and concluded that acetic acid concentrations greater than 17 g/kg dry matter of the mixed ration significantly reduced dry matter intake. However, the observed correlation between elevated levels of acetic acid and reduced dry matter intake may be confounded by other factors. Increased levels of acetic acid can be attributed to a suboptimal fermentation process, which may result in the production of other metabolites that can reduce dry matter intake. Acetic acid is a significant means of preventing aerobic damage to silages (Arriola et al., 2021). Aerobic deterioration of silage results in a reduction in the nutritive value and causes dry matter losses (Borreani et al., 2018).

2.2 The effect of aerobically damaged silages on performance and feed intake

Aerobically damaged silages are characterised by the development of moulds and yeasts (Wilkinson & Davies, 2013). High yeast concentrations in silages can impair the performance of dairy cattle. Santos et al. (2015) investigated the effect of the yeast *Issatchenkia orientalis* on rumen fermentation *in vitro*. The yeast *Issatchenkia orientalis* is involved in fermentation during aerobic deterioration of silages. The results demonstrate that an elevated yeast concentration in the mixed ratio resulted in a reduction in fibre digestibility and an elevation in propionate and acetate concentrations in the rumen fluid. It was observed that the ingestion of aerobically damaged grass silages (Gerlach et al., 2014a) and alfalfa (Gerlach et al., 2014b) had a negative impact on dry matter intake and on the goat’s preference for that intake.

2.3 The impact of silage composition and quality on rumen microorganisms

The rumen microflora varies based on the type of silage used in the mixed ration (Lengowski et al., 2016). The rumen microflora may be influenced by the representation of bacterial strains used in the inoculant, which may have a probiotic effect. Ellis et al. (2016) observed differing responses according to the type of lactic acid bacteria and the type of substrate employed, with consequences for the digestibility of organic matter and gas production during *in vitro* ruminal fermentation.

2.4 The impact of the fermentation process on the nutritional value of the resulting silage

It has been demonstrated that the nutritional value of silage, in combination with the fermentation products produced during fermentation, influences the feeding behaviour and dry matter intake of dairy cattle (Oliveira et al., 2017). The nutritional value of the resulting silage is influenced by the losses incurred during the ensiling, which depend on the technological practices employed by farmers. These deficiencies in the ensiling process can, among other things, create conditions for the development of undesirable microorganisms (Borreani et al., 2018). These microorganisms are responsible for significant losses of dry matter and energy in the resulting silage, as evidenced by the results presented in Table 1 (Rooke & Hatfield, 2003). The process of dry matter adjustment represents a crucial aspect of preventing significant nutritional losses of the final silage. The high moisture content of the silage material results in a notable loss of the nutritional value of the final silage (Liu et al., 2018). Another factor that can be influenced by technological processes is the digestibility of starch from corn grain. For example, the digestibility of starch increases with the length of corn silage storage (Hoffman et al., 2011; Ferraretto et al., 2015). Furthermore, the temperature during the ensiling period exerts a significant influence on the outcome. This has been demonstrated to affect the *in vitro* degradation of maize silage in the rumen fluid (Ali et al., 2015). The composition of microorganisms in the silo can vary based on regional climatic conditions. These microorganisms subsequently influence the fermentation of the silage (Guan et al., 2018).

Table 1 The acidification and fermentation efficiency of the fermentation pathways of the silage microorganisms (Rooke & Hatfield, 2003)

Microorganism	Metabolic pathway	Substrate	Fermentation end products	DM (% of original)	Energy (% of original)
LAB	Homofermentative	Glucose	2 lactates	96.9	100
LAB	Heterofermentative	Glucose	1 lactate + 1 acetate	79.6	83
LAB	Heterofermentative	Glucose	1 lactate + 1 ethanol	97.2	83
Yeast		Glucose	2 ethanol	97.4	51
Clostridia		Glucose	1 butyrate	77.9	66
Enterobacteriaceae		2 Glucose	2 lactate + 1 acetate + 1 ethanol	88.9	83

