Silage and total mixed ration hygienic quality on commercial farms: Implications for animal production

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Abstract

Implications of silage hygienic quality for animal production were investigated on 45 dairy farms in south-west England. Samples of grass and maize silages and of total mixed rations (TMR) were obtained together with information on silage technology, herd size and animal production. Samples were analysed for mycotoxins, bacteria, yeasts, moulds and chemical composition. Thirteen mycotoxins were assayed, but none were detected in the samples of grass silage. However, mycotoxins were found in 0.9 of all maize and other silage samples, with deoxynivalenol and zearalenone predominating. There were no relationships between mycotoxin concentrations and mean lactation milk yield per cow. Enterobacteria counts tended to be higher in maize silage than in grass silage and higher still in TMR - a cause for concern. There were no relationships between mould counts and mycotoxin concentrations in silages, implying that mycotoxins may have been produced in the field pre-ensiling.

Keywords: silage, total mixed ration, composition, mycotoxins, bacteria, yeasts, moulds

Introduction

Silage, the main forage source for dairy and beef cattle in many regions of the world, may be a potential source of infection and a risk to animal health, about which there has been comparatively little research (Wilkinson and Davies, 2012). For example, there is evidence that contamination of forages with moulds and mycotoxins can affect ruminant animal health and productivity (Fink-Gremmels, 2008; Whitlow et al., 2010), but there is no epidemiological evidence to indicate the extent of the problem. A survey of large animal veterinary practices showed a wide range (from 0.10 to 0.80 of all herds in the practice) in the incidence of mycotoxicosis in dairy and beef herds, with higher incidence associated with sub-standard, aerobically spoiled maize and grass silage when fed with cereal-based rations (Roderick et al., 2014).

Signs of mycotoxicosis in ruminant animals include loss of appetite, reduced milk yield or poor weight gain, feed refusal, diarrhoea, pyrexia, pruritis, bleeding and ill thrift (Krogh, 1978). Early veterinary diagnosis of mycotoxicosis is difficult due to a lack of specific symptoms and overlapping symptoms of other metabolic diseases such as acidosis. The problem does not end in animal disease and production loss, as mycotoxins in the feed of lactating dairy cows can lead to their presence in milk (Farber et al., 1988;

Pietri et al., 2009), dairy products (Pintado et al., 2004; Riahi-Zanjani and Balali-Mood 2013) and infant formula milk (Tavares, 2013), which pose risks to human health, particularly for infants.

The extent of contamination of silage and total mixed rations (TMR) with potentially pathogen microorganisms and microbial metabolites is unknown. Thus the study reported here was a collaborative investigation to determine, on commercial dairy farms, the extent to which silages and TMR were contaminated with moulds, mycotoxins and other undesirable components such as enterobacteria and *Listeria* spp*.* and their possible implications for animal production. The work was an attempt to establish relationships between silage composition and milk production, taking account of possible contamination of the diet by other feeds.

Material and methods

Forty-five dairy farms in the South West of England, from an initial random sample of 51 dairy farms selected from 1,345 farms that participated in a regional development programme (Healthy Livestock, 2015) collaborated in the study. The region is one of the major dairying areas of the United Kingdom and its environmental conditions are typical of UK dairying with a high proportion of grassland-based farms. The Healthy Livestock programme was funded by the European Agricultural Fund through the Rural Development Programme for England and was led by the Rural Business School of Duchy College as part of the South West Healthy Livestock Initiative. The programme was open to all farmers in the South West of England to support training and mentoring in relation to priority diseases of livestock.

Samples were collected of two silages (usually one of grass and one of forage maize) from each participating farm between 13 March and 8 May 2014, towards the end of a winter feeding period lasting about six months, during which the herds were housed. Ten sub-samples were taken at random in a 'W' pattern across the core of each exposed bunker silo feed face, mixed thoroughly and then divided into three separate samples, each of 300 to 500 g fresh weight, which were submitted immediately by courier to three laboratories for analysis of mycotoxins (Micron Biosystems Ltd, Bridgwater, Somerset, UK), microbial counts (School of Veterinary Sciences, University of Bristol, Langford, Somerset, UK) and chemical composition (AuNIR Ltd, Towcester, Northamptonshire, UK).

On the same day as the silage samples were taken, on 39 farms that were operating the total mixed ration (TMR) feeding system, a single composite 800 to 1000 g sample was taken of the TMR from the feed trough, on average 4.5 hours (range 0.5 to 9.5 hours) after the TMR was mixed. Each composite sample was divided into three 300g sub-samples and submitted immediately by courier to the three laboratories for analysis. Ten TMR samples contained grass silage as the only type of silage and 29 TMR samples contained grass silage together with maize or other silage (mainly whole-crop wheat, *Triticum aestivum*). Technological aspects of silage production, storage and feed-out, the age of grass sward, period of field-wilting, time to harvest, silo dimensions and mean speed of daily feed-out progression were recorded together with a visual assessment of wastage at the exposed silo face (on a scale 0 = no waste to 5 = excessive waste) on the same day as the samples of silage and TMR were taken. Where available, the quantities of individual raw material feeds used to produce the TMR were also recorded. Information concerning herd size, milk production, milk composition and reproductive performance was obtained either prior to (by telephone) or at the same time as the samples of silages and TMR were collected.

