Fungal survival in ensiled swine faeces

E. Serrano-García, F. Castrejón-Pineda, M.A. Herradora-Lozano, A.H. Ramírez-Pérez, S. Angeles-Campos, S.E. Buntinx

Department of Animal Nutrition and Biochemistry, Faculty of Veterinary Medicine and Animal Production, Universidad Nacional Autónoma de México, Cd. Universitaria, D.F., 04510 Mexico, Mexico

Department of Animal Production: Swine, Faculty of Veterinary Medicine and Animal Production, Universidad Nacional Autónoma de México, Cd. Universitaria, D.F., 04510 Mexico, Mexico

Received 18 May 2006; received in revised form 19 June 2007; accepted 19 June 2007

Abstract

The survival of several genera of fungi was determined in the ensiled solid fraction of swine faeces after 0, 7, 14, 28 and 56 days of ensiling. The experiment had two treatments, un-ensiled and ensiled manure, in a split-plot design. The manure was distributed into 50 containers; samples, taken at the specified times, were cultured in agar potato dextrose medium, incubated, and colony forming units (CFU/g) were counted and log-transformed. The ensiling process decreased the number of CFU after 56 days. Five fungal genera were identified (Absidia spp., Aspergillus spp., Penicillium spp., Rhizopus spp. and non-fructiferous fungi), and their vulnerability to the ensiling conditions varied, although most of them slowed their growth or disappeared after 14 days of ensiling.

Keywords: Ensiling process; Fungus; Survival; Swine manure

1. Introduction

One of the most important problems in swine production is the generation of manure and its impact on the environment, not only due to its direct effect on soil, air and water, but also due to annoyance factors, such as foul odours and insects (Pérez, 1999; Gutiérrez and Preston, 1995; Salazar and Cuarón, 2000).

In Mexico, there is an ever-increasing production of swine manure, coming from pig farms with large animal numbers. Although there has been a real effort to use these organic wastes as fertilizer, the reduction of suitable land for agriculture, due to a growing urban population, has made this means of disposal a less efficient alternative than expected. Moreover, because of soon-to-be enforced environmental laws in Mexico, almost 80% of pig farms in the country now use systems that separate solid wastes from liquid wastes. The liquid wastes are sent to oxidation lagoons and in 23% of the pig farms that have this separation scheme, the solid wastes are used, without any further treatment, in the feeding of pigs and ruminants (Pérez, 1997). This is, of course, a very risky and undesirable practice, that may recycle microbial pathogens and cause disease in animals thus fed. Several research groups in the country are studying treatment methods that reduce the possibility of such an outcome (Iníguez et al., 1990; Salazar, 1994a,b; Ramírez et al., 2005), only as a preventive and temporary measure, while other more sustainable and efficient uses of swine manure are found and tried (El-Hage and Hattam, 2003).

Some studies indicate that one pig produces approximately 6.2 kg of dejections per day, 45% of which correspond to urine and 55% to faeces. Faecal matter contains 88% humidity, and close to 90% of solids are excreted in faeces. The content of solids and of organic matter of
faeces depends on the type of feeding and on ambient conditions, which influence water consumption (Pérez, 1999; AGRORED, 2000). It is the organic matter content of faeces that is an excellent breeding ground for bacteria and fungi and these latter organisms are important because of the various toxic compounds they produce, known as mycotoxins (Gimeno, 1999). The presence of mycotoxins in faeces probably would not be of great consequence were it not for the fact that swine manure is increasingly being used in animal feeding, as has already been mentioned.

One of the simplest and cheapest ways of treating swine manure solids before feeding them to animals is through ensiling. It has been shown that the ensiling process kills manure solids before feeding them to animals is through used in animal feeding, as has already been mentioned. The internal temperature of the material was taken immediately upon opening each jar. The material was then sampled twice: 10 g were used to measure its pH and 2 g were placed in a forced-draft oven at 60 °C for 24 h to determine dry matter (DM) content.

2.3. Sampling and culturing of samples for fungal identification and quantification

Containers were opened at the specified times and samples taken from five different strata in each repetition. The samples from each container were then placed in a sterilized plastic bag, mixed, and a composite sample taken for each repetition. A 100-g sub-sample from this composite sample was placed in a sterile glass jar and transported to the laboratory at 4 °C.

Once in the laboratory, the jars were opened in a sterile environment, and 1 g of the material was deposited into a 150-ml culture tube with a bakelite screw top. Dilutions (10⁻¹, 10⁻² and 10⁻³) were obtained and from each dilution 1 ml was cultured in disposable Petri dishes with 10 ml of potato dextrose agar (PDA) medium. The Petri dishes were left to cool down at ambient temperature and then were incubated at 35 °C for 72 h. After this time had elapsed, the Petri dishes were checked every day for five consecutive days (Cervantes, 1996; Pitt and Hocking, 1997). Once fungal colonies appeared, the Petri dishes were removed from the incubation oven and kept at 4 °C before observation under a stereoscopic microscope for genus identification. Whenever there was doubt about identification, the genus was confirmed using a cotton blue lactophenol stain, observing the colony under a compound microscope and comparing it with published information (Barnett and Hunter, 1972; Hawksworth et al., 1996; Pitt and Hocking, 1997; Thomas, 1998). Quantification of colony forming units per gram (CFU/g) was performed on the 10⁻² dilution using a stereoscopic microscope and following the specifications of the Official Mexican Norm (Norma Oficial Mexicana) NOM 111-SSA1-1994. In each replication, the CFU/g from the different genera was added up and the resulting number was log-transformed. Therefore, the total amount of CFU/g (log-transformed), irrespective of genus, was used for the statistical analysis.