DM – dry matter, LAB – lactic acid bacteria

2.5 The effect of silage additives on the performance of dairy cows

The results of scientific studies on the effect of silage additives on dairy cow performance are inconsistent in the literature (Muck et al., 2018). A recent meta-analysis by Arriola et al. (2021) compared the results of 12 papers on silage treatments using a strain of *Lentilactobacillus (L.) buchneri*, *L. buchneri* in combination with other strains and untreated silage samples in relation to selected dairy cow performance parameters. The results indicated that *L. buchneri* treatment had no effect on dry matter intake, dry matter digestibility, and feed conversion. In a meta-analysis of 31 papers, Oliveira et al. (2017) compared the effect of homofermentative and facultatively heterofermentative lactic acid bacteria treatments on the performance of dairy cows in different silage types. Their results indicated that treatment with selected strains of lactic acid bacteria increased milk yield and dry matter intake but did not affect feed conversion and dry matter digestibility. The authors found no significant difference in results when using different concentrations of lactic acid bacteria with 10^5 CFU per gram of forage or 10^6 CFU per gram of forage. They attributed the increase in feed intake to a reduction in the accumulation of compounds, butyric acid, ammonia, and biogenic amines, in the treated silages. Wang et al. (2022) compared the effect of corn silages treated with a mixture of bacterial strains *Lactiplantibacillus plantarum, Pediococcus pentosaceuss* and *L. buchneri* to that of using the bacterial strains individually on rumen fermentation *in vitro*. Their results indicated that the use of a mixed inoculant facilitated microbial digestion of lignified fibres and shifting rumen fermentation toward greater propionate production. Zhang et al. (2019) compared a mixture of heterofermentative and homofermentative lactic acid bacteria, organic salts, and a mixture of bacteria with salts as a treatment for corn silage on rumen fermentation of growing bulls. Their study demonstrated that all treatments resulted in increased dry matter intake and more efficient rumen fermentation of protein. The addition of a mixture of lactic acid bacteria to silage also resulted in an increase in daily gain and a physiological blood serum response, in contrast to treatments with salts and combinations of salt with lactic acid bacteria, where this affect was not confirmed.

3 The presence of pathogenic microorganisms in silage

The presence of pathogenic microorganisms in silage may result in adverse effects on animal health. These microorganisms include *Enterobacteriaceae, Bacillus* spp., *Clostridium* spp. and *Listeria monocytogenes.* The negative effects of poor-quality silages range from mild diarrhoea and reduced feed intake to death and abortion (Queiroz et al., 2018). The prevention of pathogens in silages can be achieved through a number of methods, including the rapid acidification of the mass, the maintenance of an anaerobic environment, good agronomic practices in forage cultivation and harvesting, and the use of silage additives (Driehuis et al., 2018). Bacterial silage inoculants have been demonstrated to reduce the concentrations of pathogenic microorganisms such as fungi, *Salmonella spp., Clostridium (C.) perfringens, and Listeria spp.* (Zielińska et al., 2015). However, the interactions between lactic acid bacteria and pathogens are not fully understood (Fabiszewska et al., 2019). In contrast to the spores of undesirable bacteria, lactic acid bacteria used in silage inoculants do not have the potential to contaminate raw milk (Gagnon et al., 2020).

