ONTARIO PORK

Ontario Pork Research Final Report (# 17-022) Executive Summary

Reporting Date: March 31, 2022

Introduction: The mycotoxin deoxynivalenol (DON) contamination in animal feed may cause reduction in growth performance and compromise the immune system of farm animal. Sodium metabisulfite (SMBS) is a promising chemical agent to detoxify DON in animal gastrointestinal tract. However, when used with feeding SMBS can quickly decay at acidic conditions in the stomach, releasing sulfur dioxide which may lead to lose of its detoxifying ability and feed intake reduction. Therefore, protection of SMBS is necessary to prevent its release before arriving at the small intestines. Detoxification by bacteria is another promising approach. In our previous research a bacterial isolate, LS100, was found to have the detoxification function to DON in the lab condition. To apply this bacterial isolate directly in pigs requires further characterization.

Objectives: 1. Develop an effective and practically feasible microencapsulation method for effective delivery of sodium metabisulfite (SMBS) to detoxify DON in the gut of weaned piglets. **2.** Determine the effects of a previously isolated microorganism on DON detoxification, gut barrier function, and nutrient transporter expression in weaned piglets.

Materials and Methods:

1. Preparation and characterization of encapsulated SMBS:

Among the three methods investigated, a two-stage hot melt granulation technology with two fats of different melting points as encapsulating materials was developed to encapsulate SMBS. The in vitro release profile of the microcapsules in simulated gastrointestinal fluids are determined, and storage stability of encapsulated SMBS was tested at room temperature. The detoxification ability of encapsulated SMBS in comparison to non-encapsulated SMBS was evaluated at a range of SMBS to DON ratios in order to identify a dose-response range of SMBS to detoxify DON.

2. Characterization of bacteria for detoxification function

The growth and detoxifying ability of the isolate LS100 were evaluated in simulated gastrointestinal conditions and in the presence of pig digesta and absence of oxygen.

Results and Discussion:

1. An encapsulation method was developed for SMBS targeting delivery to pig intestines. The product can deliver over 90% SMBS to the intestines when tested in simulated gastrointestinal conditions. The product exhibited good storage properties. The encapsulation material and the process did not compromise the detoxification function of SMBS. Sufficient amount of encapsulated SMBS product was prepared and sent to University of Manitoba to be tested in animal trials.
2. Under anaerobic conditions, the LS100 was found to be stabile for more than 12 times subcultures without lose of its detoxifying function, indicating the potential to be used within animal body. LS100 also exhibited tolerance to oxygen to a certain degree, that made it convenient for the handling of the isolates for feed use. When tested in simulated gastrointestinal fluids, the bacteria isolate LS100 was shown to be sensitive to both bile salts and low pH environment, the detoxifying activity of which could lose partially or completely. This indicates protection is necessary to achieve the full detoxifying function when used with feeding.

Conclusions:

This research indicates both SMBS and bacteria isolate LS100 can potentially be used with feeding to detoxifying DON, however, protection such as microencapsulation is necessary to reach their full detoxification functions.

ONTARIO PORK

Ontario Pork Research Final Report (Project # 17-022)

(maximum of 6 pages double-spaced)

Date: March 31, 2022

Introduction:

Mycotoxin deoxynivalenol (DON) contamination in animal feed may reduce growth performance and compromise the immune system of swine. Sodium metabisulphite (SMBS) has been tested on farm to treat the affected feed ingredients before feed making, or recently, added to feed during feeding to reduce the adverse effects of DON on animal growth (Frobose et al., 2017; Yang et al., 2020). The detoxification mechanism of SMBS was mainly attributed to its ability to reduce DON to its sulfonate forms, which are less toxic (Natskoulis et al., 2018; Shawk et al., 2019b). However, over-dose of SMBS can also exhibit toxicological effects in different body organs (Adebayo & Adenuga, 2012; Vally & Misso, 2012) and the nervous system (Lai et al., 2018). Particularly, when SMBS is added to the feed it may rapidly decompose to produce sulfur dioxide under the acidic conditions in the stomach, which may upset the animals and reduce the feed intake. For this reason, encapsulation of the SMBS is required to prevent the release of SMBS from the encapsulated microparticles in the mouth and stomach and ensure the delivery of SMBS to the intestines of animals to detoxify DON.

