MICROENCAPSULATION OF BASIL OIL AND ITS EFFECT ON REPLACEMENT OF ANTIBIOTIC GROWTH PROMOTER, ANTIOXIDANT STATUS AND GUT FUNCTIONS OF BROILERS

Miss Sureerat Thuekeaw

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Applied Animal Science
Department of Animal Husbandry
FACULTY OF VETERINARY SCIENCE
Chulalongkorn University
Academic Year 2022

การห่อหุ้มแบบจุลภาคของน้ำมันโหระพา และผลของการเป็นสารเสริมทดแทนยาปฏิชีวนะ ต่อสมรรถภาพการเจริญเติบโต สถานะการต้านอนุมูลอิสระ และการทำงานของลำไส้ไก่เนื้อ

น.ส.สุรีรัตน์ ถือแก้ว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาสัตวศาสตร์ประยุกต์ ภาควิชาสัตวบาล
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2565

Thesis Title	MICROENCAPSULATION OF BASIL OIL AND ITS EFFECT ON REPLACEMENT OF ANTIBIOTIC GROWTH PROMOTER, ANTIOXIDANT STATUS AND GUT FUNCTIONS OF BROILERS
By	Miss Sureerat Thuekeaw
Field of Study	Applied Animal Science
Thesis Advisor	Associate Professor Dr. CHACKRIT NUENGJAMNONG, Ph.D.
Thesis Co Advisor	Associate Professor Dr. KRIS ANGKANAPORN, Ph.D.
	Professor Dr. SUWABUN CHIRACHANCHAI, Ph.D.

Accepted by the FACULTY OF VETERINARY SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the FACULTY OF VETERINARY SCIENCE

(Professor Dr. SANIPA SURADHAT, Ph.D.)

DISSERTATION COMMITTEE

..... Chairman

(Professor SOMCHAI CHANPONGSANG)

..... Thesis Advisor

(Associate Professor Dr. CHACKRIT NUENGJAMNONG, Ph.D.)

..... Thesis Co-Advisor

(Associate Professor Dr. KRIS ANGKANAPORN, Ph.D.)

..... Thesis Co-Advisor

(Professor Dr. SUWABUN CHIRACHANCHAI, Ph.D.)

..... Examiner

(Assistant Professor Dr. HATAIRAT PLAIMAST, Ph.D.)

..... Examiner

(Associate Professor SUPHACHAI NUANUALSUWAN)

..... External Examiner

(Associate Professor Dr. Keatisak Soisuwan, Ph.D.)

สุรีรัตน์ ถือแก้ว : การห่อหุ้มแบบจุลภาคของน้ำมันโหระพา และผลของการเป็นสารเสริมทดแทนยาปฏิชีวนะ ต่อสมรรถภาพการเจริญเติบโต สถานะการต้านอนุมูลอิสระ และการทำงานของลำไส้ไก่เนื้อ. (MICROENCAPSULATION OF BASIL OIL AND ITS EFFECT ON REPLACEMENT OF ANTIBIOTIC GROWTH PROMOTER, ANTIOXIDANT STATUS AND GUT FUNCTIONS OF BROILERS) อ.ที่ปรึกษาหลัก : รศ. น.สพ. ดร. จักรกริศน์ เนื่องจำนงค์, อ.ที่ปรึกษาร่วม : รศ. น. สพ. ดร.กฤษ อังคนาพร,ศ. ดร.สุวบุญ จิรชาญชัย