Samples were screened immediately on arrival at the laboratory for presence or absence of mycotoxins which were quantified within the next 24 hours in positive samples by liquid chromatography (UPLC) and mass spectrometry (Waters Corporation, Milford, MA, USA) by calibrations developed in-hou against laboratory standard mycotoxins of known concentrations (Sigma-Aldrich Co. Ltd, Gillingham, Dorset, UK). The following mycotoxins were assayed, with limit of detection (μg kg^{-1} at 0.12 moisture content) in brackets: Dexoxynivalenol (10.0); zearaleonone (10.0); fumonisin BI and B2 (1.0); T2-toxin (1.0); HT2-toxin (1.0); aflatoxin B1 (0.2), B2 (0.2), G1 (0.2) and G2 (0.2); ochratoxin A (0.2); sporidesmin A (1.0) and patulin (10.0). Mycotoxin concentrations were adjusted to 0.12 moisture content according to EU Commission Recommendation 2006/576/EC (Official Journal of the European Union, 2006).

Total bacteria, lactic acid bacteria, *Enterobacteriaceae*, *Listeria,* yeasts and moulds were enumerated immediately on receipt by the laboratory. Samples of 25 g fresh weight were placed in 225 ml phosphate-buffered saline and homogenized in a stomacher blender (Seward Ltd, Worthing, West Sussex, UK). Serial decimal dilutions of the homogenate were prepared in phosphate-buffered saline and 20 µl spots placed on to agar plates using the method of Miles and Misra (1938). After incubation the number of colonies counted in spots containing between 3 and 30 colonies was used to calculate the total number of target bacteria in the sample.

Enumeration of total bacteria was by culture on Plate Count Agar (all media from Oxoid, Basingstoke, UK) for 1 day at 30°C under aerobic conditions; lactic acid bacteria on de Man, Rogosa, Sharpe (MRS) agar for 3 days at 30°C under microaerobic conditions (5% O_2, 5% H_2, 5% CO_2, 85% N_2); *Enterobacteriaceae* on violet red bile glucose agar (VRBGA) for 1 day at 30°C under aerobic conditions; *Listeria* on Oxford agar at 35°C for 2 days under microaerobic conditions; yeasts on Rose-Bengal chloramphenicol agar at 30°C for up to 7 days; moulds (filamentous fungi) on Sabouraud dextrose agar at 30°C for up 7 days.

Listeria were initially identified by colony morphology and appearance on microscopy, and confirmed and speciated using an API Listeria kit (BioMerieux UK Ltd, Basingstoke, UK).

Chemical composition was determined on samples of silage and TMR immediately on receipt at the laboratory by near infrared reflectance spectroscopy on fresh samples using in-house calibrations with wet chemistry. Concentrations of dry matter (DM), crude protein (CP), ash, digestible organic matter in DM (DOMD), metabolizable energy (ME) and neutral detergent fibre (NDF) were determined on all samples. In addition starch was determined on samples of maize silage, other silage and TMR whilst ammonia-N was also determined on samples of grass silage.

Results

Herd size, milk yield and reproductive performance

All the herds comprised dairy cows of the Holstein or Holstein/Friesian breeds, with two herds also containing crossbred cows. Means and ranges of number of cows in milk, number of dry cows, heifer calvings, milk yield, length of lactation, reproductive performance and milk somatic cell count (SCC) are shown in Table 1. The range in milk yield was from 5300 to 11500 litres per lactation; 27 farms (0.60 of all 45 farms) had average milk yields between 7,000 and 9,000 litres. There was a negative relationship between mean herd milk yield and mean herd conception to first service (Figure 1), but there was no relationship between milk yield and SCC (R^2 = 0.07).

[Table 1 near here]

[Figure 1 near here]

Silage production and storage

A summary of the main features of silage production and storage on the farms is presented in Table 2. Silages were stored in walled bunker silos on all farms. There was a wide range in all assessments, mainly reflecting the range in herd size (Table 1). Notable differences between grass and maize silage were a shorter mean number of hours between the start and end of harvesting maize compared to grass, and slightly lower mean quantity of silage fresh weight removed from the silo daily for maize silage than for grass silage, giving a slower feed-out progression rate for maize than for grass. An inoculant additive was applied to grass crops on 0.38 of farms and to maize and other forage crops on 0.32 of farms.

[Table 2 near here]

Diets and total mixed rations

All 45 farms had diets that contained grass silage, 29 farms had mixtures of grass and maize silage and 6 farms had mixtures of grass and other silages (mainly whole-crop wheat silage). Thirty-nine farms made TMR. Raw material feeds in the TMR mixtures included straw, molasses, soyabean meal, soya hulls, barley grain, wheat grain, rapeseed meal, protected fat and minerals. On 22 farms a proprietary compound feed was given to the cows in addition to the TMR mix.

Mycotoxins

None of the grass silage samples tested positive for the 13 mycotoxins assayed (Table 3). Means, standard deviations and ranges of concentrations of individual mycotoxins in the samples of maize silage, other silage (mainly whole-crop wheat silage) and TMR are in Table 4. With regard to maize and other silages, only *Fusarium* spp. mycotoxins were detected, with deoxynivalenol (DON), in 30 out of a total of 35 samples (0.9 of total samples), accounting for 0.7 of the total mycotoxin concentration in maize silage and 0.9 of the total mycotoxin concentration in the other silages. Zearalenone (ZON) was present in 16 samples (0.55 of total samples), fumonisin (F) B1 and B2 were present in 7 samples (0.2), both T2 and HT2 toxins were present in one sample of other silage (an ensiled mix of moist cereal-by-products). However, no sample tested positive for all six toxins. Concentrations of DON were substantially higher than those of ZON in all silage samples. There was no detection of aflatoxin B1, B2, G1, or G2, ochratoxin A, sporidesmin A or patulin in any samples.

[Table 3 near here]

[Table 4 near here]

With regard to the TMR samples, mycotoxin concentrations were generally lower than in samples of maize silage. In TMR samples that tested positive for mycotoxins there was a similar predominance of DON (detected in 25 out of 38 samples) and ZON (detected in 15 out of 38 samples) as in the samples of maize and other silages (Table 4). There was no detection of aflatoxin B1, B2, G1, or G2, ochratoxin A, sporidesmin A or patulin in any TMR samples.

