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Running title: Microencapsulated essential oils and fatty acids for feed application

Title: Development of novel microparticles for effective delivery of thymol and lauric acid to pig intestinal tract

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ABSTRACT

Antibiotics have been widely supplemented in feeds at sub-therapeutic concentrations to prevent post-weaning diarrhea and increase the overall productivity of pigs. However, the emergence of antimicrobial-resistant bacteria worldwide has made it urgent to minimize the use of in-feed antibiotics. The development of promising alternatives to in-feed antibiotics is crucial for maintaining the suitability of swine production. Both medium-chain fatty acids (MCFA) and essential oils exhibit great potential to post-weaning diarrhea; however, their direct inclusion has compromised efficacy because of several factors including low stability, poor palatability and low availability in the lower gut. Therefore, the objective of this study was to develop a formulation of microparticles to deliver a model of essential oil (thymol) and MCFA (lauric acid). The composite microparticles were produced by the incorporation of starch and alginate through a melt-granulation process. The release of thymol and lauric acid from the microparticles was in vitro determined using simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), consecutively. The microparticles prepared with 2% alginate solution displayed a slow release 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, whereas, the microparticles without alginate showed a rapid release of thymol and lauric acid from the SSF (79.9 ± 11.8%; 84.9 ± 9.4%), SGF (92.5 ± 3.5%; 75.8 ± 5.9%) and SIF (93.3 ± 9.4%; 93.3 ± 4.6%), respectively. The thymol and lauric acid in the developed microparticles with or without alginate both exhibited excellent stabilities (> 90%) during being stored at 4°C for 12 weeks and after being stored at room temperature for 2 weeks. These results evidenced that the approach developed in the present study could be potentially employed to deliver thymol and lauric acid to...
the lower gut of pigs, although, further in vivo investigations are necessary to validate the
efficacy of the microparticles.

**Keywords:** Encapsulation; Gut, Lauric acid, Microparticles, Slow release, Thymol

1. **INTRODUCTION**

Young animals are very vulnerable to diseases, and using antimicrobials is the most cost-effective method to improve the health and productivity of food production animals raised with conventional agricultural techniques (Looft et al., 2012; Yang et al., 2015). Although this practice has been banned in Europe and other countries have also started to minimize the use of antibiotics in the animal production, it still exists in major parts of the world (Hassan et al., 2018). Therefore, replacing antibiotics with cost-effective alternatives remains crucial to ensure a sustainable food animal production.

Essential oils are considered as valid candidates to replace antibiotics in the feed industry (Li et al., 2012; Gong et al., 2014; Omonijo et al., 2018). Essential oils (e.g., thymol) are extracted from plants and can promote growth performance and health in animals because of their biological activities and antimicrobial activities (Si et al. 2006a; Edris, 2007; Del Nobile et al. 2008; Brenes et al., 2010; Puvaca et al., 2013; Rassu et al, 2014). With the identification and characterization of bioactive components in plant extracts and significant progress in mechanistic research with these components in food production animals, many research efforts have been made to use essential oils substituting antibiotics within the animal production chain (Omonijo et al., 2018). The rationales for using essential oils in animal feeds have relied on their abilities to inhibit bacterial growth, reduce virulence through quorum-sensing disruption, and regulate innate immunity of animals (Hassan et al., 2018). However, most essential oils have a high minimum inhibitory concentration (MIC) that are unlikely accepted in the industry regarding cost-
efficiency, feed palatability and government regulation (Yang et al., 2015; Omonijo et al., 2018; Hassan et al., 2018). Therefore, it is vital to ensure the delivery of essential oils to the target site for increasing their efficacy.

Essential oils have very high volatility, and their bioactive compounds are readily degradable when exposed to heat, oxygen, light, or during their interactions with other compounds, thus, negatively affecting their biological activities and antimicrobial activities (Si et al., 2006a; Zhang et al., 2016a; Gonçalves et al., 2017). Additionally, several studies are demonstrating that several essential oils including thymol and carvacrol have almost completely vanished in the upper digestive tract of pigs (Michiels et al., 2008; Zhang et al., 2016a). Therefore, unprotected essential oils can be significantly vanished at the manufacture, transportation, and storage of feeds and as well as during delivery to the pig gut, thus hindering access to the distal part of pig intestine (Omonijo et al., 2018). This serves as a major challenge to the use of essential oil in pig feeds. Thus, it is crucial to establish a useful and practical delivery approach for using essential oils in feeds.