3.1 Clostridia

Clostridia are gram-positive, sporulating bacteria. The presence of clostridia in silage is associated with soil or slurry contamination. Increased clostridia activity is common in silages with low water-soluble sugar content, high moisture (>70%), high pH (pH >4.6), temperature (>30 °C), and high silage buffering capacity (Queiroz et al., 2018). Silage may contain a variety of species belonging to the genus *Clostridium*, such as *C. tyrobutyricum, C. beijerinckii, C. butyricum, C. sporogenes,* and *C. botulinum* (Doyle et al., 2015). *C. botulinum is* a pathogenic species that can produce the neurotoxin botulinum and cause botulism (Driehuis et al., 2018). Botulism is a rare but life-threatening disease of humans and animals (Seyboldt et al., 2015). The effective prevention of clostridia in silage from wilted forage depends on the achievement of sufficient wilting and the rapid acidification of the silage (Zheng et al., 2018). Bacterial inoculants are composed of lactic acid bacteria, such as *Lactiplantibacillus plantarum*, which have the potential to improve fermentation even under less suitable harvest conditions and with subsequent ensiling at lower than recommended dry matter (Yang et al., 2020). Furthermore, the positive effect of bacteria inoculants and the use of propionic acid in low dry matter alfalfa ensiling has also been demonstrated (Liu et al., 2018). It has been demonstrated that all the previously mentioned preservatives can reduce butyric acid concentrations while maintaining nutritional value. Some chemical additives, such as formic acid and sodium nitrite, when used alone or in combination with hexamine, have been found to be effective in controlling clostridial fermentation during the ensiling of tropical grasses (Gomes et al., 2021). The activity of clostridia in silage can be identified by elevated levels of butyric acid, elevated pH values, elevated levels of acetic acid, elevated ammoniacal nitrogen and elevated soluble protein content (Kung et al., 2018). Some species of clostridia can survive in the gastrointestinal tract of cattle and produce pathogenic toxins, which can negatively affect the performance of dairy cows and cause a risk to the hygienic safety of dairy products (Dunière et al., 2013). Sánchez-Duarte & García (2017) found that the increased ammoniacal nitrogen content in alfalfa silages has a negative effect on milk production. A meta-analysis of 10 studies indicates a linear relationship between increasing ammonia nitrogen content and decreasing milk production.

3.2 Enterobacteriaceae

The Enterobacteriaceae are present in the epiphytic microflora of plants. The optimum pH for their growth is between 6 and 7, at pH 4.5 growth is stopped (Wróbel et al., 2023). The presence of *Enterobacteriaceae* in silage is associated with a reduction in silage quality. *Enterobacteriaceae* are a competitive bacterial species to lactic acid bacteria during fermentation (Wang et al., 2019). They are capable of producing acetic acid, ethanol and converting nitrate to nitrite (Kung et al., 2018). Furthermore, they are able to produce undesirable biogenic amines and ammonia (Li et al., 2022). As previously indicated in Table 1, Rooke & Hatfield (2003) delineated the considerable dry matter and energy losses associated with enterobacterial growth during the silage fermentation process. The study by Cogan et al. (2017) indicated a linear relationship between the presence of enterobacteria in silage and increased somatic cell counts in milk. A reduction in pH is known to inhibit the growth of *Enterobacteriaceae* (Queiroz et al. 2018). It has been observed that higher levels of *Enterobacteriaceae* are present in silages with high moisture content (da Silva et al., 2022). Shiga toxin-producing *Escherichia coli,* which belongs to the *Enterobacteriaceae* family, is associated with severe milkborne disease. The presence of Shiga toxin-producing *Escherichia coli* has been documented in poorly fermented silages (Driehuis et al., 2018).

3.3 Bacillus spp.

The genus *Bacillus spp.* comprises gram-positive bacteria that sporulate and are facultatively anaerobic or aerobic. Their spores are resistant to extreme temperatures, including those encountered during pasteurisation and boiling (Masiello et al., 2014). The most effective method of preventing the development of *Bacillus spp*. in silage is the avoidance of aerobic damage and the use of homolactic lactic acid bacteria in the preservative (Queiroz et al., 2018). The bacterial species *Bacillus cereus* is associated with poor food hygiene. Its endotoxins can pass through milk and cause foodborne illness (Bennett et al., 2013). Some species of the genus *Bacillus spp.* have been proposed as additives in bacterial inoculants due to their production of specific antibacterial peptides or cellulolytic enzymes (Gandra et al., 2016; Guan et al., 2018; Lara et al., 2018; Bai et al., 2020; Bai et al., 2022).

3.4 Listeria monocytogenes

Listeria monocytogenes are undesirable bacteria that can be present in silage and cause listerioses in animals. They are able to penetrate the milk of dairy cows and cause disease in humans (Dhama et al., 2015). The recommended practice for prevention of *Listeria monocytogenes* is to prevent aerobic damage and use preservatives to rapidly lower pH (Driehuis et al., 2018). Some bacterial strains that have been studied and proposed for use in silage inoculants produce bacteriocins that can replace antibiotics and have the potential to control *Listeria monocytogenes* in silages (Amado et al., 2016; Oliveira et al., 2021).