The composition of the TMR was known for 19 farms and, by taking account of the proportions of maize silage and non-forage feeds present in the TMR, the estimated contributions of these components to the TMR mycotoxin load was examined (Table 5). In the case of 11 farms the total mycotoxin concentration of the TMR was higher than would have been expected from the concentration in maize silage alone. Whilst it is difficult to draw definite conclusions based on analyses of single samples of maize silage and of

TMR, non-forage feeds appeared to be contributing to the total mycotoxin load of the TMR on these 11 farms. Conversely, maize silage appeared to be the sole contributor to the total mycotoxin concentration of the TMR on 8 farms.

[Table 5 near here]

Microbial counts

Counts of lactic acid bacteria, total non-lactic acid bacteria, enterobacteria, yeasts and moulds in silages and TMR are in Table 6. There were wide ranges in all microbial counts. Two samples of grass silage and one sample of maize silage tested zero for all microbial species and were excluded from subsequent analysis. Lactic acid bacteria (LAB) were not detected in 5 samples of grass silage, in 3 samples of maize silage and in one sample of TMR. Total non-lactic acid bacteria were not detected in 8 samples of grass silage, in 2 samples of maize silage and in one sample of TMR. No samples tested positive for *Listeria monocytogenes*; one sample of maize silage and 2 samples of TMR tested positive for *Listeria innocua*. One sample of TMR tested positive for *Listeria ivanovii*. Six samples of grass silage, 7 samples of maize silage and 28 samples of TMR had positive counts of enterobacteria. Twenty-three samples of grass silage, 21 samples of maize silage and 32 samples of TMR tested positive for yeasts. Eighteen samples of grass silage, 8 samples of maize silage and 27 samples of TMR tested positive for moulds.

[Table 6 near here]

Mean counts of LAB and total non-lactic acid bacteria tended to be lower for the grass silage samples than for maize silages, other silages and TMR. Mean counts of enterobacteria tended to be higher in the maize silage samples than in grass silage and higher still in the TMR samples. Mean counts of yeasts tended to be higher in maize silages and TMR than in grass silages. Mean mould counts were similar between the different types of silage and tended to be higher for TMR than for silages.

Mycotoxins were detected in silage samples from 24 farms that also had zero mould counts in their silage samples. For those silage and TMR samples with positive counts of both mycotoxins and moulds, there were no relationships between mould counts and total mycotoxin concentrations (Figure 2). A proprietary mycotoxin binder was added to the TMR on 9 farms (0.24 of total farms with TMR); the average total mycotoxin concentration in the TMR of these farms was 664 μg/kg (range 0 to 3085 μg kg^{-1}), compared with the average total mycotoxin concentration of all TMR samples of 251 μg kg^{-1}.

[Figure 2 near here]

There were positive relationships between counts of yeasts and counts of enterobacteria, in samples that tested positive for both yeasts and enterobacteria, for maize and other silages (R^2 = 0.47) and also for TMR (R^2 = 0.32), but not for the few grass silages (Figure 3). There were no significant relationships between counts of moulds and enterobacteria in silages or TMR.

[Figure 3 near here]

Chemical composition of silages and TMR

Mean values, standard deviations and ranges for concentrations of DM, CP, ash, DOMD, ME, pH, NDF, ammonia-N (grass silages only) are in Table 7. There were wide ranges in chemical constituents of both silages and TMR. Mean concentrations of DM, CP, DOMD, ME and pH values were similar for grass and maize silages, but mean concentrations of ash and NDF tended to be higher for grass than for maize silages. Concentrations of DM and NDF tended to be higher for other silages than for grass or maize silages.

[Table 7 near here]

Use of inoculant additive was associated with higher grass silage ME (by 0.5 MJ kg^{-1} DM, *P*<0.04) and higher herd milk yield (by 1280 litres/lactation, *P*<0.001, Table 8). Mould counts were similar for maize and other silages made with additive to those made without additive but there was a trend of lower mould counts in grass silages made with additive.

[Table 8 near here]

Relationships between silage or TMR composition and milk production

There were no significant relationships between mean herd milk yield per cow and total mycotoxin concentrations in silage or in TMR. There were weak positive linear relationships between milk yield and ME concentration of grass silage (R^2 = 0.11) and also between milk yield and ME concentration of maize silage (R^2 = 0.17). There were no significant relationships between milk yield and grass silage CP. There were also no relationships between milk yield and concentrations of ME, NDF or starch in the TMR.

There was a positive relationship between count of enterobacteria in silage and milk SCC for those herds with positive counts of enterobacteria in silage (R^2 = 0.24), but there was no relationship between count of enterobacteria in TMR and milk SCC (R^2 = 0.02, Figure 4). There were no significant relationships between total silage mycotoxins and somatic cell count (SCC), between total TMR mycotoxins and SCC, between total silage mycotoxins and conception to first service or between total TMR mycotoxins and conception to first service.

[Figure 4 near here]

Discussion

The number of cows in milk per herd, mean milk yield per cow, length of lactation, conception to first service, length of lactation and calving index (Table 1) were similar to national statistics for the United Kingdom (AHDB Dairy, 2016a). The negative relationship between mean herd milk yield and mean herd conception to first service (Figure 1) is in agreement with other work (Caraviello, 2004; Pryce et al., 2014). It has been implied that single-trait selection for milk production in the Holstein breed has achieved its goal by uncoupling the feedback loop between growth hormone (GH) and insulin-like growth factor 1 (IGF-1). Normally, pituitary GH increases IGF-1 production in the liver and IGF-1 inhibits GH secretion - a feedback loop. A side effect of this uncoupling of the feedback loop is lower fertility as both GH and IGF-1 affect the ovary and energy balance (Lucy, 2008; Grala et al., 2011)**.** Mean herd somatic cell count (162,000; Table 1) was close to the target of less than 150,000 cells ml^{-1} of milk (AHDB Dairy, 2016b) though there was a wide range between herds.