Detoxification of DON by microorganism is another promising approach. In our previous research a bacterial isolate, LS100, was found to have the detoxification function to DON in the lab condition. In order to evaluate the suitability of applying this bacterial isolate in pigs, more characterization is required which includes its oxygen tolerance and sensitivity to adverse gastrointestinal conditions.

Objectives: (original objectives from project proposal)

1. Develop an effective and practically feasible microencapsulation method for effective delivery of sodium metabisulfite (SMBS) to detoxify DON in the gut of weaned piglets. Proposed activities:

- In vitro characterization of SMBS microparticle structure

- In vitro release of SMBS from microencapsulation with simulated gastric and intestinal fluids

- In vitro evaluation of detoxification function of SMBS containing microparticles using simulated gastric and intestinal fluids

2. Determine the effects of a previously isolated microorganism on DON detoxification, gut barrier function, and nutrient transporter expression in weaned piglets. Proposed activity:

   - Assessment of tolerance to oxygen and gastric acid

   - Examination of detoxifying activity under simulated gut conditions

Note: The rest activities under Objectives 1 and 2, mainly animal trials, were undertaken by University of Manitoba under Swine Cluster Activity 9. We will happy to share the final report once the project ends.

Materials and Methods:

1. Preparation and characterization of encapsulated SMBS:

Three encapsulation procedures were tested which include hot melt granulation, hot melt granulation + secondary coating and two-step melt-solidify granulation methods (Figure 1). The efficiency of these methods were compared by the releasing profiles of SMBS in the simulated gastric fluid (SGF) and intestinal fluid (SIF). The two-step melt-congealment granulation method was selected and further optimized. Briefly, a cross-blades blender was used to disperse the SMBS fine powders in molten fatty acids of high melting point fat; the primary microparticles were formed during the cooling process. The primary microparticles were then dispersed in molten low melting point fatty acids and formed secondary microparticles during cooling. An enteric polymer Edudragit S100 that is insoluble at acidic pH and dissolves at above neutral pH was added into the molten fat to control the release of SMBS.