4 Pathogenic metabolites of microorganisms

The presence of certain microbial metabolites in dairy cow rations has been associated with adverse health effects, including decreased performance, and reduced feed conversion efficiency. Frequently occurring microorganisms such as proteolytic clostridia are capable of producing biogenic amines (Queiroz et al., 2018). Furthermore, fungi contamination may result in the production of mycotoxins. It has been demonstrated that cattle can reduce mycotoxins in the rumen microflora. Nevertheless, high concentrations or combinations of these can cause acute or subacute mycotoxicosis (Wambacq et al., 2016).

4.1 Mycotoxins

Silage can contain a plethora of mycotoxins, as detailed in Table 2 (Driehuis et al., 2013). In their study, Vaičiulienė et al. (2021) compared 119 samples of TMR, different types of grass silage, and silage from wilted forage for mycotoxin content in Lithuania. The findings indicated that the highest measured values of deoxynivalenol (DON) and zearalenone (ZEA) were observed in corn silage samples, while aflatoxin B_1 and T-2 toxin were present in grass silage samples. The authors highlighted that maize silage is more susceptible to mycotoxin contamination than grass silage. In a study conducted by Reisinger et al. (2019), 158 corn silage samples from various regions across Europe were analysed. The results demonstrated that the mycotoxins ZEA and DON were present in 67% of the samples, while more than five mycotoxin species were present in 87% of the samples. Manni et al. (2022) analysed 37 samples of grass silage and 6 samples of whole grain cereal silages in Northern Europe, which they divided into groups according to the visibility of fouling. The analysis revealed that 92% of the samples contained mycotoxins, with the visibly fouled group containing mycotoxin in all samples. The mycotoxin levels varied considerably, and the mycotoxin content was heterogeneous. The authors observed that the visibly fouled silages exhibited markedly elevated concentrations of a diverse array of mycotoxins in comparison to the samples without visible fouling, where the concentrations were comparatively lower. Mycotoxin prevention is a multifaceted process that encompasses the prevention of fungal infestation on plants at the field stage. This encompasses a number of factors, including crop rotation, variety selection, tillage, irrigation, fertiliser, and fungicide selection. Furthermore, it is important to consider proper harvest management, including the growth stage, stubble height, establishment technique, soil contamination, and the use of fungicides. Finally, silo management practices are crucial, such as limiting unwanted oxygen exposure through proper covering, compaction, silage removal methods, and the use of silage additives (Gallo et al., 2015; Wambacq et al., 2016). The negative impact of mycotoxins can be mitigated by the use of mycotoxin absorbents (Marczuk et al., 2023). The use of lactic acid bacteria to counteract the presence of mycotoxins is still not yet widely used in commercial practice, although model studies point to complex interactions between lactic acid bacteria and mycotoxins (Fabiszewska et al., 2019).

Table 2 The most prevalent mycotoxigenic fungi and mycotoxins are found in silages and silage crops (Driehuis, 2013)

Mycotoxins group	**Main mycotoxins**	**Producer (type of moulds)**	**Feed**	**Location**
Aflatoxin	Aflatoxin B_1 (M_1), B_2, G_1, G_2	*A. flavus,* *A. parasiticus*	maize	field
Trichothecenes	Type A: T-2, Diacetoxyscirpenol	*F. langsenthiae,* *F. poae, F. sporotrichioides*	maize, cereal	field
	Type B: DON,	*F. graminearum*	maize, cereal,	field

	Nivalenol	*F. culmorum*	grassland	
Fumonisins	Fumonisin B_1, B_2	*F. verticillioides* *F. proliferatum*	maize	field
Resorcylic acid lactones	Zearalenone	*F. verticillioides* *F. proliferatum*	maize, cereal, grassland	field
Ochratoxins	Ochranotoxin A	*A. ochraceus* *P. verrucosum*	cereal	field
Ergot alkaloids	Clavines, lysergic acid amide, ergotamine	*Claviceps purpurea*	cereal	field
	Lolitrem B, ergovaline	*N. lolii* *N. coenophialum*	grassland	field
Penicillium roqueforti toxins	Roquefortine C, polyphenolic acid	*P. roqueforti* *P. paneum*	all types of silage	silage
Aspergillus fumigatus toxins	Gliotixin, fumigaclavines	*A. fumigatus*	all types of silage	silage
Monascus ruber toxins	Monocolin K, citrinin	*Monascus ruber*	all types of silage	silage