การศึกษานี้มีวัตถุประสงค์ 1) เพื่อศึกษาการเตรียม และลักษณะของน้ำมันโหระพาไมโครแคปซูล 2) เพื่อตรวจสอบความเสถียรของน้ำมันโหระพาไมโครแคปซูลต่อต้านระบบทางเดินอาหารจำลองและการเก็บรักษา 3) เพื่อตรวจสอบคุณสมบัติการต้านจุลชีพ และต้านอนุมูลอิสระของน้ำมันโหระพาไมโครแคปซูล และ 4) เพื่อประเมินการเสริมน้ำมันโหระพาไมโครแคปซูลต่อประสิทธิภาพการเจริญเติบโต สถานะการต้านอนุมูลอิสระ ค่าการย่อยได้ในลำไส้ส่วนไอเลียม และสัณฐานวิทยาของลำไส้เล็กในไก่เนื้อ การศึกษานี้แสดงการใช้พอลิเมอร์สองชั้น (ไคโตซาน และโซเดียมอัลจิเนต) ที่มีประจุต่างกันนำไปสู่การตอบสนองต่อพีเอชแบบคู่ ไมโครแคปซูลห่อหุ้มแบบสองชั้นทำการศึกษาลักษณะทางกายภาพเคมี ประสิทธิภาพการบวมตัวและประสิทธิภาพการปลดปล่อยสาร ความเสถียรของกิจกรรมการต้านอนุมูลอิสระและฤทธิ์ต้านจุลชีพ ใช้ไก่เนื้อพันธุ์ Ross 308 เพศเมีย อายุ 1 วัน จำนวน 288 ตัว แบ่งเป็น 4 กลุ่ม (กลุ่มละ 6 ซ้ำ ซ้ำละ 12 ตัว) วางแผนการทดลองแบบสุ่มสมบูรณ์ อาหารทดลองมีดังนี้ กลุ่มที่ 1 อาหารพื้นฐาน กลุ่มที่ 2 อาหารพื้นฐานเสริมอะไวลามัยซิน ที่ระดับ 10 พีพีเอ็ม กลุ่มที่ 3 อาหารพื้นฐานเสริมน้ำมันโหระพาที่ระดับ 500 พีพีเอ็ม และกลุ่มที่ 4 อาหารพื้นฐานเสริมน้ำมันโหระพาไมโครแคปซูลที่ระดับ 500 พีพีเอ็ม ตามลำดับ ผลการศึกษาการวิเคราะห์ฟูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปกโตรสโคปี และเทอร์โมกราวิเมตริกพิสูจน์ได้ว่าน้ำมันโหระพาถูกห่อหุ้มโดยไคโตซาน-อัตจิเนต สามารถปรับปรุงเสถียรภาพทางความร้อน การบวมตัวแสดงประสิทธิภาพการบวมที่ดี ขณะที่ไคโตซาน-อัตจิเนตแสดงการการปลดปล่อยสะสมของน้ำมันโหระพาในกระเพาะอาหาร (63.3 ± 4.6%) และในลำไส้ (86.8 ± 4.4%) ตามลำดับ ไคโตซาน-อัตจิเนตรักษาคุณสมบัติการต้านอนุมูลอิสระ และการต้านจุลชีพของน้ำมันโหระพาภายใต้สภาวะการเก็บรักษา และสภาวะความทนทานต่อกรดน้ำดี ทริปซิน รวมถึงความร้อนได้ดี น้ำมันโหระพาไมโครแคปซูลปรับปรุงอัตราการเจริญเติบโตเฉลี่ยต่อวัน และประสิทธิภาพการเปลี่ยนอาหารเป็นน้ำหนักตัวของไก่เนื้อตลอดการทดลอง 42 วัน ($P < 0.05$) ไก่ที่ได้รับน้ำมันโหระพาไมโครแคปซูลมีการย่อยได้ของโปรตีนและพลังงานรวมที่ปรากฏในลำไส้เล็กในลำไส้ส่วนไอเลียมดีกว่ากลุ่มอื่นๆ ($P < 0.05$) จำนวนประชากรแลคโตบาซิลลัส และอีโคไลไม่ได้รับผลกระทบจากอาหารทดลอง นอกเหนือจากนี้ทั้งน้ำมันโหระพา และน้ำมันโหระพาไมโครแคปซูลมีผลในเชิงบวกต่อความสูงของวิลไล สัดส่วนของวิลไลต่อคริปท์ และพื้นที่ผิวของวิลไลในลำไส้ส่วนเจจูนัมของไก่เนื้อ เมื่อเปรียบเทียบกับกลุ่มอาหารพื้นฐาน และอาหารพื้นฐานเสริมอะไวลามัยซิน ($P < 0.05$) เอนไซม์ซูเปอร์ออกไซด์ดิสมิวเทสในเยื่อบุลำไส้ส่วนดูโอดีนัมของกลุ่มน้ำมันโหระพาไมโครแคปซูลเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P < 0.01$) ขณะที่ระดับมาลอนไดอัลดีไฮด์ลดลง ($P < 0.01$) แนวคิดพอลิเมอร์สองชั้นในรูปแบบไมโครแคปซูลสามารถขับเคลื่อนประสิทธิภาพของน้ำมันโหระพา ดังนั้นน้ำมันโหระพาไมโครแคปซูลสามารถนำไปใช้ได้เป็นสารเสริมอาหารสัตว์ สำหรับการปรับปรุงความสมบูรณ์ของลำไส้ การใช้ประโยชน์ได้ของโภชนะ นำไปสู่ประสิทธิภาพการเจริญเติบโตที่ดีของไก่เนื้อ

สาขาวิชา	สัตวศาสตร์ประยุกต์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2565	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

6075511431 : MAJOR APPLIED ANIMAL SCIENCE

KEYWORD: Basil oil, Broiler, Double-layered Microcapsule, Dual pH responsive, Growth performance, Intestinal integrity, Microencapsulation

Sureerat Thuekeaw : MICROENCAPSULATION OF BASIL OIL AND ITS EFFECT ON REPLACEMENT OF ANTIBIOTIC GROWTH PROMOTER, ANTIOXIDANT STATUS AND GUT FUNCTIONS OF BROILERS. Advisor: Assoc. Prof. Dr. CHACKRIT NUENGJAMNONG, Ph.D. Co-advisor: Assoc. Prof. Dr. KRIS ANGKANAPORN, Ph.D.,Prof. Dr. SUWABUN CHIRACHANCHAI, Ph.D.