Medium-chain fatty acids (MCFA) including lauric acid (C\textsubscript{12}) and its ester derivatives also have potential to substitute antibiotics in weaning piglets (Han et al., 2011; Zentek et al., 2012; 2013; Hanczakowska et al., 2013; De Smet et al., 2016). Several studies indicated that MCFA could inhibit \textit{Salmonella} growth (Van Immerseel et al., 2004; Messens et al., 2010). Synergistic antimicrobial activities between oregano oil and caprylic acid were observed with several strains including \textit{Salmonella} (Hulánková and Bořilová, 2011). Similarly, Vande Maele et al. (2016) demonstrated in an \textit{in vitro} study that a combination of lauric acid and cinnamaldehyde had synergistic effects in inhibiting the growth of \textit{Brachyspira hyodysenteriae} that causes swine dysentery. The use of MCFA is popular both in the food and feed industries. However, some
MCFA and their ester derivatives can compromise feed palatability and acceptance and reduce feed intake in pigs due to their unpleasant odors (Omonijo et al., 2018). Thus, it is also essential to develop a useful and practical delivery approach for using MCFA in feeds.

Microencapsulation has been becoming one of the most popular and practical approaches to mask the unpleasant taste/odor, and deliver bioactive compounds in food production animals (Piva et al., 2007; Chitprasert et al., 2014). Ideal microencapsulation should not only stabilize essential oils but also release them specifically in the targeted regions of the intestine (Chen et al., 2017; Omonijo et al., 2018). Therefore, the objective of the present study was to develop a formulation of microparticles containing both thymol and MCFA for effective delivery to pig intestinal tract.

2. MATERIALS AND METHODS

2.1. Materials

Thymol (≥ 98.5%), lauric acid (LA), palmitic acid (PA, C₁₆), stearic acid (SA, C₁₈), amylase, sodium alginate (low viscosity), pepsin originated from porcine and pancreatin originated from porcine were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). Cornstarch was purchased from Cargill (Cargill Inc., Minneapolis, MN, USA) and pre-gelatinized starch (1500) from Coloran (West Point, PA, USA).

2.2. Selection of a suitable fatty acid

Three fatty acids including lauric acid, palmitic acid, and stearic acid were used in this experiment because those have a melting point above a melting point (42°C) of thymol and have been used to deliver bioactive compounds (Ma et al., 2016; Pitigraisorn et al., 2017). The melting points of lauric acid, palmitic acid, and stearic acid are 43°C, 63°C and 69°C, respectively. Ten grams of each fatty acid was mixed with 10 g of thymol, respectively. The mixtures were then
melted in a water bath at 70°C. After melting, the mixtures were stirred for 30 min. The molten mixture of each fatty acid with thymol was left to stay at 55°C without stirring for 2 h before placing at room temperature (23°C) up to 6 h to allow for solidification.

To observe the crystal morphology of thymol, lauric acid, and their mixture, an emulsion of thymol, lauric acid and the mixture of thymol and lauric acid (ratio 1:1) were prepared. Lauric acid and thymol were melted at 70°C individually or mixed at a ratio of 1:1, and then added into the water at 10% with 1% tween 80 as a surfactant. The mixture was mixed using a Polytron (PT10-35GT, Kinematica AG, Switzerland) for 2 min at 13,000 rpm to make an emulsion. Then, three emulsions were stored at 4°C overnight allowing the emulsions to crystallize. The crystal morphology was examined under a microscope (Eclipse Ci, Nikon, Japan).

