Development of novel microparticles for effective delivery of an antimicrobial essential oil to pig intestinal tract

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Young piglets have a high susceptibility to various stressors, including bacterial pathogens, oxidative stress and inflammation, leading to reduced growth performance, high mortality and morbidity rates, and compromised animal welfare. Antibiotic growth promoters (AGP) have been routinely used in pig diets, especially in nursery diets, for decades to reduce incidences of post-weaning diarrhea and improve growth performance. In 2010, total consumption of antimicrobials in food animal production worldwide was estimated at 63,151 tonnes with an increasing trend. The annual consumption of antimicrobials for pigs is 148 mg/kg body weight.

Mounting concern over this increasing use, coupled with the possible role of AGP use in the emergence of antimicrobial resistant bacteria, has coincided with an increasing amount of research to identify possible alternatives to AGP. Essential oils such as thymol are widely recognized as an alternative to AGP due to their antimicrobial, anti-inflammatory and anti-oxidative properties. However, as thymol is highly volatile, it is not fully effective when used in its natural state. To overcome this limitation, a method of protecting the essential oil until it reaches the lower gut is required.

Project objective

Our aim was to develop a novel microencapsulation technique that is affordable and can easily deliver thymol to the target site. In an *in vitro* experiment, low melting point fat microparticles were developed via a melt-granulation technique. Lauric acid was selected as a good carrier for thymol because of its miscibility at a molten state and at room temperature (23°C). Moreover, lauric acid has significantly reduced the melting point of thymol which provides the convenience of processing thymol at room temperature (23°C) in liquid form. To develop a molten oil mixture, lauric acid and thymol were separately weighed into a closed vial and melted in a water bath, mixed and stirred together with a stirring bar. A starch mixture was prepared using corn starch and pre-gelatinized starch mixed at a ratio of 3:1. Two per cent of the distilled water was calculated to denote the amount of polymer to be weighed. The molten oil mixture was mixed with the starch mixture by hand stirring in a container before adding a polymer solution in distilled water. The solid particles produced were immediately inserted into an ice-water bath and allowed to solidify overnight after which they were granulated. Using gas chromatography, *in vitro* release of thymol from low melting point fat microparticles in simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) was determined. The stability of the microparticles was measured by gas chromatography after 12 weeks of storage at 4°C.

Our findings

As shown in Figure 1, our results show that the low melting point fat microparticles with 2 per cent polysaccharide solution exhibited a slow release rate (%) of thymol and lauric acid in the SSF (21.2±2.3; 36 ±1.1), SGF (73.7 ± 6.9; 54.8 ± 1.7) and SIF (99.1±1.2; 99.1±0.6), respectively. The microparticles produced had good stability (> 90%) when stored at 4°C for 12 weeks (Figure 2).

Recommendation

The formula and method developed for encapsulating thymol in this research is simple to practice, affordable and can be potentially used to deliver essential oils effectively to the pig intestinal tract. Also, the method developed can be used by researchers to successfully deliver other essential oils to the pig gut.

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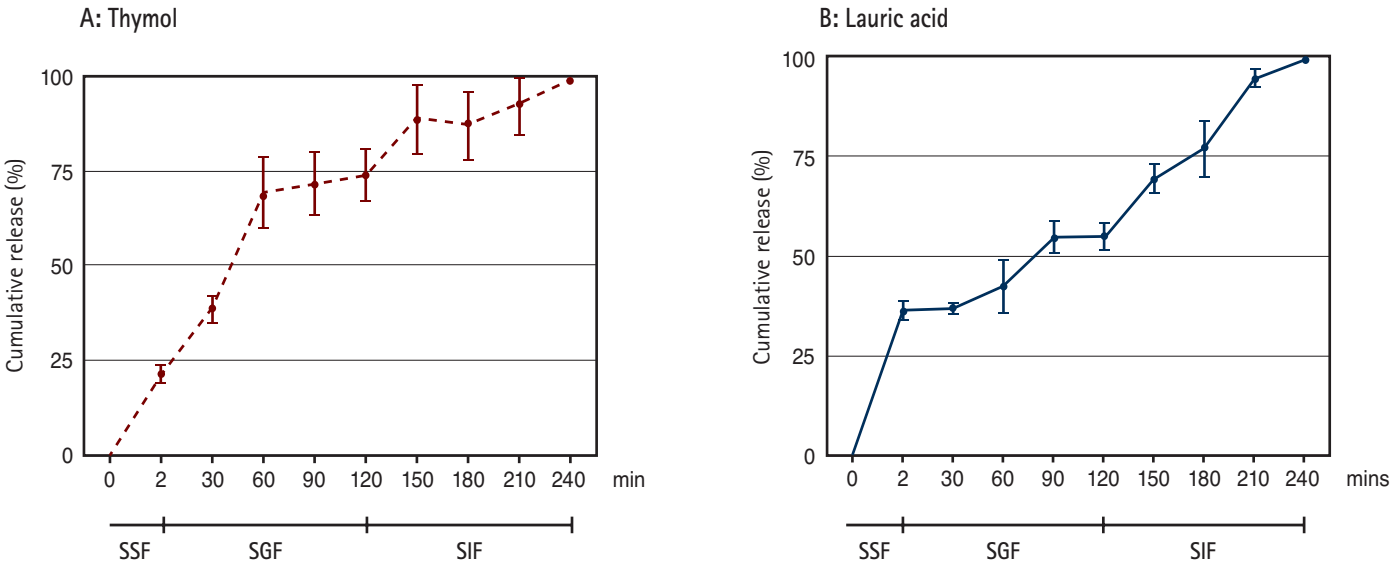
Figure 1. *In vitro* release profile of thymol and lauric acid from microparticles with two per cent polymers using simulated fluids.



In vivo studies will be conducted to verify the effectiveness of the low melting point fat microparticles. This formula and encapsulation method will be further optimized for better controlled release by investigating the physicochemical and molecular property of the low melting point fat microparticles and the retention of encapsulated thymol during feed processing, which will be simulated by steaming for different time periods and validated in a real pelleting process.

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Figure 2. Stability of microparticles of thymol and lauric acid with two per cent polymers stored at 4 °C for 12 weeks.