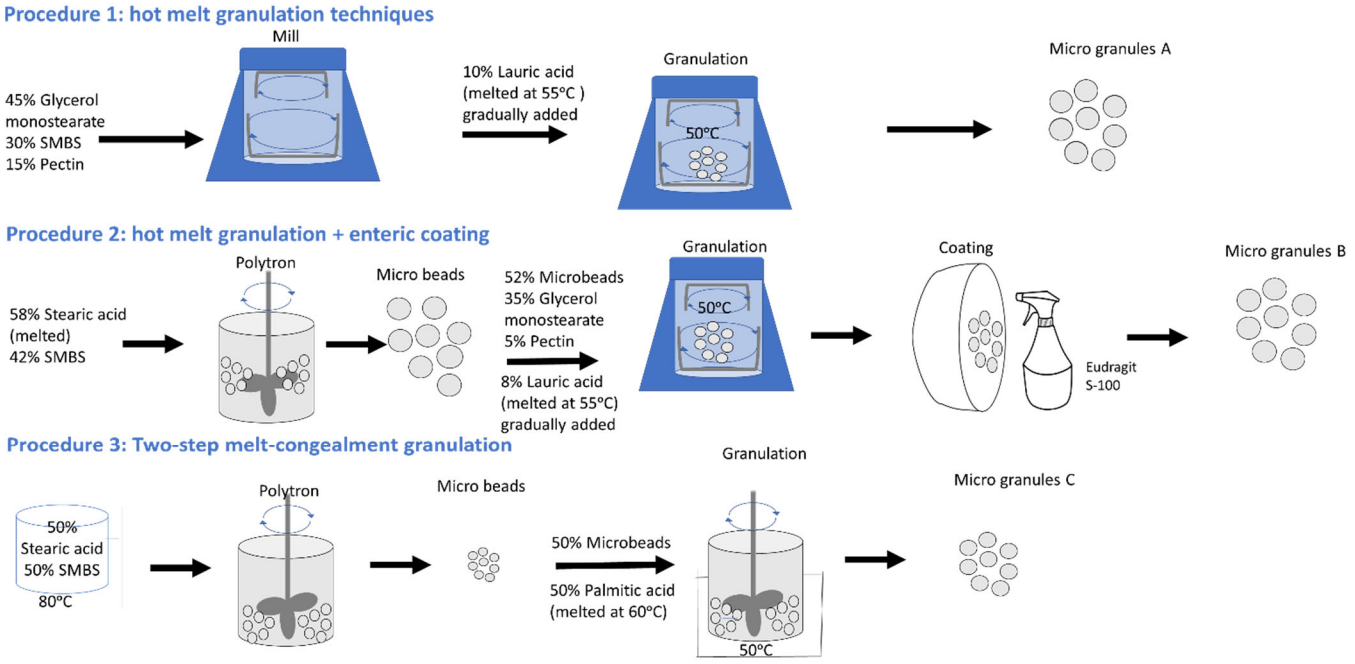

Figure 1 Schematic diagram of three encapsulation procedures.

Results and Discussion: (results and discussion should address objectives or state why these objectives could not be met)

1. **Preparation and characterization of encapsulated SMBS:**

1.1. The microgranules prepared were in the size range of 0.8 mm -1.2 mm, in round or irregular shapes (Figure 2). The releasing profiles of encapsulated SMBS produced from the three encapsulation procedures in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) are shown in Figure 3. Procedure 3, the two-step melt-congealment method was found the most promising in delivering SMBS to the intestines (Granules C), thus was selected for further optimization.

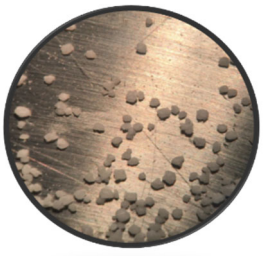

Figure 2. Microgranules of SMBS.

Upon optimization, an enteric Eudragit S-100, which is insoluble at acidic pH and soluble in above neutral pH, was added to reduce the release rate in stomach. This formula contained stearic acid 23.8%, SMBS 21.4%, palmitic acid 47.6%, and Eudragit S-100 7.2%. It released less than 30% of SMBS in SGF and completely released the encapsulated SMBS within 4 hours in SIF (Granule D, Figure 3). In the next step, we replaced 23% of stearic acid with carnauba wax which has a

higher melting point of 82°C. This resulted in a significant reduction in gastric release to less than 10%, suggesting over that 90% of the encapsulated SMBS could likely be delivered to the intestines (Granule E).

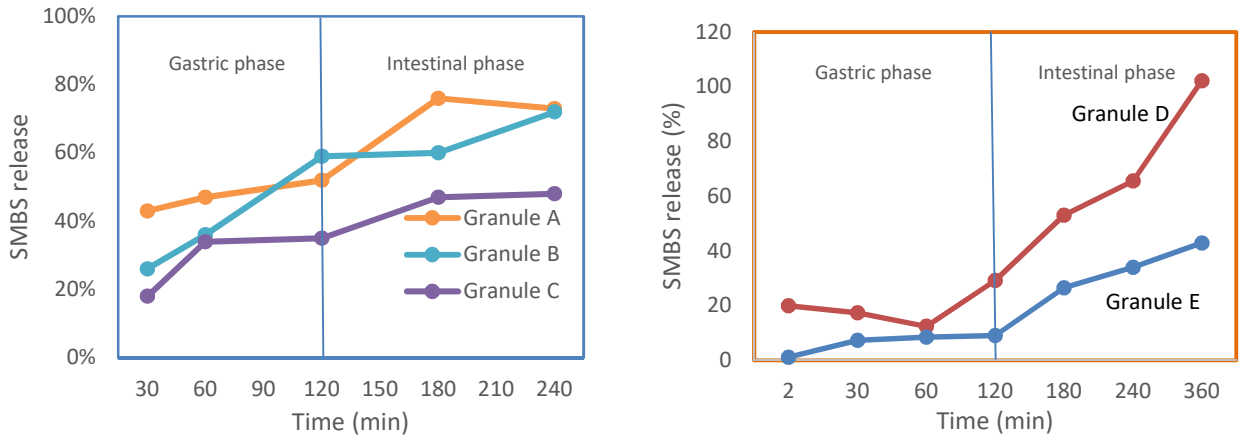

Figure 3 Release profiles of SMBS from microgranules prepared by three different methods in simulated gastric and intestinal fluids.