The objectives of this work were 1) to study the preparation and characterization of microencapsulated basil oil (MBO), 2) to determine the stability of MBO against simulated gastrointestinal tract and storage 3) to investigate the antimicrobial and antioxidant properties of MBO and 4) to evaluate the supplementation of MBO on growth performance, antioxidant status, ileal digestibility and intestinal morphology in broiler chickens. The present work demonstrates the use of double-layered polymers (chitosan, CS and sodium alginate, SA) with opposite charges leading to a dual pH responsiveness. The double-layered microcapsule was studied on physicochemical characteristics, the swelling performance as well as the release efficiency and the antioxidant and antimicrobial stabilities under the storage. A total of 288 one - day - old female broilers (Ross 308) were randomly allocated into 4 groups (6 replicates of 12 birds), based on a completely randomized design. Dietary treatments were (i) basal diet (NC), (ii) basal diet with avilamycin at 10 ppm (PC), (iii) basal diet with free basil oil (FBO) at 500 ppm, and (iv) basal diet with MBO at 500 ppm, respectively. Fourier transform infrared (FTIR) and thermogravimetric analyses (TGA) proved successful loading of BO in CS-SA and improved its thermal stability. Swelling showed great performance, whereas CS-SA displayed the cumulative release of BO in gastric stage (63.3 ± 4.6%) and in intestinal stage (86.8 ± 4.4%), respectively. CS-SA retains the antioxidant and antimicrobial activities of BO under storage in addition to a great tolerance of acids, bile, trypsin, including the thermal conditions. MBO improved average daily gain (ADG), and feed conversion ratio (FCR) of broilers throughout the 42-d trial ($P < 0.05$). The broilers fed MBO diet exhibited a greater AID of crude protein (CP) and gross energy (GE) compared with those in other groups ($P < 0.05$). *Lactobacillus* spp. and *E. coil* populations were not affected by feeding dietary treatments. Both FBO and MBO had positive effects on jejunal villus height (VH), villus height to crypt depth ratio (VH:CD) and villus surface area (VSA) of broilers compared to NC and PC groups ($P < 0.05$). Superoxide dismutase (SOD) level in the duodenal mucosa of MBO group was significantly increased ($P < 0.01$), whereas malondialdehyde (MDA) level was significantly decreased ($P < 0.01$). The concept of double-layered polymer in microcapsule form could be considered as a promising driver of the BO efficiency, consequently MBO could be potentially used as a feed additive for improvement of intestinal integrity and nutrient utilization, leading to better performance.

Field of Study:	Applied Animal Science	Student's Signature .....
Academic Year:	2022	Advisor's Signature .....
		Co-advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to a number of people who supported and inspired my work on this dissertation without them, this would not have been possible.

Assoc. Prof. Dr. Chackrit Nuengjamnong, my advisor, for allowing me to discover the value of independent work, thinking and research, for his assistance in the experimental studies during my graduate study.

Prof. Dr. Suwabun Chirachanchai and Assoc. Prof. Dr. Kris Angkanaporn, co-advisors, for their guidance, kindness, concern in my work and technical advice during my work and their confidence in my abilities.

I would also like to thank my colleagues of the M.S., Ph.D. students, instructors and all the staff members in Department of Animal husbandry, Faculty of Veterinary Sciences, Chulalongkorn University for sharing experiences and knowledge during the time of study.

I would like to thank Assoc. Prof. Dr. Wilmer Javier Pacheco and all staff members of the Department of Poultry Science, Auburn University for sharing experiences and knowledge during the time in The U.S.

I would like to thank Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Food Risk Hub, Research Unit of Chulalongkorn University and The Petroleum and Petrochemical College, Chulalongkorn University for providing research facilities.

Furthermore, I would like to thank Chulalongkorn University, Thailand in collaboration with Thailand Science Research and Innovation (TSRI) and Nano Artech Company, Thailand through the research and researcher for industries (RRI), Thailand (Code: PHD61I0002) for financial support.

Finally, I would like to thank my father Suwin Thuekeaw and my mother Rattanaporn Thuekeaw, who supported me in every aspect of my education. I want to acknowledge their love and devotion to provide me with the opportunity to have the best education possible.

Sureerat Thuekeaw

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

High environmental temperature is a principal limiting factor for broiler production, which can cause heat stress (HS), especially in tropical regions. HS adversely affects physiology status and the productivity of birds (Attia *et al.*, 2018). Therefore, the means to increase the bioavailability of certain feed additives is one of the most crucial strategies to boost gastrointestinal function and animal performance (Celi *et al.*, 2019). Essential oils (EOs) have already been recognized as a group of functional feed additives with a great benefit to reduce the negative effects of antibiotic growth promoters (AGPs) in animal feed (Chitprasert and Sutaphanit, 2014; Attia *et al.*, 2017). It is well known that AGPs can promote growth performance and heath in animals. Oppositely, the use of AGPs has led to antimicrobial resistance in food chain. Due to pharmacological properties of EOs particularly antimicrobial, antioxidant and anti-inflammatory activities, it could be considered as an alternative to AGPs (Yarmohammadi *et al.*, 2020).

Basil oil (BO, *Ocimum basilicum* Linn.), is one of EOs and originally cultivated to use as a flavoring agent. Methyl chavicol (estragole), linalool, eugenol, and methyl cinnamate are main pharmacological constituents (Rawat *et al.*, 2017; Ahmed *et al.*, 2019). BO containing 92.48 % of methyl chavicol had a better 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Bunrathep *et al.*, 2007). In addition to it antioxidant capacity, BO has been also reported for its digestive stimulants, antibacterial, anti - inflammatory activities as well as immunomodulatory effects (Pu *et al.*, 2020). However, inconsistent results of BO efficiency in poultry nutrition have been reported.