2.3. The melting point of thymol, lauric acid, and their mixture

Among the three fatty acids tested, lauric acid was selected for further study because its mixture with thymol remained a homogeneous liquid at room temperature for 6 h. Before use, 1 g of thymol and lauric acid each were kept at -80°C for 30 min and then mixed by vortexing for 30 sec at 3,000 rpm. The mixture was kept in -80°C for 3 h and then ground to a fine powder using a grinder. The grinder was kept -20°C for 3 h before use to avoid increasing temperature to higher than the melting temperature of thymol and lauric acid. The melting temperature of the thymol, lauric acid, and their mixture (50: 50 wt%) was measured by differential scanning calorimetry (DSC). For the measurement, 12.1 mg thymol, 13.1 mg lauric acid, and 10.7 mg mixture were weighed into individual Tzero Aluminum hermetic pans. The pan was placed in the chamber of DSC (Q Series DSC, TA Instrument). The DSC was programmed as follow: 1) Equilibrate at
25˚C; 2) Jump to -10˚C; 3) Ramp 10˚C/min to 80˚C (1st run); 4) Cooling; 5) Equilibrate at -
10˚C; 6) Isothermal for 5 min; and 7) Ramp 10˚C/min to 80˚C (2nd run).

2.4. Preparation of microparticles

For preparing microparticles without adding 2% alginate solution, 5 g of lauric acid and 5 g of
thymol were weighed into a closed vial separately and melted at 70˚C in a water bath, mixed
together and stirred for 30 min. Thirty grams of cornstarch and 5 g of pre-gelatinized starch (a
ratio of 6:1) were weighed separately and then mixed in a container by hand shaking. The molten
thymol and lauric acid mixture was added into the starch mixture and then mixed by hand
stirring. Fifteen milliliters of distilled water (3 times of pre-gelatinized starch) was added to the
mixture. The containers were immediately placed into an ice-water bath for 1.5 h and kept in a
refrigerator (4˚C) overnight for solidification. The solid particles were then granulated into
micro-particles with a granulating machine (UAM Pharmag, Germany) at 90 rpm using a pore
size of 0.1 mm and dried at room temperature (23˚C) for 1 h before being stored in a refrigerator
(4˚C).

For preparing microparticles with alginate, a total of 0.3 g of alginate was weighed and dissolved
in 15 mL of distilled water to make a 2% (w/v) alginate solution. The same protocol described
above was used to make the microparticles except for replacing the 15 mL of water with the 2%
alginate solution.

2.5. Morphology of microparticles

The morphology of the microparticles produced with or without adding a 2% alginate solution
was determined with a light microscope (Axio Cam 105, Carl-Zeiss, Switzerland; Nikon eclipse,
Japan) at 10 × magnification and the Zen Image Software (2012) was used to determine the
surface diagram of the microparticles.

2.6. In vitro release of thymol and lauric acid from the microparticles

In vitro release of thymol and lauric acid from the microparticles was determined with simulated
digestive fluid using previously published procedures with some modifications (Minekus et al.
2014). The simulated salivary fluid (SSF) contained 15.1 mmol/L KCl, 13.6 mmol/L NaHCO3,
3.7 mmol/L KH2PO4, 0.15 mmol/L MgCl2(H2O)$_6$ and 0.06 mmol/L (NH4)$_2$CO3. The simulated
gastric fluid (SGF) contained 47.2 mmol/L NaCl, 25 mmol/L NaHCO3, 6.9 mmol/L KCl, 0.9
mmol/L KH2PO4, 0.5 mmol/L (NH4)$_2$CO3 and 0.1 mmol/L MgCl2(H2O)$_6$. The simulated
intestinal fluid (SIF) contained 85 mmol/L NaHCO3, 38.4 mmol/L NaCl, 6.8 mmol/L KCl, 0.8
mmol/L KH2PO4 and 0.33 mmol/L MgCl2(H2O)$_6$. The pH of SSF, SGF and SIF was adjusted
using HCl or NaOH to 7.0, 3.0 and 7.0, respectively. The final digestion mixtures of the
electrolyte solution for SSF, SGF and SIF contained 1.5, 0.15 and 0.6 mmol/L of CaCl2(H2O)$_2$,
respectively. Respective enzymes were also added to simulate digestion in pig digesta. Alpha-
amylase originated from human saliva was included in the SSF final digestion mixture at a
concentration of 75 U/mL. Pepsin originated from porcine gastric mucosa was added to the SGF
final digestion mixture at a concentration of 2000 U/mL and pancreatin originated from porcine
pancreas was added to the SIF final digestion mixture at a concentration of 100 U/mL.