A - *aspergillus*, F – *fusarium, N – neotyphodium, P - penicillium*

4.2 The effect of mycotoxins on dairy cattle health

It has been demonstrated that ruminants are capable of degrading certain mycotoxins due to the rumen microbiota present in their rumen. A study published by Valgaeren et al. (2019) revealed a correlation between the composition of the rumen microbiota and the toxicity of DON. The bioavailability of DON was found to be 50.7% in calves with an undeveloped rumen, in contrast to 4.1% observed in calves with a developed rumen. This indicates that the inactivation of mycotoxins is closely related to rumen function. The incidence of rumen disease increases when cow production is high. In an *in vitro* study utilising realistic mycotoxin levels, Debevere et al. (2020) demonstrated the role of the functional rumen in mycotoxin detoxification. The study revealed that in the case of rumen acidosis or lower rumen microorganism activity, which is typical for non-lactating cows, detoxification of enniatin B, DON, and nivalenol was reduced. Mycotoxins can interfere with a wide range of physiological functions in cattle. Non-specific signs of mycotoxicosis may include reduced feed intake, diarrhoea, weight loss, lameness, increased somatic cell count in milk and ovarian cysts. In addition, mycotoxicosis is associated with impaired protein metabolism (Marczuk et al., 2023). In their study, Hartinger et al. (2022) investigated the effects of short-term exposure to ZEA or fumonisins (FUM) on the rumen ecosystem and health parameters of cattle. In their study, six cows were exposed to 20 mg of FUM, or 5 mg of ZEA for two days, followed by a seven-day purge cycle. Both ZEA and FUM affected the composition of the rumen microbiome. Immediate hepatotoxic effects were observed in the cow's exposure to FUM, accompanied by changes in heart and respiratory rates. Exposure to ZEA resulted in a decreased in rumen pH and an increase in cow temperature to mild fever. Bailey et al. (2019) investigated the effect of the common mycotoxins DON, ZEA, fumonisin B_1 (FB_1) and patulin (PA) on the function and metabolism of bovine kidney epithelial cells *in vitro*. FB 1 demonstrated no cytotoxic effect, whereas ZEA exhibited weak cytotoxicity at concentrations above 100 µg/ml. In contrast, PA and DON demonstrated cytotoxic effects at concentrations of 1 a 2 µg/ml, respectively. Following exposure to a subtoxic dose of DON, a significant change in intracellular metabolism was observed in bovine kidney epithelial cells. The hypoxic, hypertonic and ribotoxic mechanism of action of DON on these cells has been described (Fig. 1). The mechanism of action elucidates how chronic exposure to low levels of to DON leads to symptoms associated with mycotoxicosis.