The natural compounds of EOs are more fragile and volatile during feed processing (at 70 °C - 85 °C), storage conditions (e. g. oxygen, heat, light, and time etc.), and gastrointestinal conditions (e. g. aqueous conditions, endogenous enzymes and pH) (Chitprasert and Sutaphanit, 2014). These harsh environments cause the failure of the use of BO in livestock; consequently, the development of feed additives to boost gastrointestinal function and animal performance is necessary.

Microencapsulation is recognized as a process to fabricate the microcapsules covering the active species with polymers (Saini *et al.*, 2020). The microcapsules have potential for the delivery system of EOs into animal body with a minimum damage. Numerous microencapsulation techniques have been proposed e.g. spray drying, emulsions, and coacervation methods etc. (Ydjedd *et al.*, 2017). Among these techniques, spray-drying is practical because of its ease to scale up, low cost, and less time consumption in comparison with other techniques (Celebioglu and Uyar, 2019). On the other hand, there are several drawbacks as a consequence of the high inlet temperature (typically 200 °C - 250 °C) (Fournaise *et al.*, 2020). The problematic points of microcapsules are the encapsulation efficacy (%, EE), the stability, and the active ingredient content in the finished products (Celebioglu and Uyar, 2019; Sun *et al.*, 2020). For example, it was reported that antioxidant and antimicrobial activities of the microencapsulated carvacrol in pectin/sodium alginate were decreased when increasing the inlet temperature (Sun *et al.*, 2020). According to the abovementioned cases, the microencapsulation technique for EOs with effective and efficient delivery in animal body should be investigated.

Alginate (AG) is a natural polysaccharide with biocompatible, biodegradable, and non-toxic properties. AG consists of β-(1, 4) linked D-mannuronic acid (M) and **α**-(1,4) linked L-guluronic acid (G). In general, AG is in the form of sodium alginate (SA). It is an anionic polymer which can be fabricated in various forms such as gel, membranes,

scaffolds including porous foams (Kang *et al.*, 2019). In similar to SA, chitosan (CS) is the second most abundant polysaccharide with the promising characteristics such as biocompatibility, biodegradability, and non-toxicity. CS is composed of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units derived from chitin. CS exhibits high solubility under acidic condition (Sabourian *et al.*, 2020). On the structural viewpoint, SA contains carboxylic acid groups, leading to a negatively charged polymer, whereas CS carries the amino group to perform positively charged chains. Therefore, the combination of SA and CS results in the complementary charged biopolymers and potentially forms the polymer complexes (Keawchaoon and Yoksan, 2011; Cuadros *et al.*, 2015). Gawad and Fellner (2019) demonstrated the foam prepared from the mixture of SA and CS was stable in the gastric condition to release glycerol in intestinal condition. It was reported that Babchi essential oil loaded in SA microporous foam showed 87.70% ± 1.20% EE with good photostability (Wadhwa *et al.*, 2019). However, the long-term holding capacity of porous structure is limited due to its open pore network (Peretz *et al.*, 2013). It is important to note that animal feed with encapsulated active ingredients has to deal with the specific conditions, i.e., pH (at pH 2 and pH 7.0 for stomach and intestine, respectively), temperature (at the body temperature 39.5 ºC), and endogenous enzymes.

On this viewpoint, in order to release active ingredients at the targeted intestine, the pH responsiveness might be the key function to add up. To our idea, as CS and SA are complementary and responsive to pH, the tailoring of CS-SA microcapsules in the double-layered structure is a way to achieve effective encapsulation while favor the stabilization of EOs during the delivery through the stomach until the release at the targeted intestine. The fabrication of SA in porous microcapsules followed by the encapsulation of active ingredients via absorption process before coating with CS, the double-layered microcapsules can be obtained.

Based on the double-layered structure of two types of pH responsive polymers, it can be expected that when the CS-SA microcapsules enter the gastric stage, CS will be gradually solubilized under acidic environment while SA functions in protecting the active ingredients. In the step of entering the intestine, SA eventually dissolved in the neutral to the basic conditions to allow the release of the active ingredients.

The present work, therefore, double-layered microencapsulation was considered in manufacturing of microencapsulated basil oil (MBO) for delivery system of BO in the intestine via the dual pH responsive CS-SA microcapsules. The work demonstrates how to fabricate the MBO along with the studies on physicochemical characteristics, the swelling performance as well as the release efficiency under simulated gastrointestinal fluid including the antioxidant and antimicrobial stabilities through the storage. Additionally, this was conducted to evaluate the effects of MBO supplementation on productive performance, ileal nutrient digestibility, bacterial population, jejunal histomorphology, and antioxidant capacity of broilers.

