RESEARCH ABSTRACT

ENVIRONMENT

Title: Effect of soybean peroxidase activity on phenolic compounds, ammonia, and other organic compounds in swine manure - **NPB #06-115**

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Scientific Abstract

An in vitro manure system was used to evaluate the efficacy of soy peroxidase (SoyP) in swine manure for the control of phenolic and other odorous compounds in head space air and manure. The SoyP was added to the manure columns at increasing amounts of 7.5, 15, and 30 mg (200 u/mg) and calcium peroxide (CaP) levels increased from 0, 12.5, to 25 mmoles in a factorial approach of treatments plus 2 controls, a 0-0 SoyP-CaP and 0-25 SoyP-CaP treatments. Manure was collected from a 6 ft deep pit where the pigs had been placed in the facility for 8 -12 weeks during this experiment. The manure was thoroughly mixed and subsampled in 350 mL aliquots into 500 mL flasks that served as bench top manure containers. Rubber stoppers with a hole in the top were placed on the flasks and covered with parafilm for 2 hr prior to sampling of headspace air samples. A solid-phase micro extraction fiber (SPME; Supelco, Inc. Bellefonte, PA) was inserted through the parafilm and hole in each rubber stopper on each flask and the fiber exposed for 1 hr to absorb volatile organic compounds in the headspace of each flask. After the SPME fibers were removed, the flasks were mixed and a 50 mL manure sample was obtained and frozen for later analysis of ammonium nitrogen, short chain volatile fatty acids, pH, dry matter, and peroxidase enzyme activity analysis. Sampling of the manure flasks and air head space was at 0 (initial manure slurry); 2, 24, 48, and 120 hr. In between sampling times the manure flasks were left open to naturally vent inside a laboratory hood. The soybean peroxidase activity was determined using a monoclonal-antibody assay specific for soybean peroxidase. The air head space GC fibers and manure samples were analyzed on a GC for phenolic and VFA compounds. All data were analyzed using the GLM procedures of SAS. The base manure had no appreciable levels of SoyP as indicated by the near zero detection in the two control treatments that did not have additional SoyP added to these manure columns. As the SoyP was added to the manure columns at increasing amounts the average analyzed levels of SoyP increased linearly ($P<0.001$; 0.49, 0.68, and 0.78 purpurogallin units/µL, respectively). Adding CaP to the manure resulted in a linear decline ($P<0.01$; 0.69, 0.66, 0.59 purpurogallin units/µL, respectively) in the average level of SoyP analyzed levels of activity. The average SoyP levels were at their highest levels at the 2 hr sampling and then declined to 48 hrs and remained at this level to 120 hr (Time $P<0.01$; 0.74, 0.65, 0.60, 0.60 purpurogallin units/µL,

respectively). Manure pH, ammonium N, manure ash content as a % of manure DM were not affect by SoyP levels (P>0.31), however manure DM declined slightly as SoyP increased (P<0.02; 7.98, 8.00, 7.87%). As CaP levels increased, manure pH (P<0.001; 6.54, 7.00, and 7.25, respectively), manure DM (7.42, 8.03, 8.40%) and ash as a % of DM (16.7, 18.2, 20.0%) linearly increased (P<0.001) and manure ammonium N linearly decreased (P<0.001; 3492, 3310, 3293 ppm). Increasing levels of SoyP achieved only non-significant changes in most manure phenolic compounds on its own. Manure phenol declined slightly (14.7, 11.3, 13.2, respectively) compared to the 0-0 control at 18.1 ppm with increasing SoyP. The manure compounds indole and 4-ethyl phenol combined declined (P<0.04; 1.35, 1.05, 0.61 ppm, respectively) along with total phenolic compounds (16.0, 12.4, 13.8 ppm) with increasing SoyP. The addition of SoyP had linear numerical reductions in head space air phenol (13.2, 9.0, 6.0 ppm, respectively; P<0.16), total phenolics (16.3, 11.2, 8.0 ppm respectively; P<0.14) as SoyP levels increased in the manure columns. By contrast, as CaP increased, all manure phenolic compounds linearly declined. Phenol (P<0.002), 3 and 4 methyl phenol (P<0.001), indole and 4-ethyl phenol (P<0.06), and total phenolic compounds (20.7, 13.6, 7.9, respectively; P<0.001) were all reduced with increasing CaP. Increasing levels of CaP also linearly decreased head space air phenol (14.5, 8.1, 5.7 ppm, respectively; P<0.09), total phenolics (16.0, 11.8, 7.7 ppm, respectively; P<0.13); while decreasing and then slightly increasing in a quadratic fashion indole and 4-ethyl phenol (Quad., P<0.05). Manure acetic acid (P<0.15) increased slightly while butyric (P<0.14) and isovaleric (P<0.09) decreased slightly with increasing SoyP enzyme levels. While increasing levels of CaP increased manure acetic acid (P<0.001), decreased butyric (P<0.001), and increased and then decreased isobutyric (Quad., P<0.04) and isovaleric acids (Quad., P<0.005), However, neither Soy P nor CaP had any effect on manure total VFA's. The addition of SoyP had a quadratic response in head space air Total VFA's (926, 621, 913 mmoles; P<0.08) as SoyP levels increased in the manure columns. Increasing levels of CaP linearly decreased head space air combined acetic and valeric acids (461, 67, 74 mmoles, respectively; P<0.001) and total VFA's (1365, 528, 566 mmoles/g, respectively; P<0.001) while decreasing and then slightly increasing in a quadratic fashion isovaleric acid (110, 43, 79 mmoles respectively; P<0.03). Careful evaluation of the data clearly shows that it is the combination of SoyP enzyme and CaP that clearly reduces the phenolic compounds. Comparing the 25 mmole level of CaP with no SoyP enzyme, having a manure total phenolics level of 15.6 ppm, and when any level of the SoyP is added to the 25 mmole level of CaP the total phenolics in the manure are reduced on average by 50% to 7.9 ppm (P<0.10). Also the greatest reduction in phenol and total phenolics (from 13.1 to 4.8 ppm) in manure column head space air occurred at the highest inclusion rate of SoyP (30 mg) in combination with CaP (25 mmoles). The use of soybean peroxidase in combination with a catalyst (calcium peroxide) may be a viable option to reduce both manure content and air emissions of phenolic compounds, however the increased losses of ammonium N with the increased manure pH as CaP was added to the manure is a potential negative result of this research. Further research is needed for application of this technology to be viable for the swine industry to use to control phenolic compounds in swine manure storage systems.