Silage-making procedures reflected normal practice in northern Europe (Wilkinson and Toivonen, 2003), though the proportion of farmers that applied an additive at harvest was relatively low at 0.38 for grass and 0.32 for maize and other silage crops. Mean score for the amount of visible waste on the exposed silo feed-out face tended to be higher for maize than for grass silage, possibly reflecting the lower mean quantity of silage removed daily and slower feed-out progression rate for maize than grass silage (Table 2).

None of the samples of grass silage contained detectable levels of the mycotoxins assayed (Table 3). Although not assayed in this study, roquefortine C, a neurotoxic metabolite of *Penicillium roqueforti* (Häggblom, 1990), was found to be the predominant fungal contaminant of grass and maize silages in several studies (Nout et al., 1993; Auerbach et al., 1998; O'Brien et al., 2005). McElhinney et al. (2016) found in a two-year study in Ireland of 300 silages, of which 290 were grass silages, that the mycotoxins of highest incidence were enniatin B and enniatin BI, whilst those of highest mean concentration were andrastin A, enniatin B, mycophenolic acid and roquefortine C. Auerbach et al. (1998) concluded that the count of *P. roqueforti* could be used as an indicator of the likely contamination of silages by mycotoxins formed by *Penicillium* species. It is possible that roquefortine C was present in the grass silages sampled in the present study, but the level of ingestion of this toxin required to cause acute toxicity in cattle is likely to be relatively high (Scudamore and Livesey, 1998). Further, the mean mould count of the grass silage samples was relatively low (2.32 log cfu g^{-1}; Table 6) with 0.67 of all grass silage samples having no moulds detected. The low level of contamination of grass silages with mould may have reflected the procedure of taking samples of silage from the freshly exposed silo feed-out face, which most likely had had relatively few days of exposure to air prior to sampling.

By contrast, with the same silage sampling procedure at the same time of the year, 0.9 of all maize silage samples, 0.7 of all other silages and 0.7 of all TMR samples contained mycotoxins (Table 3). The relatively high proportion of TMR samples that tested positive for mycotoxins reflected the high proportion of farms (0.75 of total) with TMR that contained mixtures of maize and/or other silages together with grass silage. Only *Fusarium* spp. mycotoxins were detected in maize silage, with DON accounting for 0.7 of total mycotoxins, in agreement with Driehuis et al. (2008).

The literature is conflicting regarding the likely risk of clinical effects of mycotoxicosis at the concentrations of DON found in the samples of maize silage and TMR in this study (Fink-Gremmels, 2008; Whitlow et al., 2010). The guideline concentration of DON in feeds for ruminants set by the United States Food and Drug Administration (FDA) is 10 ppm (10,000 μg kg^{-1}) and the ingredients should not exceed 0.4 of the diet (Whitlow et al., 2010). The upper guidance value for DON in ‘maize by-products’ set by the European Commission (EC) is 12 ppm (12,000 μg kg^{-1}; Official Journal of the European Union, 2006). The mean concentration of DON found in the maize silages analysed in the present study was 603 μg kg^{-1} and the highest recorded concentration of DON was 7111 μg kg^{-1} (Table 4); both these concentrations are below the FDA and EC guideline levels. It is unlikely, given that the mean concentration of DON in the TMR samples (154 μg kg^{-1}) was only 0.26 of the mean concentration of the maize silages (603 μg kg^{-1}), that the cows were at risk of clinical mycotoxicosis.

The mean total mycotoxin concentration in maize silage was 0.37 higher than that of the mean DON concentration of maize silage samples, with the highest total mycotoxin concentration recorded at 11,012 μg kg^{-1} (Table 4), which, if given in excess of 0.4 of the total diet ingredient would have exceeded the US guideline. The mean total mycotoxin load of TMR was 0.59 higher than that of the mean DON concentration of the TMR samples and there was evidence that the total mycotoxin concentration in some TMR samples was greater than that predicted from the proportion of maize silage in the ration (Table 5), implying inclusion of other non-forage feeds that were contaminated with mycotoxins. A survey of 38 large animal veterinary practices in the same region (Roderick et al., 2014) revealed that 0.50 of respondents were of the opinion that mycotoxicosis was increasing in incidence and 0.45 of respondents indicated that diagnosis of mycotoxicosis was confirmed *ex post* by observation of the response of animals to the addition of a mycotoxin binder to the diet. In the present study there were no relationships between concentrations of total mycotoxins in silage or in TMR and milk yield per cow. Addition of a mycotoxin binder was found to increase milk yield in cows given feeds contaminated naturally with mycotoxins at concentrations in TMR comparable to those detected in this study (Kiothong et al., 2012) and use of a proprietary mycotoxin binder on some of the farms may have obscured any relationship between mycotoxin concentrations in silage or TMR and milk production.

The wide range in counts of bacteria, yeasts and moulds (Table 6) may have been due in part to deterioration between time of sampling and analysis with loss of viable organisms. However, steps were taken to minimize the period of time that elapsed between sampling and analysis by shipping samples by courier to the laboratory immediately after the samples were collected. Mean counts of LAB (10^5 cfu/g^{-1} for grass and 10^6 cfu/g^{-1} for maize silage) were lower than counts reported by other workers (Driehuis et al., 2001; Jalč et al., 2009; Kristensen et al., 2010), which may reflect the relatively low proportion of crops on the farms in this study that were inoculated with LAB.