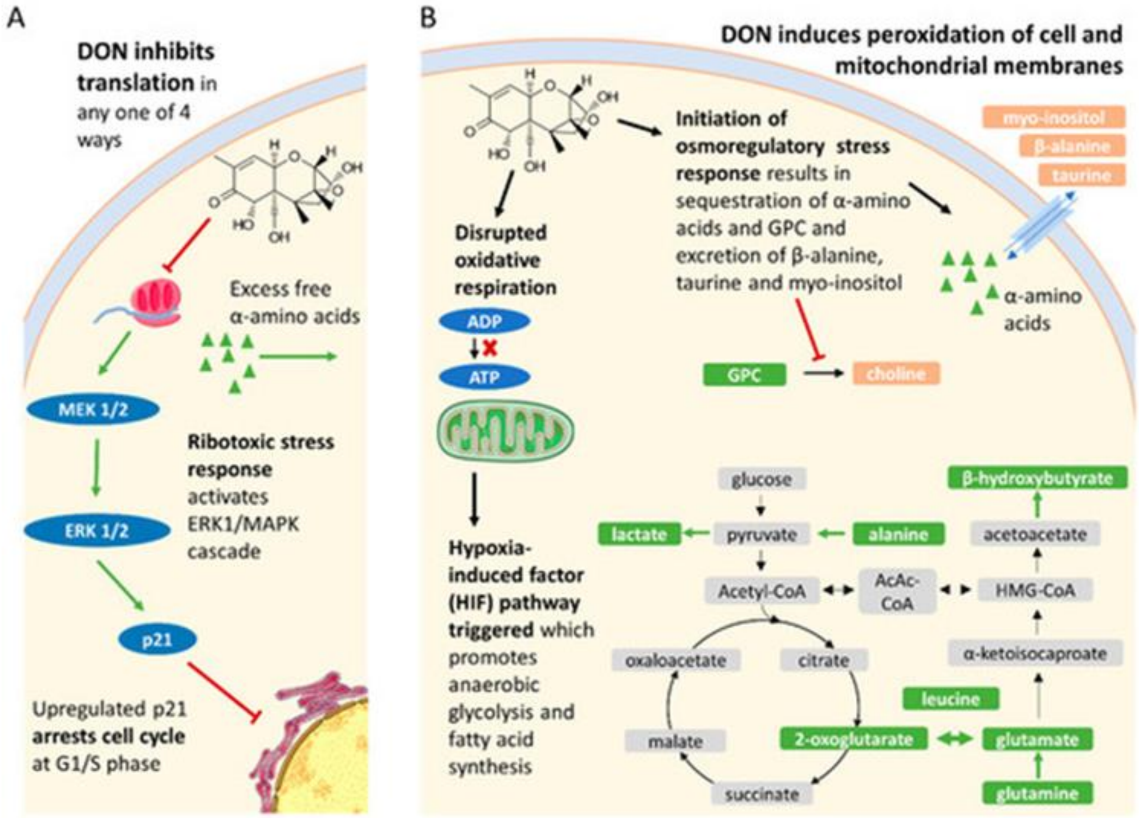

Fig. 1 Mechanism of chronic low-level deoxynivalenol action on renal epithelial cells (Bailey et al., 2019)

4.3 *Biogenic amines*

High concentrations of ammonia and biogenic amines are typical of high-protein silages such as alfalfa silage (Scherer et al., 2015). Histamine and tyramine have high biological toxicity, which can negatively affect animal performance and can also adversely affect the hygienic quality of dairy products (Li et al., 2022). Biogenic amines and ammonia may also be present in well-made silage. The concentration of these compounds is significantly reduced by the use of silage additives (Mlejnkova et al., 2016; Skladanka et al., 2017). According to a metagenomic sequence analysis of alfalfa silage, the authors Li et al. (2022) identified dominant microbial species associated with ammonia production, dominant species associated with rotting, and dominant species producing biogenic amines (Table 3).

Table 3 A metagenomic analysis of the relationships between bacteria and functional enzymes in alfalfa silage (Li et al., 2022)

The dominant species responsible for the formation of ammonia	*Enterobacter cloacae* *Klebsiella oxytoca*
The dominant species associated with rot	*Escherichia coli* *Enterobacter cloacae* *Citrobacter spp.*
The dominant species responsible for cadaverine and tyramine production	*Escherichia coli* *Klebsiella oxytoca*
The dominant species dominating the bacterial community	*Enterobacter cloacae* *Escherichia coli* *Klebsiella oxytoca*

5 Conclusion

This article examines the relationship between silage quality and dairy cow health and performance. The silage production technique is not standardised, with farmers directly influencing the quality of the resulting silage through technical practices. These practices can influence the amount of silage loss, the potential health risk, and the feed intake of dairy cows. Technological recommendations for improving silage quality such as avoiding air exposure and harvesting hygiene are already well described. Consequently, the literature is now focusing, for example, on examining the effect of silage additives on dairy cattle performance and dry matter intake. Further studies could examine the synergistic effect of bacteria when using multi-component bacterial inoculants on their effect on rumen microflora, the effect on mycotoxin content and the resulting palatability of silage.

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