Excellent stability of encapsulated SMBS at room temperature was observed for over four months. The SMBS contents only slightly reduced from 17.5% to 16.2% (Figure 4). This result indicates that fat based substances are suitable materials for preventing oxidation of SMBS.

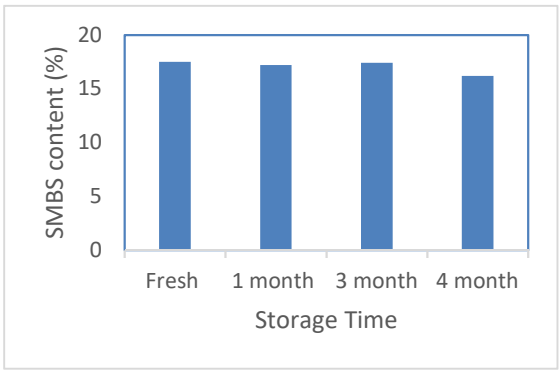

Figure 4 Storage property of encapsulated SMBS at room temperature (21 °C)

1.2. *Detoxification function of SMBS and encapsulated SMBS*

We identified a linear dose-response range of SMBS to detoxify DON as shown in Figure 5; For example, when the concentration of DON was 25 ppm, the detoxification effects increased with SMBS concentration in the range of 125 ppm-1250 ppm. This corresponds to the SMBS to DON ratio of 5 to 50. Interestingly, within this concentration range, the detoxification function of SMBS was not significantly affected by the pH levels, which is contradictory to the results reported in the literature. We believe this is because that the decomposed SMBS also has the ability to detoxify DON.

Nevertheless, preventing the release of SMBS in the stomach could reduce the discomfort in the animal stomach caused by formation of surfer dioxide.

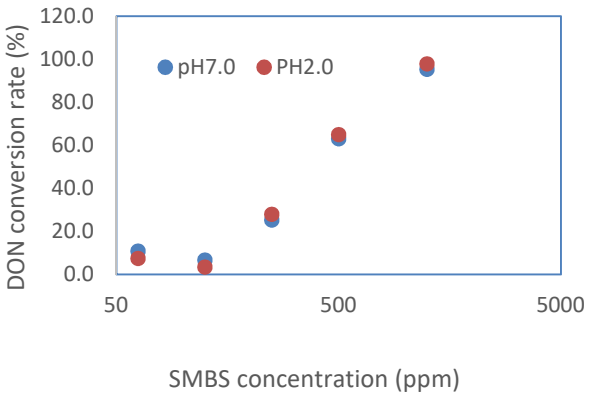

Figure 5 Detoxifying function of SMBS at different concentration showing the linear dose response range at pH 7 and pH 2, respectively.

The materials used for encapsulation of SMBS had no influence on the detoxifying function. Encapsulated SMBS demonstrated a similar detoxifying capacity to the non-encapsulated SMBS when tested in simulated digestive fluids, although is slightly lower. This can be explained by the fact that a small amount of SMBS was not released from the microgranules.

2. **Determine the effects of a previously isolated microorganism on DON detoxification, gut barrier function, and nutrient transporter expression in weaned piglets_ in vitro characterization**

The tolerances of bacterial isolate, LS100, to the presence of oxygen was assessed. Under anaerobic conditions, the initial culture and subsequent 12 subcultures of LS100 were all able to fully detoxify vomitoxin, indicating the stability of this isolate. After a full growth of the isolate under anaerobic conditions, the initial culture were then kept at room temperature aerobically and tested for the detoxification activity (once a day) by culturing the oxygen-exposed initial culture under anaerobic conditions. The culture maintained the detoxification activity for up to 11 days before a significant decrease of the activity. This indicates the isolate could tolerate oxygen to a certain degree, that made it convenient for the handling of the isolates for feed use.

The tolerance of bacteria isolate LS100 to bile salts was investigated at different concentrations of bile salts. Little growth of the isolate was detected in the presence of bile salts, suggesting that LS100 was sensitive to bile salts. The

tolerance of LS100 to different pH from 2.0 to 5.0 was also examined. When tested at different pH conditions, the isolate reached a full growth at near neutral pH; however, the growth was reduced more than 12% at pH 5.0 and over 70% at pH 2.0. These results suggest that LS100 may need protection for delivery and survival in the pig guts.