Despite a wide range between samples, mean counts of enterobacteria in samples of silage were relatively low, reflecting the high proportions of grass silages (0.92) and maize and other silages (0.76) with zero counts of enterobacteria. Ostling and Lindgren (1995) were unable to detect enterobacteria in grass silage 4 days after ensiling, following inoculation with a mixed culture of enterobacteria at 10^6 and 10^8 per gram fresh crop at the time of ensiling. The low counts of enterobacteria in samples of silage with detectable enterobacteria most likely reflected the relatively low pH of the silages (Table 7) since Pahlow et al. (2003) reported a rapid decline in population of enterobacteria in wilted grass silage as pH decreased below pH 4.3. Ostling and Lindgren (1995) found enterobacteria were absent in grass silages after a 125-day ensiling period.

Although there was a linear relationship between enterobacteria and milk SCC in silages with positive counts, suggesting a possible route for contamination of milk, there was no such relationship for TMR (Figure 4). Nevertheless, the trend of a higher mean count of enterobacteria in samples of TMR than in silages (Table 6) and greater proportion of positive samples of TMR (0.72 of all TMR samples), compared with only 0.12 of all grass silage and 0.25 of all maize silage samples is a cause for concern meriting further investigation. Possible reasons for contamination of TMR with enterobacteria include poor hygiene of mixing equipment or accidental inclusion of aerobically deteriorated silage containing elevated concentrations of enterobacteria (Lindgren et al., 1985; 1988). A further possibility is that growth of yeasts and/or moulds in TMR in the period between mixing and sampling (average 4.5 hours), with reductions in concentrations of undissociated fermentation acids in the silages, may have stimulated growth of enterobacteria. There were positive relationships between counts of yeasts and counts of enterobacteria in maize and other silages, and also in TMR, but not in the few grass silage samples with positive counts of both enterobacteria and yeasts (Figure 3). Further evidence implicating yeasts was the lack of significant relationships between counts of moulds and enterobacteria. Yeasts, rather than moulds, are considered to be primarily responsible for the early phase of the aerobic deterioration of silage (Pitt et al., 1991; Wilkinson and Davies, 2012). It is possible that deterioration of TMR with high yeast counts was accelerated following the addition of readily available substrates in the form of cereal grains and by-product feeds, with additional aeration during mixing.

Listeria spp. were detected in only one sample of silage and three samples of TMR. High numbers of *Listeria* spp. have been detected in silages but their development is usually associated with aerobically deteriorated material with pH values above 5 in peripheral areas of the silo (Fenlon, 1986; McDonald et al., 1991; Donald et al., 1995). In this study, samples of silage were taken at random across the exposed silo face and absence of *Listeria* spp. most likely reflects lack of pre-sampling exposure of the silage to oxygen.

Mean counts of yeasts and moulds in the silages (Table 6) were lower than reported elsewhere (Jonsson and Pahlow, 1984; Kristensen et al., 2010), probably reflecting lack of prolonged exposure of the silages to oxygen. However, 5 samples of grass silage, 13 of maize silage, 2 of other silage and 18 samples of TMR had yeast counts of 10^5 cfu/g^{-1} or above and would be likely to be aerobically unstable (Borreani and Tabacco, 2010). Similar numbers of samples had mould counts of 10^5 or above. There was, however, no relationship between silage or TMR mould counts and total mycotoxin concentrations, suggesting that mycotoxin formation possibly occurred either pre-ensiling or immediately post–ensiling. Teller et al. (2012) found increased concentrations in maize silage following physical damage to plant ears pre-harvest and Schmidt et al. (2015) were unable to relate the temperature of the exposed silo feed-out face to concentrations of mycotoxins in maize silages and concluded that the pre-harvest period was the most likely source of mycotoxin contamination of silage.

The majority of the silages were well preserved (Table 7), with relatively low mean pH values and, in the case of the grass silages, low concentrations of ammonia-N (McDonald et al., 1991). Mean concentration of DM in the samples of grass silage was higher and those of ME, CP, ash and NDF were lower than typical values for ‘good’ grass silage in the United Kingdom (Thomas, 2004). Similarly, the mean concentration of DM in the samples of maize silage was higher and those of ME, CP, ash, NDF and starch were lower than typical values for the UK (Thomas, 2004). The differences in composition may have reflected the season, the stage of maturity of the crops at harvest, the restricted geographical area, or the method of analysis. Despite the wide range in composition between silage samples, there was only a weak positive relationship between the concentration of ME in silage and mean herd milk yield per cow and no relationship with respect to TMR. Nor was there any relationship between either NDF or starch in TMR and milk yield, probably indicating a significant role of concentrate supplementation in the diet of the cows. However, this in itself is of concern regarding mycotoxicosis as the low rumen pH resulting from high concentrate diets reduces the ability of the rumen to detoxify mycotoxins and increases the risk of clinical mycotoxicosis (D'Mello et al. 1999).

Associations between use of inoculant additive and silage composition revealed no differences in mean concentration of total silage mycotoxins between silages made with additive and those made with no additive (Table 8), supporting the possibility that mycotoxins were already present on the crop at the time of harvest. There was a trend for grass silages treated with additive to have a lower mould count but no such trend was evident for the maize silages. Mean herd milk yield per cow was significantly higher for herds where an additive was used than for those where no additive was applied, probably reflecting higher grass silage ME concentration. These results do not imply cause and effect.

Conclusions

This study demonstrated that maize and other cereal silages are major sources of mycotoxin contamination of conserved forages, with contamination possibly occurring pre-harvest. The absence of the same mycotoxins in grass silages requires confirmation with respect to other *Penicillium* and *Fusarium* mycotoxins not assayed in the present study but known to be associated with grass silages. These findings, though based on a regional study, have relevance to other areas where similar silages comprise the principal forage feeds grown for dairy cows.