2.4. Experimental design and statistical analyses

The experiment was a split-plot design, where the main plot was the treatment (control or ensiling) and the small plot was time of opening (0, 7, 14, 28 and 56 days), with five repetitions per treatment-time combination. An analysis of variance was performed on the total amount of CFU/g (log-transformed), and pH and temperature data were correlated (separately for the control and ensiled treatments) with the CFU data (JMP, 1996).

3. Results and discussion

The pH in the microsilos decreased steadily from 4.6 at day 0 to 3.6 by day 28 of the experiment, increasing slightly to 3.8 by day 56, with minimal variation among repetitions.
These values were in sharp contrast with those of the control treatment, where the pH had an abrupt increase to 6.8 by day 14, stabilizing at pH 6.6 for the remainder of the experiment. The variation was a little larger in this treatment than in the microsilos. The ensiled swine manure mixture achieved a pH consistent with an optimal ensiling process (McCullough, 1978) and the values coincided with those of Toledo (1996) and Martínez-Gamba et al. (2001).

Temperature in the control jars (Fig. 2) oscillated markedly throughout the experiment, whereas in the microsilos temperature decreased steadily from day 0 to day 14 (from 24 to 16°C). However, by the end of the experiment, the temperature in the microsilos and in the control jars was similar to that registered at day 0. The temperature inside the containers was outside the range (35–37 °C) reported by McCaskey and Anthony (1979) and Iníguez (1991) as favourable for the development of microorganisms. Nonetheless, temperature is not such an important factor in the inhibition of fungal growth because some genera withstand cool temperatures (Webster, 1986).

Dry matter content of the ensiled material decreased 6% units from day 0 to day 7 and hovered around 40% for the remainder of the experiment (Fig. 3). McCaskey and Anthony (1979) and Iníguez (1991) have indicated that a moisture content of 60% induces a good fermentation process. In the control treatment, the DM concentration of the material averaged 44.6% from day 0 to day 28, increasing abruptly to 60% by day 56 (Fig. 3), probably because of a lower ambient relative humidity. It must be kept in mind that control jars were not sealed, but just covered with micropore tape, and the water content from the faecal mixture probably evaporated.

The analysis of variance for the log-transformed data of CFU/g revealed a treatment × day interaction (P < 0.002), depicted in Fig. 4. The number of log CFU/g in the ensiled treatment decreased from 3.5 in day 0 to 2.6 in day 7 and then again, to 0.6 by day 28. In contrast, the control treatment did not have any effect on the number of CFU/g. Variation, however, was much higher in the ensiled than in the control manure mixture. The correlation analysis for the ensiled treatment indicated that there was a highly
significant positive correlation between CFU/g and pH \((r = 0.64, P = 0.0005, n = 25)\). As pH decreased (Fig. 1), the number of CFU/g decreased (Fig. 4). The microenvironment created by the acidic conditions and the absence of oxygen were detrimental to fungal survival.

The fungal genera identified were: Absidia spp., Aspergillus spp., Penicillium spp., Rhizopus spp. and non-fructiferous fungi. Figs. 5 and 6, respectively, depict their behaviour in control jars and microsilos. In all cases, there was considerable variation in the number of CFU/g found at each time of opening. All the genera identified, except for the non-fructiferous fungi, are considered environmental contaminants, frequently isolated from air, decomposing organic matter, plants and feedstuffs (Bonifaz, 1994; Deacon, 1994). In the case of Absidia spp. and non-fructiferous fungi, the number of CFU/g decreased progressively with time in the ensiled material, but the fungi did not completely disappear. In contrast, Rhizopus, Aspergillus and Penicillium colonies could not be found in the ensiled material after 7, 14 and 28 days of ensiling, respectively. Because the genera Rhizopus and Penicillium present their maximum activity at acidic pHs (Pitt, 1991; Webster, 1986), the probable explanation for their disappearance in the microsilos is a lack of oxygen. Aspergillus spp. fungi, on the other hand, are sensitive to acidic pHs (Samson and Pitt, 2000) and this is most likely the reason for the absence of growth in the microsilos.

4. Conclusion

The ensiling process, through a combination of low pH and low oxygen concentration, decreased the number of fungal colony forming units after 56 days. Five fungal genera were identified (Absidia spp., Aspergillus spp., Penicillium spp., Rhizopus spp. and non-fructiferous fungi), and their vulnerability to the ensiling conditions varied, although most of them slowed their growth or disappeared after 14 days of ensiling.

Acknowledgements

The laboratory work of this study was carried out at the National Centre of Reference from the General Office of Plant Health (Ministry of Agriculture, Livestock Production, Fisheries and Feeding, SAGARPA) and it would not have been possible without the assistance of Biol. David Bonilla-López and the Centre’s personnel. The authors also kindly acknowledge Dr. Roberto A. Cervantes-Olivares’ help in reviewing the manuscript.

References
