Mycotoxins in haylage
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# Introduction

Silage with high dry matter content, also referred to as haylage, is a common forage in feed rations for horses in Sweden (Enhäll *et al*., 2012). As water activity is low in haylage, lactic acid fermentation is restricted resulting in high content of residual water soluble carbohydrates and no or a very small pH-decrease as opposed to silage (Finner, 1966; Müller, 2005). If haylage bales are not sufficiently air-tight, this environment may favour mould growth in the forage. Mould growth may increase the risk for mycotoxin presence in the feed as well, but there is scarce information about formation of mycotoxins in haylage and in horse feeds in general (Liesener *et al.,* 2010).

# Materials and Methods

Haylage samples from in total 100 farms in Sweden and Norway were analysed for chemical composition, mould growth and mycotoxins. Sampling of haylage was performed at 77 different Swedish farms during two years (2010 and 2011), and at 23 Norwegian farms during 2011. The farms were evenly distributed over the countries. Samples for mould cultivation were taken in three ways as described by Schenck et al. (2013). In short, they were: i) direct plating of samples taken from visible fungal growth on bale surface, ii) direct plating of plant material from drilled core samples and iii) dilution plating where core samples were mixed with peptone water and the dilutions cultured.

Correlation calculations between presence of mycotoxins and moulds in haylage samples were performed using PROC CORR statement (SAS, 2014). The probability to find mycotoxins was also tested with PROC LOGISTIC model (SAS, 2014), where variables such as presence of mould (any species or *Fusarium* spp. in particular) and of chemical composition such as dry matter, crude protein, ash, NDF, pH, ammonia nitrogen, lactic acid, acetic acid, propionic acid, butyric acid, ethanol and 2,3-butanediol were included. In all statistical calculations, farm was used as experimental unit and effects were considered as statistically significant when *P* < 0.05.

# Results and Discussion

One or more mycotoxin(s) were found in haylage from fifty farms, while no mycotoxins were detected in the remaining haylage samples (50) (Figure 1). The most frequently detected mycotoxin was ENN-B (31 farms) followed by BEAU (16 farms) and DON (12 farms).These mycotoxins are all known to be produced by *Fusarium* species. Minimum, maximum and mean mycotoxin concentrations are reported in Table 1.

**Figure 1** Mycotoxins detected in haylage sampled from 100 farms in Sweden and Norway and number of farms where each mycotoxin was present.

Mould was detected in haylage samples from 49 farms with method ‘’', 74 farms with method ‘ii’, and 56 farms with method ‘iii’. The most common mould genera were *Pencillium* spp. and *Artrinium* spp. *Fusarium* spp. were only detected in haylage from five farms.

**Table 1** Mycotoxins detected in haylage samples from 100 Swedish and Norwegian farms (µg/kg). Values only represent samples where the mycotoxins were detected

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Mycotoxin | Minimum | Maximum | Mean  | SD |
| Patulin | Nd | Nd | Nd | Nd |
| NIV | Nd | Nd | Nd | Nd |
| DON | 69 | 479 | 238 | 134.7 |
| 15- ACDON | 70 | 288 | 179 | 154.1 |
| Gliotoxin | 44 | 57 | 51 | 9.2 |
| Alternariol | 11 | 1452 | 212 | 501.7 |
| HT-2 | 19 | 78 | 35 | 28.8 |
| T-2 | 8 | 11 | 9 | 1.5 |
| ZEA | 8 | 8 | 8 | - |
| BEAU | 11 | 988 | 248 | 376.8 |
| ENN B | 10 | 283 | 56 | 83.9 |

Nd = value below lower limit of detection (see text for lower detection limits)

There was no correlation between mycotoxin presence and mould occurrence (r = 0.03). Thus, finding visible mould at the bale surface or from core samples will not mean that mycotoxins are present. An analysis of the magnitude of total mould growth (log CFU/g) established with the dilution method, and the prevalence of mycotoxins, resulted in an indication that the risk of finding mycotoxins was higher when mould numbers were higher (r = 0.25; *P* < 0.05). However, higher mould counts were not correlated to higher mycotoxin concentrations (r = 0.03). This is in contrast to the findings of Legzdina and Buerstmayr (2004) who studied *Fusarium* head blight and mycotoxin presence in barley, where the percentage of visually infected barley spikes was positively correlated with the content of DON and 15-ACDON.

# Conclusions

Mycotoxins were detected in 50% of sampled haylage bales in Sweden and Norway. *Fusarium* spp. mycotoxins were the most common mycotoxins present. Neither conventional mould analysis by culturing nor chemical variables were able to indicate if mycotoxins were present. However, in bales where moulds occurred, higher mould counts correlated weakly with the presence of mycotoxins.

# References

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Unpublished data and personal communications