Research is needed to develop novel diagnostic techniques to help veterinarians differentiate between mycotoxicosis and other metabolic diseases such as sub-acute rumen acidosis. The relatively high proportion of TMR found to contain enterobacteria is a cause for concern requiring further investigation.

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Table 1 Means and ranges for size of herd, milk yield per lactation, reproductive performance and milk somatic cell count.

	n*	Mean	Minimum	Maximum
Cows in Milk	45	159	40	530
Dry Cows	45	28	0	108
Heifer calvings per annum	44	51	12	240
Milk Yield (litres lactation^{-1})	45	8217	5300	11500
Length of lactation (days)	34	353	305	450
Conception to first service (%)	29	45	25	65
Calving index (days)	38	412	365	561
Milk somatic cell count (‘000 cells ml^{-1})	45	162	69	310

* In this and subsequent tables and figures n = number of herds or number of samples.

Table 2 Means and ranges for silage making procedures, silage storage and feed-out

Grass (n=41)	**Mean**	**Minimum**	**Maximum**
Age of sward (years)	4.6	1	15
Wilting period (hours[§])	25	24	48
Harvesting period (hours[§])	32	2.5	72
Silo length (metres)	33.3	9	100
Silo width (metres)	14.4	5	24
Silo height (metres)	3.4	1.8	5
Number of covering sheets	1.8	1	3
Amount of visible waste (none= 0, excessive = 5)	1.5	0	3
Silage removed from silo (t fresh weight d^{-1})	4.1	1.0	11
Feed-out progression rate (m $week^{-1}$)	1.42	0.5	2.0
Maize and whole-crop cereal silage (n=32)			
Harvesting period (hours[§])	19	4	48
Silo length (metres)	29.3	6	40
Silo width (metres)	13.6	4	25
Silo height (metres)	3.4	1.2	6
Number of covering sheets	1.85	1	3
Amount of visible waste (none= 0, excessive = 5)	1.9	0	4
Silage removed from silo (t fresh weight d^{-1})	3.6	0.8	10
Feed-out progression rate (m $week^{-1}$)	1.16	0.5	2.5

[§]Number of hours between start and end of wilting or harvesting

Table 3 Mycotoxin incidence by type of sample.

Type of sample	**Number received**	**Number of samples with mycotoxins detected**	**Proportion of positive samples**
Grass silage	51	0	0
Maize silage	29	26	0.90
Other silages[§]	6	4	0.67
TMR	38	27	0.71

[§] Comprises 4 whole-crop wheat silages, 1 mixture of maize and whole-crop wheat silage, 1 mix of ensiled moist feed and brewers' grains.

Table 4 Means, standard deviations (SD) and ranges of concentrations of mycotoxins (μg kg^{-1}, adjusted to 880 g DM kg^{-1} fresh weight)

	DON	**ZON**	**FB1**	**FB2**	**T2**	**HT2**	**Total**
Maize silage (n=29)							
Mean§	603	209	10.4	2.50	0	0	825
SD	1370.0	723.7	27.15	5.85	-	-	2057.1
Minimum	0	0	0	0	0	0	0
Maximum	7111	3901	107	24	0	0	11012
Other silages (n=6)							
§Mean	80	0	4.0	0.83	1.17	4.17	90.2
SD	70.7	-	9.80	-	-	-	90.1
Minimum	0	0	0	0	0	0	0
Maximum	182	0	24.0	5.00	7.00	25.0	243
TMR (n=38)							
§Mean	154	84.2	11.5	3.95	0	0	251
SD	294.3	257.13	27.9	9.39	-	-	533.4
Minimum	0	0	0	0	0	0	0
Maximum	1654	1431	119	48.0	0	0	3085

§Mean of all samples including those with zero concentrations. DON= Deoxynivalenol, ZON = Zearalenone, FB1 = Fumonisin B1, FB2 = Fumonisin B2.

Table 5 Estimated contribution (μg kg^{-1}) to the total mycotoxin concentration (μg kg^{-1}) of TMR samples from maize silage and non-forage feeds.

Farm No.	**7**	**12**	**14**	**15**	**16**	**18**	**21**	**22**	**27**	**29**	**31**	**33**	**34**	**36**	**39**	**40**	**42**	**48**	**51**
Total mycotoxins in maize silage	206	201	1163	294	596	36	0	570	418	225	239	874	767	749	3326	238	689	209	661
Total mycotoxins in TMR	274	146	1033	279	546	99	49	92	58	42	527	317	299	520	938	45	321	189	190
Expected total mycotoxin concentration based on maize silage inclusion	59	110	547	145	322	12	0	228	109	112	143	357	170	225	1292	75	434	102	198
Potential contribution of non-forage feeds	215	36	486	134	224	87	49	0	0	0	384	0	129	295	0	0	0	87	0

Table 6 Means, standard deviations and ranges in counts of lactic acid bacteria, total non-lactic acid bacteria, enterobacteria, yeasts and moulds (Log_{10} colony forming units g^{-1} fresh weight)

	Lactic acid bacteria	**Total non- lactic acid bacteria**	**Enterobacteria**	**Yeasts**	**Moulds**
Grass silage (n=49)					
Mean	5.00	4.41	0.52	2.04	2.32
SD	2.15	2.66	1.44	2.51	3.48
Min.	ND	ND	ND	ND	ND
Max.	10.6	10.0	5.70	8.70	9.70
Maize silage (n=28)					
Mean	6.03	5.46	1.13	3.90	1.64
SD	2.01	1.85	2.09	2.54	2.98
Min.	ND	ND	ND	ND	ND
Max.	9.70	9.70	6.70	6.70	9.70
Other silage (n=6)					
Mean	5.67	5.57	1.40	2.00	2.35
SD	2.87	1.12	2.37	3.10	2.65
Min.	ND	4.0	ND	ND	ND
Max.	8.00	6.70	5.70	6.30	5.70
TMR (n=39)					
Mean	6.70	6.67	3.19	4.27	3.50
SD	1.85	2.04	2.40	2.26	2.89
Min.	ND	ND	ND	ND	ND
Max.	10.7	10.4	10.3	7.70	9.70

ND not detected

Table 7 Chemical composition of samples of silage and TMR.

