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Short communication

Presence of mycotoxins in sugar beet pulp silage collected in France

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ABSTRACT

Sugar beet pulp, a major by-product of the sugar industry, is a common feed component in cattle diets that is preserved on-farm as silage. This study was designed to investigate if sugar beet pulp silage could be a vehicle of common mycotoxins found in silages and other regulated mycotoxins. Samples ($n = 40$) favouring mouldy spots, if present, on the front face of open silages were collected in 2011 from 5 regions representing the main French sugar beet producing areas. Mycotoxins were extracted by QuEChERS procedure without any further clean-up and analyzed by liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS). The mycotoxins monitored were: aflatoxin B1, deoxynivalenol, gliotoxin, ochratoxin A, mycophenolic acid, patulin, penicillic acid, roquefortine C and zearalenone. Matrix-matched calibrations were used, yielding acceptable levels of recovery ranging from 64 to 168%, except for gliotoxin and roquefortine C for which recovery was lower (21 and 34%, respectively). Eight samples out of 40 (20%) were found to be positive. Mycophenolic acid and zearalenone were the most predominant of the mycotoxins studied. Mycophenolic acid was found in five of 40 samples at levels ranging from traces up to 1436 μg/kg. Zearalenone was found in three samples at concentrations of 1023, 4862 and 6916 μg/kg. The last 2 samples were at concentrations above the recommended limit of 2000 μg/kg. Ochratoxin A was detected in one sample at 15 μg/kg, which is below the recommended EU limit of 250 μg/kg. Roquefortine C was also detected but at low levels. To our knowledge, this study is the first to report on the presence of mycotoxins in sugar beet pulp silage. Contamination for the tested mycotoxins was low and did not seem to present a health risk for animals or consumers for the tested mycotoxins.

1. Introduction

Sugar beet (*Beta vulgaris* L.) is an important industrial crop in temperate regions that provides about a third of all sugar consumed in the world. France is one of the main producers of sugar beet in Europe, with 34 million tonnes harvested in 2009 (http://www.labetterave.com). Due to its nutritional value and availability, the sugar beet pulp by-product of the

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sugar industry is widely used by farmers in production areas. The sugar beet pulp is ensiled on the farm and incorporated into cattle diets. One common risk affecting silage conservation and quality is the development of fungi which reduces the nutritive value of feeds and animal performances (Morgavi et al., 2008; Boudra, 2009). In addition, fungal development can be accompanied by production of mycotoxins that can affect animal health. The most common infestation of sugar beet in the field is caused by *Fusaria*, and some species are capable of producing mycotoxins in the field (Hanson, 2006; Nitschke et al., 2009; Hill et al., 2011) and *in vitro* (Bosch and Mirocha, 1992; Burlakoti et al., 2008; Christ et al., 2011). In addition to *Fusarium* spp. that cause losses in the field, *Aspergillus* and *Penicillium* species can grow when silage is not appropriately conserved (Binder et al., 2007; Boudra, 2009). Information on sugar beet contamination with mycotoxins is sparse. Bosch and Mirocha (1992) reported that 6 out of 25 moldy sugar beet samples collected in the field contained zearalenone (ZEA) in concentrations ranging between 12 and 391 μg/kg. Zearalenone was also detected in 31 out of 75 sugar beet fiber samples (13 to 47 μg/kg) (Bosch and Mirocha, 1992). To our knowledge, there is just a single report on fungal contamination of stored sugar beet pulp silage (Nout et al., 1993), but the presence of corresponding mycotoxins was not investigated. The aim of the study reported here was to assess the risk of mycotoxin contamination in sugar beet pulp silage samples collected from the 5 main areas of sugar beet production in France. The method developed in this study targeted mycotoxins that were frequently found in silages: patulin (PAT), penicillic acid (PENI), gliotoxin (GLIO), roquefortine C (ROQ C), and mycophenolic acid (MYCO) (Auerbach et al., 1998; Richard et al., 2007; Mansfield et al., 2008; Rasmussen et al., 2010; Van Pamel et al., 2011), in addition to aflatoxin B1 (AFB1), ochratoxin A (OTA), deoxynivalenol (DON), and zearalenone (ZEA) that are regulated in feeds in the European Union (European Commission, 2006).