Forty microparticle samples (each 0.5 g) were employed to mimic digestion within the mouth,
stomach and small intestine in pigs. Four samples were taken from each sampling point (0, 2, 30,
60, 90, 120, 150, 180, 210 and 240 min) with points between 0 to 2 min representing the
digestion in the mouth, 2 to 120 in the stomach and 120 to 240 min in the small intestine. All
simulated solutions were maintained at 37°C. The SSF was added to each of the samples at a
ratio of 1:1 and placed in the incubator with shaking (Innova TM. 4200, New Brunswick Scientific, Edison/ NJ. USA) for 2 min. The pH was adjusted to 3 with 1M HCl before SGF was added. At the end of the SGF stage, the pH was adjusted to 7 with 1M NaOH followed by addition of the SIF. To measure the concentration of thymol and lauric acid, 5 mL of oil extraction solvent (hexane) was added to each of the supernatants, shaken (IKA Vibrax VXR Basic, U.S.A) for 20 min and allowed to stay for 30 min. Each of the supernatant from each point was diluted 10 times and the diluent was filtrated using a syringe-driven filter unit (polytetrafluoroethylene, 0.22 nm) and further analyzed by gas chromatography (GC) following the method explained below. Two replicates for each sample were used.

The column installed was SUPELCO WAX™ 10 (fused silica capillary column; 60 m × 0.25 mm × 0.50 nm film thickness and the temperature limits from 35-280°C). Thymol and lauric acid were identified by comparing the retention time with the standard thymol and lauric acid and their concentrations were calculated by comparing the total peak area of thymol and lauric acid with the standard curve. Released thymol or lauric acid content = thymol or lauric acid concentration in GC vial × 5 (volume of added hexane) × dilution times/thymol or lauric acid in the dry samples × 100%.

**2.7. Determining the stability of thymol and lauric acid in the microparticles**

The stability of thymol and lauric acid in the microparticles with or without alginate was measured after being stored at room temperature (23°C) for 2 weeks and during the storage at 4°C for 12 weeks. The recovery rate of thymol and lauric acid were determined with the procedure described as below. Samples were taken at different time points (1 week, 3 weeks, 6 weeks and 12 weeks) for analysis. Each sample (0.5g) was suspended in 15 mL of distilled water
containing Pancreatin (100 U/mL). The mixture was incubated and analyzed as described above. Each of the samples was measured in triplicate.

3. RESULTS AND DISCUSSION

3.1. Selection of a fatty acid

There was no visible phase separation for all three mixtures at the molten state (Fig. 1A). After being placed at room temperature (23˚C) for 6 h, the molten mixture of thymol and lauric acid was still in a clear liquid state without having phase separation, however, the other two molten mixtures (thymol / palmitic acid and thymol / stearic acid) solidified and formed a gel-like mixture (Fig. 1B).

These results are consistent with the DSC measurements. As shown in Fig. 2, the mixture of lauric acid and thymol exhibited a single melting peak with a value of 30.6˚C, which is lower than both that of thymol (52.8˚C) and lauric acid (47.4˚C). This suggested that the mixture of lauric acid and thymol was in a eutectic solution, that is, a mixture of two or more pure chemicals at certain ratios, in which the chemicals inhibit the crystallization process of one another, resulting in a system having induced melting point depression (Washburn, 1924).