OBJECTIVES OF STUDY

1. To study the preparation and characterization of MBO
2. To determine the stability of MBO against simulated gastrointestinal tract and storage
3. To investigate the antimicrobial and antioxidant properties of MBO
4. To evaluate the supplementation of MBO on growth performance, antioxidant status, ileal digestibility and intestinal morphology in broiler chickens

CHAPTER 2

LITERATURE REVIEW

1. Essential oils (EOs) as feed additives in poultry nutrition

The negative effects of antibiotic growth promoters (AGPs) in animal feed are well documented. It is well known that AGPs can promote growth performance and heath in animals. Oppositely, the use of AGPs has led to antimicrobial resistance in food chain. Due to the concern regarding AGPs in livestock, alternative additive strategies have been investigated to boost gastrointestinal function and animal performance**.** A wide variety of phytochemicals (PEAs) such as essential oils (EOs) and purified components have been reported as green additives with several benefits to reduce resistant bacteria in livestock, leading to food chain contaminations. EOs are natural bioactive compounds with economically viable for several fields such as food, aromatherapy, pharmaceuticals and medical. EOs are volatile lipophilic substances obtained from different parts of plant as secondary metabolites. The variable chemical compositions of EOs consist of monoterpenes, sesquiterpenes, triterpenes, flavonoids and aromatic compounds. They are useful compounds to protect themselves from insects, animals and microorganisms. The application of EOs has been interesting as valuable alternatives to AGPs due to beneficial characteristics, particularly antimicrobial, antioxidative and anti-inflammatory effects (Amiri *et al.*, 2021)**.** Carvacrol, thymol, citral, eugenol and cinnamaldehyde are commonly used in animals (Omonijo *et al.*, 2018b). However, some variations in EOs depend on plant genetics, growing conditions, development stage at harvest, drying technology, storage periods and extraction processes. EOs are usually added into animal diets at recommended levels and can have a broad range of bioactive properties, including feed palatability, digestive functions, feed utilization, as well as modifier of gut microbial fermentation

and reduction of methane emission (Gholami-Ahangaran *et al.*, 2022), leading to improvement of production performance and quality of animal products (Tolve *et al.*, 2021). The different researches have published the effect of EOs on poultry performances as follow. For example, the supplementation of phytonutrients in low energy diet improved feed conversion and intestinal morphology of broilers (Yan et al., 2019). Betancourt *et al.* (2019) found that Colombian oregano essential oil (*Lippia origanoides* Kunth) with its high thymol showed a positive impact on body weight (BW) and the feed conversion ratio (FCR) of broiler challenged with a mixed inoculum of *Eimeria* spp. It was also reported that a commercial product (3.05% thymol, 2.3% carvacrol, and 0.26% cinnamaldehyde) in broiler diet improved growth rate by increasing nutrient digestibility and, modulating intestinal morphology, immunity and antioxidant ability (Su *et al.*, 2021). Additionally, licorice (*Glycyrrhiza glabra*) essential oil at the recommended level (200 mg/kg) not only improved thyroid hormones but also reduced *E.coil* in the GI tract leading to better growth performance (Geravand *et al.*, 2021). Broilers under temperature stress received a blend of EOs in drinking water, resulting in a decrease in liver weight and increase in abdominal fat. The blend of EOs had no effect on the thiobarbituric acid-reactive substances (TBARS), which is used to define lipoperoxidation in meat (Tekce *et al.*, 2020). However, the inconsistent results of EOs efficiency have been reported in both laboratory and field studies because of the variations of product compositions, extraction methods, available dosages and experimental design (e.g., the dose and duration of the consumption, concentrations of the included active substances, animal age, genetics, feeds and health status).

2. The mode of action of EOs in poultry nutrition

2.1 Antimicrobial effect of EOs

The constituents in EOs can help animals against pathogens which are typically colonized in GI tract. Pathogens cause the lower performance and poor health. Thus, the balance between pathogenic and beneficial microbial communities is necessary. There are numerous studies that EOs have antimicrobial property. Abers *et al.* (2021) indicated that rosemary (1,8-cineole, α-pinene, and camphor) and thyme (thymol) are the highly effective EOs which have both antibacterial and antifungal activities. Antimicrobial effect is linked to the hydrophobicity, functional groups of bioactive constituents in EOs, namely monoterpene hydrocarbons (Tanhaeian *et al.*, 2020) and bioavailability in the GI tract of animals. Generally, the accepted mechanisms of antimicrobial activity associated with disturbances of the bacterial cell wall. It is possible that its phenolic components are able to disintegrate the outer and inner mitochondrial membranes of harmful microorganisms, resulting in releasing lipopolysaccharides, increasing the permeability of the cytoplasmic membrane and losing some functions in their cells. Due to hydrophobic characteristic, they are easy to transport cross the phospholipids of cell membrane which involves pH disturbance and dysfunction of protein synthesis (Zeng *et al.*, 2015). Likewise, bioactive compounds in EOs may coagulate cytoplasm and reduce proton motive force, as well as prevent the synthesis of flagellin (Gholami-Ahangaran *et al.*, 2022), causing the negative influence to the growth of pathogenic microorganisms. Gram-positive bacteria are more susceptible to EOs than gram-negative bacteria because of their hydrophobic molecules, which increase cell permeability and then damage on both cell wall and cytoplasm. The thick cell wall of gram-negative bacteria increases the resistance to EOs (Tanhaeian *et al.*, 2020). There are several studies demonstrating the supplementation of EOs in poultry diets. It was comprehensively reviewed that birds

receiving diet with BO increased the proportion of beneficial bacteria in the gastrointestinal tract. In contrast, the proportion of pathogenic bacteria decreased on account of antimicrobial effect (Riyazi *et al.*, 2015). Furthermore, the antimicrobial effect is reported to be depended upon several factors i.e. high variation of bioactive compounds in each product, bacteria strains, available dosage and physiological functions of host (Turek and Stintzing, 2013).