2. Materials and methods

2.1. Sample collection

Sugar beet pulp silage samples were collected from 5 regions: Haute-Normandie ($n = 11$), Ile-de-France ($n = 9$), Picardie ($n = 7$), Nord-Pas-de-Calais ($n = 8$), and Centre ($n = 5$), representing the main areas of sugar beet production in France (http://agreste.agriculture.gouv.fr/). Silages from each region were from one sugar production plant. All production plants used high pressure technology to extract sugar from beet root and the pulp was transported immediately to farms for ensiling within the same day. Samples were taken from silos that were in current use at five places on the front face, on a diagonal line from one upper corner to the opposite bottom corner. While respecting this sampling procedure, operators were instructed to favour moldy spots if present on the front face. This was done as the objective was to assess the risk of mycotoxins contamination from this feed resource. Samples were then pooled and a subsample of 150 g was sent to the laboratory within 24 h for mycological and mycotoxin analysis. Farms included in this study had bunker silos with capacities of between 100 and 750 m^3 and did not use preservatives for silage making. The dry matter (DM) of sugarbeet silage was determined in triplicate by drying at 105 °C for 24 h, and ranged from 19 to 28%.

2.2. Mycological analysis

Ten grams of fresh sample were transferred into a sterile plastic bag, suspended in 90 ml of sterile 0.05% Tween 80, and homogenized for 5 min in a laboratory Stomacher blender (BayMixer 400, Interscience, Saint Nom la Breteche, France). Serial dilutions from 10^{-2} to 10^{-5} were made and 0.1 ml of each was inoculated in duplicate in two different culture media: 2% Malt extract with and without sodium chloride. The plates were incubated at 25 °C in the dark, and molds and yeasts were counted after three days of incubation. The results were expressed as colony-forming units (CFU)/g DM. The remainder of the sample was dried in a forced draft oven at 50 °C for two days, ground through a 1 mm sieve and stored at +4 °C until mycotoxin analysis.

2.3. Mycotoxin analysis

Extraction of selected mycotoxins was performed by the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method according to the original Waters procedure based on the AOAC method in two different steps. Briefly, six grams of silage were placed in a 50 ml-DisQuE™ tube (186004837, Waters) containing trisodium citrate dihydrate (1 g), disodium hydrogencitrate sesquihydrate (0.5 g), NaCl (1 g) and $MgSO_4$ (4 g). Five milliliter of distilled water containing 1% of acetic acid were added. After complete absorption of water, 10 ml of 1% of acetic acid in acetonitrile were added and tubes were shaken vigorously for 1 min, and then centrifuged (2000 g, 5 min). One ml aliquot of the upper phase (corresponding to 0.4 g of silage) was transferred to a second 2-ml-DisQuE™ tube (186004837, Waters) containing 0.15 g MgSO4 and 0.025 g PSA (primary secondary amine). We added 7.5 mg of Graphitized Carbon Black (DisQuE™, 186004837, Waters) to each tube in order to remove pigments such as carotenoids and chlorophyll from the samples. Samples were shaken vigorously for 1 min and then centrifuged (2000 g, 5 min). The upper phase was filtered through a 0.45 μm filter, and 10 μl were injected into the LC system. The chromatographic system was an Alliance 2695 module (Waters Corporation, St-Quentin-en-Yvelines, France). Separation was performed at room temperature on a C_{18} RP column (Luna, 50 × 2 mm, 3 μm, Phenomenex, Paris, France) using a gradient solvent system (solvent A = 0.1% formic acid–ammonium acetate 0.5 mM adjusted to pH 3.5, and solvent B = Acetonitrile–0.1% formic acid). The gradient conditions were as follows: the initial percentage of solvent B was