Once cooling the emulsions, thymol crystalized in irregular shapes (Fig. 3A), whereas lauric acid crystalized in round shapes (Fig. 3B). The resulted mixture of the two crystalized into somewhat ovular shaped particles without visible distinctions between the two individual components (Fig. 3C). This observation indicates that thymol and lauric acid co-crystalized together. Both results from DSC and microscopy observation showed that thymol and lauric acid form a pair of a good candidate for a formulation of antimicrobial microparticles for the following reasons. Firstly, since lauric acid significantly reduced the melting point of thymol, it served as a liquid carrier for thymol at room temperature for a period up to 6 h. This property provides an excellent
convenience for processing of thymol products such as in the present study. This is because when at a liquid state, thymol and fatty acids can be easily mixed and better absorbed by the starch granules which helps to ensure even distribution and better protection of the core ingredients within the encapsulation matrix. Secondly, a combination of thymol with lauric acid in one product may provide additional protective benefits to the animals. An *in vitro* study demonstrated that lauric acid could effectively inhibit the growth of *Brachyspira hyodysenteriae* with a MIC value less than 1.5 mM (Vande Maele et al., 2016). Dietary fats with a considerable level of lauric acid and myristic acid increased broiler growth performance that may be related to lauric acid’s antimicrobial properties (Zeitz et al., 2015). Most recently there was a study showing that lauric acid can reduce *Campylobacter* spp. in broiler meat (Zeiger et al., 2017). Lauric acid’s ester derivatives (e.g., monolaurin) are also known for their protective biological activities as antimicrobial agents (Seleem et al., 2016). The exact mechanism of lauric acid antimicrobial effect is still unclear. However, it is believed that some MCFA can damage the cell membrane, therefore, causing bacterial death (Desbois et al. 2010). It has been believed that the amphipathic structure of MCFA allows them to cause pores with a different size in the cell membrane. MCFA also could cause bacteria death by reducing enzyme function, blocking nutrient absorption and producing toxic compounds for bacteria (Desbois et al. 2010). Therefore, in this study lauric acid is not only a suitable carrier for thymol but also a bioactive compound with antimicrobial properties.

3.2. **Morphology of microparticles**

The compositions of microparticles with/without alginate include 66.22%/66.67% cornstarch, 11.03%/11.11% pre-gelatinized starch, 11.03%/11.11% thymol, 11.03%/11.11% lauric acid and 0.7%/0% alginate. The average particle sizes of the microparticles were 800 µm in diameter, and
this was similar to the average size of 890 µm for microparticles produced by Benavides et al. (2016) through the method of ionic gelation of alginate. There is no difference in the average particle size between the microparticles produced with or without alginate; however, the shapes and surfaces of the two types of microparticles were different (Fig. 4). The microparticles with alginate were mostly spherical with a relatively smooth surface, whereas those without alginate had irregular shapes with rough edges and coarse surfaces.

Many kinds of polymers have been employed to encapsulate and deliver bioactive compounds in both food and feed applications (Almeida et al., 2013; Zhang et al., 2016a; Chen et al., 2017). For applications in animal feeds, it is better to use natural polymers that have been approved for use in feeds. Starch is popularly used for microencapsulation because it is biodegradable, edible, commonly available at low cost, nonallergic, easy to use and thermo-processable (Zhu, 2017). Starch consists of both amylose and amylopectin (Tester et al., 2004; Udachan et al., 2012). Pre-gelatinized starch has undergone processing under intense heat conditions by cooking, drying and making into fine powder thus, leading to better solubility in water and being readily solubilized at room temperature (Romano et al., 2018; Fiorda et al., 2015). The combined use of cornstarch and pre-gelatinized starch in this study increases the water retentivity (Romano et al. 2018), thus promotes hydrogen bonding and the formation of the network in the encapsulation matrix. As a natural polymer derived from brown seaweed, alginate is a linear and anionic polysaccharide (Dragan, 2014). At room temperature, alginate is soluble in water allowing the formation of gel without heating and cooling cycles, which make alginate as an attractive microencapsulation material for feed applications (Benavides et al., 2016; Agüero et al., 2017). The inclusion of alginate to the starch matrix improved the shape and surface properties. This
could be attributed to its remarkable crosslinking capability and excellent film-forming properties.