	DM [§]	CP	Ash	DOMD	ME	pH	NH_3-N	NDF	Starch
	g kg^{-1} fresh weight	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	MJ kg^{-1} DM		g kg^{-1} total N	g kg^{-1} DM	g kg^{-1} DM
Grass silage (n=51									
Mean	378	119	77.3	660	10.6	4.18	35.6	447	ND
SD	74.3	17.4	11.1	66.4	1.06	0.353	22.22	51.9	-
Min.	220	65.6	39.9	446	7.13	3.70	37.0	370	-
Max.	535	149	122	764	12.2	5.65	116	668	-
Maize silage (n=29)									
Mean	343	117	33.1	658	10.5	4.00	ND	414	239
SD	64.0	7.64	7.92	36.9	0.55	0.209	-	44.5	53.3
Min.	264	94.1	20.5	562	9.2	3.57	-	211	151
Max.	619	128	70.7	710	11.6	4.36	-	463	369
Other silage (n=5)									
Mean	432	105	40.6	588	9.58	4.21	ND	355	177
SD	79.4	18.2	9.08	28.1	0.46	0.206	-	125.0	58.6
Min.	333	82.2	31.9	548	8.77	4.03	-	220	119
Max.	501	124	46.3	617	9.86	4.55	-	472	249
TMR (n=39)									
Mean	361	142	ND	627	10.4	ND	ND	477	98.0
SD	50.5	33.3	-	62.4	1.26	-	-	57.8	65.7
Min.	274	100	-	455	7.30	-	-	394	10.0
Max.	466	286	-	717	15.1	-	-	672	226

[§]DM = Dry matter, CP = Crude protein, DOMD = Digestible organic matter in DM, ME = Metabolizable energy, ND = Not determined, NDF = Neutral detergent fibre, NH_3N = Ammonia N.

Table 8 Comparisons between silage samples from farms that used a silage additive and those that did not (number of herds or samples in brackets).

	No additive	**With additive**	**s.e.d.**	**Sig.**
Total silage mycotoxins (µg kg^{-1})	94 (21)	416 (10)	541.5	NS
Grass silage mould count (log_{10} cfu g^{-1})	3.29 (28)	1.91 (14)	1.081	0.12
Maize and other silage mould count (log_{10} cfu g^{-1})	1.40 (21)	2.18 (10)	1.174	NS
Grass silage ME (MJ kg^{-1} DM)	10.4 (33)	10.9 (16)	0.279	0.04
Maize and other silage ME (MJ kg^{-1} DM)	10.4 (21)	10.3 (10)	0.251	NS
Mean herd milk yield per cow (litres $lactation^{-1}$)	7773 (26)	9053 (16)	349.0	<0.001

Figure 1 Relationship between mean herd milk yield per cow and mean herd conception to first service (n=29).

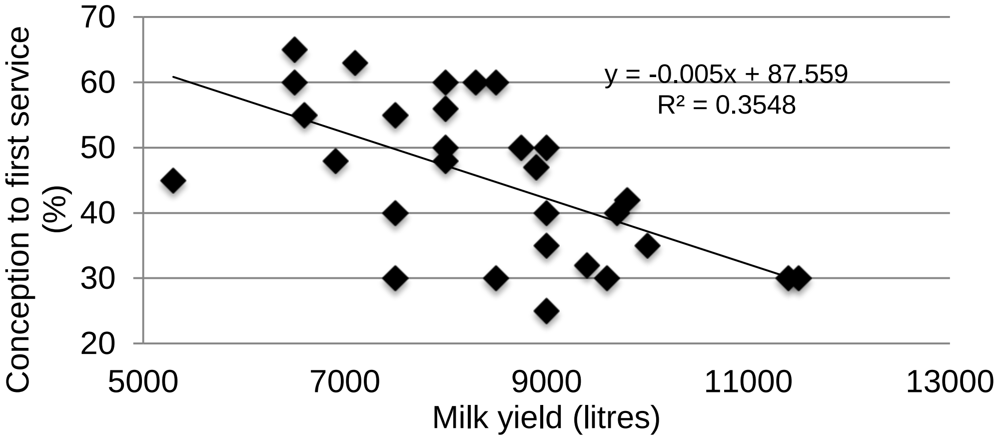

Figure 2 Mould counts and total mycotoxin concentrations in silage (n=9) and TMR (n=18) for samples that tested positive for both moulds and mycotoxins.