The detoxifying activity of LS100 was found completely lost after being incubated in simulated gastric conditions for 2 hours. To sustain its detoxifying activity, the LS100 was encapsulated in alginate based microcapsules by the extrusion technology. The detoxification activity of encapsulated LS100 was then tested at different storage time after encapsulation. The detoxifying activity of encapsulated LS100 in wet particles fully retained after 10 days storage at 4°C, whereas the dried particles retained full activity in 8 days, but lost all the activity in 10 days. The wet beads after 14 days storage were put into simulated gastric fluid for 2 hours, significant detoxifying activity of LS100 was present, with some loss of activity. Further optimization of the encapsulation formula could be helpful.

We further investigated the DON-detoxification ability of LS100 in the presence of digestive fluids obtained from pigs. The results demonstrated that pre-treatment of LS100 with stomach fluid from pigs demolished the bacterial DON-detoxification activity. The digesta from the jejunum also partially reduced the detoxification activity; however, the presence of ileal digesta did not affect the detoxifying ability. This result is consistent with the previous result that acids and bile salts were detrimental to the detoxification ability of LS100. These results further confirmed that encapsulation of LS100 to protect them from contacting stomach acids and bile salts might be useful to preserve its detoxification function.

Conclusions:

The following conclusions can be drawn from the current study:

1) This research identified a dose responsive range for SMBS to detoxifying DON in simulated gastrointestinal conditions. This information provided an useful guidance for further testing the encapsulated SMBS in animal trials. The two-step melt-congealment granulation method using fats of different melting points was shown to be an effective and convenient method to encapsulated SMBS. Such prepared SMBS product exhibited good storage stability. When tested in simulated gastrointestinal conditions, over 90% SMBS could be delivered to the intestines. The encapsulating material did not negatively affect the detoxification function of SMBS.

2) The bacteria isolate LS100 was shown to be stable in the presence of some levels of oxygen, which made it feasible for it being used as a feed additive. When tested in simulated gastrointestinal fluids, the bacteria isolate LS100 was shown to be sensitive to both bile salts and low pH environment, the detoxifying activity of which could lose partially or completely. This result was further confirmed in the stomach fluids collected from pigs, which demolished the bacterial detoxification activity completely; The detoxification activity of LS100 was not affected by the presence of ileal digesta from pigs, but the digesta from jejunum partially reduced the bacterial detoxification activity. The result also demonstrated that encapsulation could effectively protect the LS100 from deactivation by the adverse gastrointestinal conditions.

References:

Frobose, H.L., Stephenson, E. W., Tokach, M.D., DeRouchey, J.M., Woodworth, J. C., Dritz, S.S., Goodband, R.D. 2017. Effects of potential detoxifying agents on growth performance and deoxynivalenol (DON) urinary balance characteristics of nursery pigs fed DON-contaminated wheat. Journal of Animal Science, 95 (1), 327–337.

Natskoulis, P.I., Lappa, I.K., Panagou, E.Z., 2018. Evaluating the efficacy of turbimetric measurements as a rapid screening technique to assess fungal susceptibility to antimicrobial compounds as exemplified by the use of sodium metabisulfite. Food Res. Int. 106, 1037–1041.

Shawk, D.J., Dritz, S.S., Goodband, R.D., Tokach, M.D., Woodworth, J.C., DeRouchey, J.M., 2019. Effects of sodium metabisulfite additives on nursery pig growth. Transl. Anim. Sci. 3, 103–112.

Yang, C., Song, G., Lim, W., 2020. Effects of mycotoxin-contaminated feed on farm animals. Journal of Hazardous Materials. 389, 122087.

Knowledge Transfer:

1. A poster and abstract were presented in the First Annual Canadian Poultry Research Forum, June 21-23, 2021 (virtual). Poster is attached.
2. Sharmila Durairaj, Qian Guo, Qi Wang, Aicheng Chen. Sensitive Electrochemical Detection of Metabisulphite in Gastrointestinal Fluids. Submitted to Biosensors and Bioelectronics.
3. Another manuscript on the encapsulation methods is in preparation and will be submitted soon.