3.3. *In vitro release profiles of thymol and lauric acid from the microparticles*

As shown in Fig. 5A, both thymol and lauric acid encapsulated in the microparticles with alginate exhibited slow release profiles in the simulated gastrointestinal fluids. The cumulative release (%) of thymol and lauric acid increased gradually to 21.2 ± 2.3 and 36.0 ± 1.1 in the SSF, 73.7 ± 6.9 and 36.8 ± 0.6 in SGF. Both thymol and lauric acid were completely released in the SIF within 240 min. However, as shown in Fig. 5B, the microparticles produced without alginate had a rapid release of thymol (79.9 ± 11.8%) and lauric acid (80.8 ± 5.9%) after incubation in the SSF for 2 min. When the microparticles were placed in the SGF for 120 min, the cumulative release rates reached 92.5 ± 3.5% and 75.8 ± 5.9% respectively for thymol and lauric acid. The rest of thymol and lauric acid were released from both types of microparticles in less than 40 mins after they were placed in the SIF.

The goal of a current delivery method is to release thymol and lauric acid at a low percentage in the mouth and stomach but have a sustained release as it passes through the intestine (Piva et al. 2007). The fast release of thymol and lauric acid in SSF from the microparticles without alginate is primarily due to the presence of alpha-amylase in the SSF, an enzyme that is known to digest starch quickly. The excellent solubility of pre-gelatinized starch could also have contributed to the fast release of the active components. The inclusion of alginate to the starch matrix markedly reduced the release rate in the SSF. This is mainly due to the existences of carboxylic groups in alginate molecules and calcium ions in the simulated digestive fluids. Calcium ions may form crosslinks between carboxylic groups in addition to hydrogen bondings, leading to enhanced
networks of encapsulation matrix, therefore, retard the dissolution of starch molecules and slow
the release of thymol and lauric acid. The globular shaped and smooth surface of microparticles
with alginate would have a smaller specific surface area compared to the irregular shaped and
rough surface of microparticles without alginate. This may be another factor contributing to the
better release property of alginate containing microparticles. Notably, alginate also effectively
reduced the release of active components in the SGF which can be explained by the pH
sensitivity of alginate molecules. When it is under very acidic conditions (e.g., pH at stomach)
that are lower than its pKa, the carboxylic groups are not ionized and stay as COOH resulting in
an insoluble structure (Agüero et al., 2017). When pH is close to 7 which is similar to the
intestinal pH, the carboxylic groups became ionized (COO-) resulting in that the polymer chain
significantly expands and the hydrophilic alginate matrix enlarges (Agüero et al., 2017). In this
study, the results indeed demonstrated that alginate significantly decreased the release of thymol
and lauric acid in SGF and increased their release in the SIF. Many studies have shown that
alginate matrix prevented a quick release of active components in the acidic environment of the
stomach and allowed a prolonged release under the intestinal conditions (Zastre, 1997, Zhang et
al. 2016a). However, compounds that are highly soluble and have a low molecular weight cannot
be prevented from releasing in the mouth and stomach even though the granules matrix does not
erode or swell. The alginate-containing microparticles developed in this study need to be further
optimized to reduce the release rates in the SSF and SGF.

Although the release behavior of thymol and lauric acid from the microparticles provides
precious information, it is challenging to precisely demonstrate release behavior in pig gut
because of the complexity of gut physiological environments. This was supported by the study
indicating that the rate of release of encapsulated carvacrol in the pig stomach via in vivo studies
was 25% higher than the rate obtained from *in vitro* studies (Zhang et al., 2016a), which may be due to the phenolic binding to other components such as fats and hydrophobic compounds present in the diet (Lallès et al., 2009). Therefore, *in vivo* release behavior of the microparticles has to be determined eventually in the gastrointestinal tract of pigs.

3.4. The stability of microparticles with/without alginate during storage

As shown in Fig. 6, thymol and lauric acid had good stabilities (> 95%) in both types of microparticles with or without alginate after being stored at room temperature (23°C) for 2 weeks. As shown in Fig. 7, thymol and lauric acid had good stabilities (> 90%) in both types of microparticles with or without alginate after being stored at 4°C for 12 weeks. Durante et al. (2012) showed that the encapsulation of wheat bran oil into 2% (w/v) sodium alginate beads significantly increased the stability of wheat bran oil at 4°C. This was also found in the research conducted by Otálora et al. (2016), that the encapsulation of betalain with calcium-alginate had good stability when stored at low relative humidity.