2.2 Antioxidant effect of EOs

Normally, reactive oxygen/nitrogen species (ROS/RNS) are generated by normal metabolism process, resulting in oxidative stress and lipid peroxidation. Natural antioxidants are interesting property of EOs in modulating oxidative stress. Free radicals can damage other structures in the gut, especially intestinal epithelium as the first barrier between intestinal issue and its luminal environment. A poor function of intestinal epithelium often triggers loss of immune function, and malabsorption. Malondialdehyde (MDA) as an indicator of lipid peroxidation and antioxidant status in gastrointestinal tract. In poultry industry, stressors including disease, mycotoxin, malnutrition as well as heat stress may be induced the over production of ROS/RNS.

The plant extracts as natural antioxidants have been positively correlated with antioxidant status in the gut as well as the shelf life of animal products owing to radical scavenging ability of phenolic constituents (Stevanovic *et al.*, 2018). The phenolic constituent in EOs acts as free radical scavenger by donating its phenolic OH groups to ROS/RNS, thereby potentially reducing oxidative stress, tissue damage, and lipid peroxidation. Gholami-Ahangaran *et al.* (2022) reported that the *Lamiaceae* family (oregano, thyme, basil, mint, rosemary, sage, savory, marjoram, hyssop and lavender) has good antioxidant activity. Total phenolic component of crude extract of Thai basil oil was found approximately 45.38 mg. gallic acid equivalents/g (GA/g) (Naidu *et al.*, 2016). Furthermore, total phenols in *Ocimum basilicum* could be 5 times than those

in ginger (*Zingiber officinalis*) (147 and 23.5 mg GA/g, respectively) (Hinneburg *et al.*, 2006). Antioxidant property of EOs can prevent villi damage by eliminating free radicals and stimulating antioxidant enzymes (Abd El-Hack *et al.*, 2022). Therefore, EOs can modulate the balance of antioxidant defense system in animal body leading to the reduction of lipid peroxidation. Elecampane (*Inula helenium* L.) rhizome inhibited MDA concentration whereas it simulated the activity of antioxidant enzymes in the intestine of broiler (Abolfathi *et al.*, 2019). For an action of EOs on meat quality, the supplementation of EOs has been interesting due to side effects of synthetic preservatives such as butylated hydroxytoluene (BHT) and hydroxyanisole (BHA) (Zeng *et al.*, 2015). Lipid oxidation of chicken meat can cause unpleasant odor, taste, appearance and short shelf life, which are the important factors for customers consideration. For instance, the combination of capsaicin, carvacrol and cinnamic aldehyde improved sensorial characteristics of poultry meat. The possible reason might be the high activity of radical scavenging which stopped the oxidation process.

Additionally, the impact of EOs on lipid metabolism is discussed. The supplementation of EOs (cineole, borneol, citral, menthone, geraniol, mentol, fenchyl alcohol, fenchone, and ionone) can reduce activity of hepatic 3-hydroxy-3-ethylglutaryl coenzyme A (HMG–CoA) reductase, which is an important enzyme in cholesterol synthesis (Abd El-Hack *et al.*, 2022). Birds receiving 300 ppm of EOs had a lower cumulative percentage of fat in the abdominal than those in the control (Jamroz, 2003). Besides, EOs can prevent oxidation during storage, transportation and undesirable conditions. The oxidation can result in low qualities of diets and reduce feed palatability.

3. Absorption, metabolism, elimination and toxicity of bioactive components of EOs

The application of EOs in animal feed is necessary to know their pharmacokinetics and side effects on animals. EOs are considered generally regarded as safe (GRAS) in mammals (Horky *et al.*, 2019). However, some EOs can irritate the gastrointestinal mucosa causing inflammation (Abd El-Hack *et al.*, 2022). Metabolism, elimination and toxicity of bioactive components in EOs are necessary for the evaluation of animal safety. There are three main routes in which EOs enter the body including inhalation, skin permeability and oral administration with considering the objective of application, the problems to be solved and appropriate route are crucial steps to observe maximal outcomes. A majority of these substances are eliminated by the kidneys and lung as in the form of glucuronides and CO_2, respectively. A minority was found in urine and feces. Their accumulation in the body issue is unlikely because of highly volatile character and short half-lives (Kohlert *et al.*, 2000). Carvacrol, thymol, eugenol, and trans-cinnamaldehyde have the half-life between 1.84 and 2.05 h (Horky *et al.*, 2019).

For the pulmonary delivery of volatile molecules in poultry via inhalation, aromatics are breathed in the top of the nose and olfactory mucous membrane with its receptors. The receptors identify the smell and the sensory stimulation is passed through the olfactory bulb, the olfactory nerve and directly into the limbic system of the brain. Theses constituents are absorbed through the respiratory tract and lungs and then circulated through the bloodstream. Thus, an increased rate and depth of breathing will enhance the uptake of EOs. This route always uses for preventing or ameliorating respiratory diseases such as infectious bronchitis. Inhalation is not only fastest way to get EOs into the blood circulation, but also safety way to animal.

For dermal delivery, lipophilic character of EO compounds encourages skin permeability by sample diffusion. The advantages of this route are the reduction of

the side effect and no interference with other parts of body because EOs act only at the local site of application. The efficacy of dermal absorption depends on skin area, concentration of administrated constituents, time of administration.