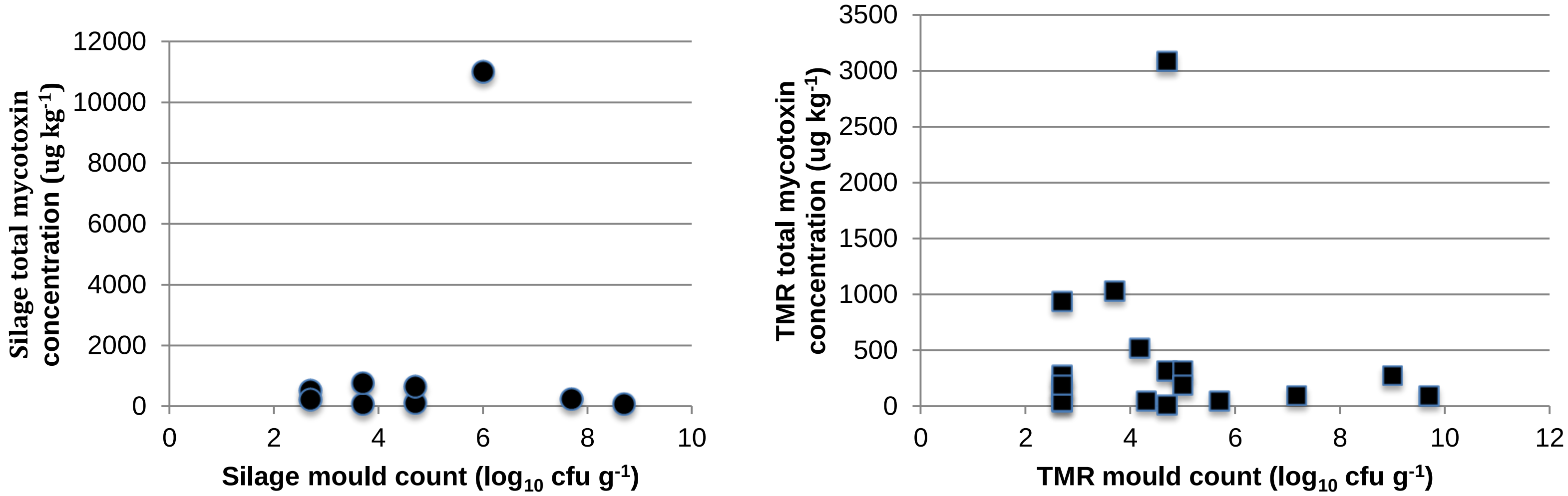

Figure 3 Relationships between yeasts and enterobacteria in samples that tested positive for both yeasts and enterobacteria.

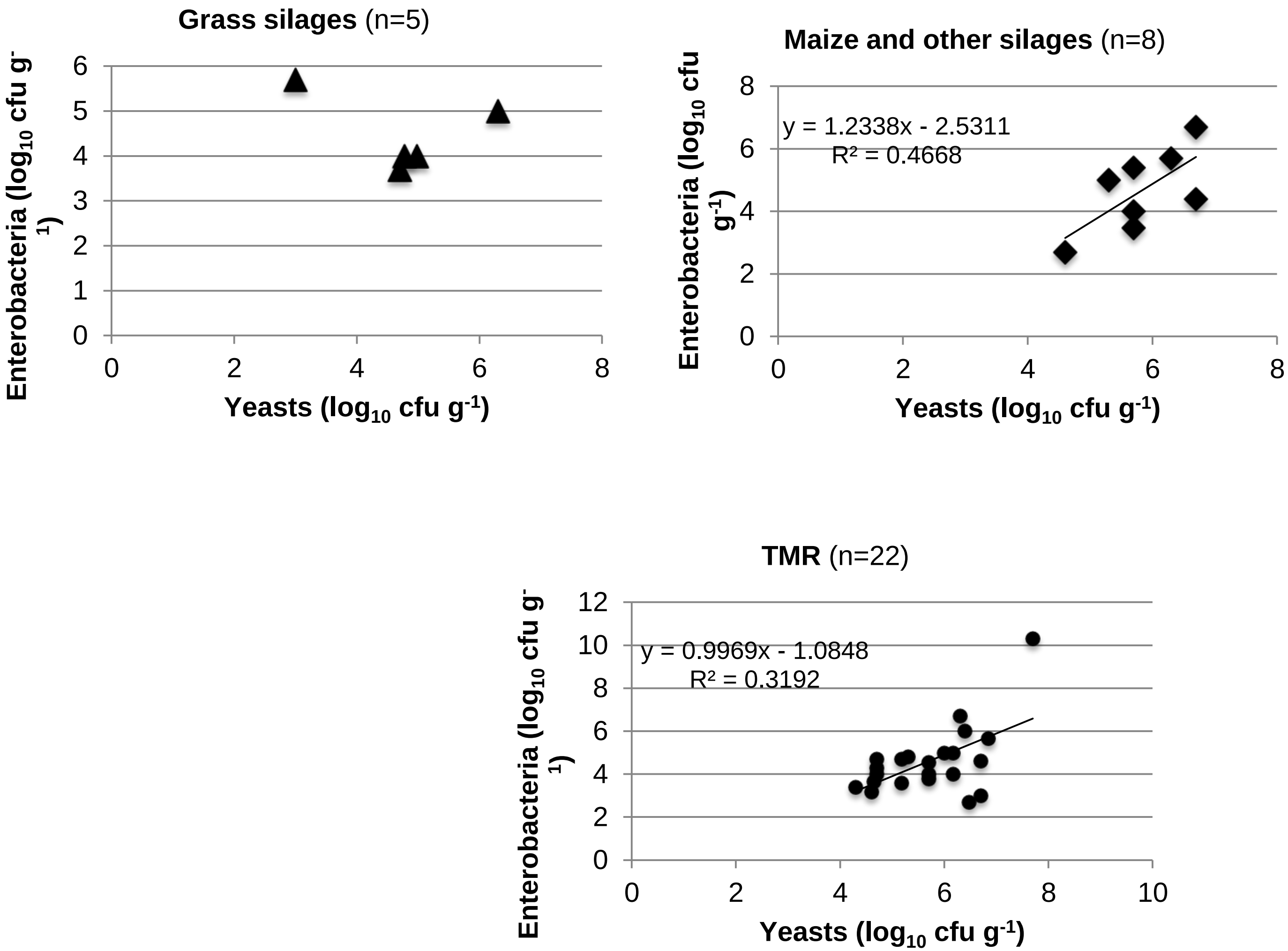

Figure 4 Relationship between enterobacteria in silages (n=12) and TMR (n=27) and milk somatic cell count in samples that tested positive for enterobacteria.