Stability during storage is an essential factor that should be considered for a feed additive. Feed additives have a 1-2 year shelf life under current industry practice. Our preliminary data demonstrated that the current microparticles are stable during short-term storage. However, the stability of long-term storage (e.g. 1-2 year) must be further investigated. The inclusion of antioxidants in the formula may be considered to enhance the stability of encapsulated thymol and lauric acid. In conclusion, the formulation and method established in this study for the encapsulation of thymol and lauric acid in microparticles are relatively simple and can be used as a potential method to effectively deliver essential oils and MCFA to the pig intestinal tract. This unique essential oil formula will be further optimized for better-controlled release though
investigating the physicochemical and molecular property of the microparticles. Retention of
encapsulated thymol and lauric acid during feed processing will be mimicked by the treatments
of steam for different time periods and validated in a real pelleting process. Further
investigations are needed to confirm the efficacy of the microparticles with \textit{in vivo} studies.

Notes
The authors declare the following competing financial interest (s): Drs. C. Yang, S. Liu, and M.
Nyachoti have a patent application in process for the developed microparticles.

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**Figure legends**

Fig. 1. A) Pictures showing the molten mixture of thymol and fatty acids at 0 min at room temperature (23°C). B) Pictures showing the molten mixture of thymol and fatty acids at 6 h at room temperature (23°C). FA1- mixture of thymol and lauric acid, FA2 – mixture of thymol and palmitic acid; FA3 – mixture of thymol and stearic acid.

Fig. 2. Differential scanning calorimetry (DSC) of (A)Thymol, (B) Lauric acid, and (C) Mixture of thymol and lauric acid (50: 50wt%). The second run with heating rate 10 °C/min from -10°C to 80°C.

Fig. 3. Morphology of crystals of thymol (A) and lauric acid (B) and a mixture of thymol and lauric acid (C) after crystallization. The measuring bar in the pictures were 1µm.

Fig. 4. Morphology and surface diagram of the microparticles of lauric acid and thymol with and without 2% alginate observed with a light microscope. (A) Morphology of microparticles with alginate; (B) Morphology of microparticles without alginate; (C) Surface diagram of microparticles with alginate and (D) Surface diagram of microparticles without 2% alginate.

Fig. 5. *In vitro* release profile of thymol and lauric acid from the microparticles with (A) and without (B) alginate using simulated fluids (SSF - simulated salivary fluid, SGF - simulated gastric fluid and SIF - simulated intestinal fluid). (Mean ± SD, n = 4).
Fig. 6. Stability of the microparticles of: (A) thymol in the microparticles with alginate, (B) lauric acid in the microparticles with alginate, (C) thymol in the microparticles without alginate and (D) lauric acid in the microparticles without alginate. Samples were stored at room temperature (23°C) for 2 weeks. (Mean ± SD, n = 4).

Fig. 7. Stability of the microparticles of thymol and lauric acid with (A) and without (B) alginate stored at 4°C for 12 weeks. (Mean ± SD, n = 4).
Fig. 1. Omonijo et al. (2018)
Fig. 2. Omonijo et al. (2018)

![Graph showing heat flow and temperature.]

- Heat flow (Exo up)
- Temperature (°C)
- Lines: A, B, C
- Key temperatures: 30.6°C, 47.4°C, 52.8°C
Fig. 3. Omonijo et al. (2018)
Fig. 4. Omonijo et al. (2018)
Fig. 5. Omonijo et al. (2018)
Fig. 6. Omonijo et al. (2018)

A: Microparticles with 2% alginate solution: Thymol

B: Microparticles with 2% alginate solution: Lauric acid

C: Microparticles without 2% alginate solution: Thymol

D: Microparticles without 2% alginate solution: Lauric acid
Fig. 7. Omonijo et al. (2018)

A: Microparticles with 2% alginate solution

B: Microparticles without 2% alginate solution
**Step 1**  
Molten mixture of thymol and lauric acid

**Step 2**  
Mix thymol/lauric acid with starch and alginate solution

**Step 3**  
Solidify in an ice water bath

**Step 4**

**Step 5**  
Grind with a granulator

**Step 6**

Final granules

Harden at 4 °C overnight