Oral ingestion is the most widely accepted route of EOs delivery in animals. For livestock production, the oral administration is an optimum way to prove the proposed objective of the replacement of AGPs and easy to farm management. EOs with diets are degraded and metabolized by a chemical process in stomach. Moreover, the amount of absorbed compounds depend on their degrees of solubility, type of molecules and the activity of digestive enzymes. EOs are partially absorbed via the proventriculus into the bloodstream (Horky *et al.*, 2019). In intestine, lipophilic molecules of EOs with other lipids are formed as micelles by the bile function. Before EOs transported the bloodstream, they are absorbed into intestinal epithelial cell by passive diffusion.

There are several factors influencing the toxicity of EOs such as type of constituents, route of exposure, time of exposure and availability. Several studies have clearly established that toxic effects of EOs based on *in vitro* and *in vivo* studies. Poultry are continuously fed EOs without withdrawal periods. EOs may be accumulated in various tissues, resulting in their impact on sensory quality of poultry meat. Moreover, masking techniques and encapsulation could be considered to reduce the risk of EOs to animal health.

4. Different types and levels of the major bioactive compounds in EOs

The achievement of EOs supplementation in poultry production must consider functional properties of active components and the dosage of EOs. Each individual constituent has its own characteristic on animals. To illustrate, carvacrol and thymol were found in oregano oil and they acted as antimicrobial substances. They affected

the lipopolysaccharides of gram-negative bacteria, resulting in cell membrane permeability and ATP loss. The combined carvacrol and *p*-cypnene induced the synthesis of heat shock proteins (HSPs). Linalool can permeate and degrade fatty acyl chains of membrane lipid bilayers of biofilm. Methyl chavicol, a main chemotype in basil oil, acted as electron donor to peroxyl radical in the initiation step. However, some bioactive constituents can be toxic and decreased animal performances (Stevanovic *et al.*, 2018). The different types and levels of the major bioactive compounds on performance of farm animals are summarized in Table 1. The main components could be responsible for antimicrobial and other biological effects. In contrast, some components can be acute or chronic, reversible or irreversible, toxic, on animals.

Toxic effects of EOs include mucosal irritation, vomiting, diarrhea, convulsions, renal failure, and depression of central nervous system which caused very high doses and long time periods. The accumulation of EOs in animals is unlikely due to their fast metabolic conversion and excretion. In poultry production, when birds continuously received diets containing EOs, they could be deposited in various tissues, resulting in their impact on sensory quality of poultry meat. However, there are many affecting factors on the potential of bioactive compounds in EOs such as i) geographic origin i.e., climate, soil and water quality, ii) ages of plant, iii) parts of plant, and iv) the extraction methods (Zeng *et al.*, 2015).

Table 1 Different types and levels of the major bioactive compounds on broiler performance

Active component	Level	Animal	Effects
Thymol, eugenol and piperine	100–200 mg/kg	Broiler	No effect on intestinal numbers of *C. perfringens*, and FCR. Reduction of FBW.
Carvacrol, thymol, Eucalyptol, lemon	125–500 mg/kg	Broiler	Modulation of intestinal microbial communities.
Oregano, laurel leaf and lavender	50 mg/kg	Broiler	Increase in BWG, Restoration of the antioxidant/oxidant system balance after the parasitic infection.
Cinnamaldehyde, carvacol, extract	250 mg/kg	Broiler	No effect on BWG, FI, FCR. Increase in *bifidobacteria* and LAB counts and reduction of *Bacteroides* spp. and *E. coli* counts.
Oregano EO	300–600 mg/kg	Broiler	No effect on BWG and FI. Improvement of FCR. Decreased faecal *Eimeria* sp. oocyst counts.
Peppermint EO	400 mg/kg	Broiler	No effect on BW, ADG, FI and FCR, faecal DMD and CPD and intestinal morphology
Thyme EO	1 g/kg	Broiler	No effect on FCR, AME, ATTD and intestinal microflora populations. Increase in BWG and FI.
Thyme EO	0.5 g/kg	Broiler	No effect on cecal and large-intestinal bacterial counts. Improvement of intestinal barrier integrity and antioxidant status

5. Basil (*Ocimum basilicum* Linn.)

Basil, a sub-class of the *Lamiaceae* family, is cultivated commercially in many countries. Basil oil (BO) has been extensively utilized in various industries such as food perfumery, pharmaceutical products and cosmetics. Structurally, EOs are categorized into 4 groups including terpenes, terpenoids, phenylpropenes and other constituents. The economically important chemotypes in *O. basilicum,* mainly are estrogole (methyl chavicol), linalool, methyl eugenol, eugenol, trans-ß-bergamotene, linalool, 1,8-cineole, camphor, methyl cinnamate, citral, camphor, geraniol, (E)-anethole (Padalia *et al.*, 2017) in addition to a high source of minerals and vitamin A (Nadeem *et al.*, 2020).

The major constituents of BO with aromatic ring are methyl chavicol (62.5-77.6%) and linalool (14.4-34.1%) (Kumari *et al.*, 2017). Methyl chavicol, a phenylpropene, consists of a benzene ring substituted with a methoxy group and an allyl group. According to the chemical structure, methyl chavicol has excellent antioxidant activity. Santos *et al.* (2018) revealed that methyl chavicol can reduce oxidative damage and lipid peroxidation. Linalool, a monoterpenoid, consists of a hydroxyl group, with an apolar structure. It is soluble in organic solvents but poorly soluble in water. Linalool is an acyclic monoterpene tertiary alcohol containing a hydroxyl group. Monoterpenes are antibacterial component, interfering in development stage of microorganism, physiological and metabolic activities (Masyita *et al.*, 2022). Besides, it might eliminate spoilage by insects or fungi in poultry feed (Beier *et al.*, 2014). These are beneficial effects for reducing campylobacteriosis and salmonellosis in commercial poultry. Methyl chavicol and linalool have been reported to exert antibacterial, antioxidant, anti-inflammatory activities and immunomodulatory effect (Riyazi *et al.*, 2015). *O. basilicum* presents *in vitro* antimicrobial properties against a wide range of microorganisms (Citarasu, 2009). Consequently, it can be applied to poultry industry due to different chemotypes. They act as natural antioxidant and

antimicrobial substances (Pandey *et al.*, 2014; Poonkodi, 2016). BO had the highest of antimicrobial activity against *Salmonella* Enteritidis when compared with cinnamon, clove, fingerroot and garlic oil (Rattanachaikunsopon and Phumkhachorn, 2010). *O. basilicum* EO also reduced 63.6% of biofilm production which produced by *P. aeruginosa* (Jugreet *et al.*, 2020). However, the variable constituents of EOs depend on several factors including climate, habitat, agricultural, environment, technology, storage time and extraction methods.

There are literature reviews of the BO bioactive compounds that impact on animals as follows. Dried BO seeds showed an improvement of fish growth and feed utilization (El-Dakar *et al.*, 2015) The BO supplementation at 600 ppm decreased *E. coli* colonies in ileum of broiler chickens, whereas the BO had no effect on *Lactobacilli* colonies (Riyazi *et al.*, 2015). Additionally, BO improved the activation of feed intake, body weight gain and feed per gain. Similarly, an improvement in nutrient digestibility of weaned pig was observed in the BO compared to the control group. Moreover, BO increased digestibility of amino acids and encouraged protein synthesis in broilers, resulting in protein accumulation in muscle, particularly breast and thigh (Riyazi *et al.*, 2015). This phenomenon is associated with the activation of digestive function (Li *et al.*, 2012). However, the inconsistent results of BO efficiency in poultry nutrition have been reported.

6. The limitations of EOs as a feed additive

The main challenge in the application of EOs is related to more fragile and volatile characteristics. It is an unstable ingredient due to their high volatility, fast decomposition, low aqueous soluble, uncontrolled release, causing low bio-accessibility, and bio-availability in addition to handling issues and undesirable sensory

effects (Delshadi *et al.*, 2020). Therefore**,** the direct integration of EOs into animal diets has shown limitations in the presence of the harsh environments i.e., feed processing, storage conditions (e. g. oxygen, heat, light, and time etc.) and a range of gastrointestinal conditions (e. g., endogenous enzymes and pH) (Chitprasert and Sutaphanit, 2014). As a result, there was a deterioration of biological activity, leading to the failure of the use of EOs in livestock**.**

In feed processing, EOs, thermal unstable compounds, are directly added into mixer (2-10 mins depending on mixer type), resulting in the negative interaction between EOs and other ingredients or other feed additives. EOs are evaporated quickly after they exposed to high temperature and steam pressure in conditioner. In storage condition, several factors i.e. high temperature, light, metals, water and oxygen induce autoxidation which negatively affected the physical stability and shelf life of EOs (Temiz and Öztürk, 2018).

In site-specific delivery to animal intestinal tract, the decomposition of EOs in GI tract appears at the beginning of the ingestion. It exposed to low pH environment and endogenous enzymes that disrupt the performance of EOs. Some reviews indicated that EOs are rapidly absorbed in the stomach as consequence of hydrophobic characteristic (Chitprasert and Sutaphanit, 2014). The movement of EOs into the hindgut is relatively difficult because of its poor solubility in GI tract. Eventually, the final amount is insufficient to active at target site. These factors significantly decrease their functional properties. The efficacy of EOs has been shown to increase when they are produced as encapsulated forms. For instance, garlic essential oil in nanoencapsulated form had better antimicrobial and antioxidant activities, leading to better performance and gut health in broiler chicken than its free form. (Amiri *et al.*, 2021)

7. Microencapsulation for animal nutrition

Microencapsulation has been increasing the application in animal nutrition. It is a coating or entrapment technology of the sensitive compounds with suitable wall materials, forming microcapsules, which consist of core materials (active ingredients) and wall materials (coating materials). Figure 1 demonstrated the basic structure of the microcapsules. These particles release the core materials in the suitable condition and at ideal time. This technology has been currently used in a wide range of industries i.e. pharmacology, cosmetics, medicine and food (Temiz and Öztürk, 2018). It is a practical technique to perpetuate functional properties of the products (Al-Kasmi *et al.*, 2017). The application of microencapsulation in animal feed can be beneficial for protecting feed additive properties under heating process in pelleting machine and storage conditions, delaying and extending release rates in the gastric and intestinal phases, respectively (Amiri *et al.*, 2021). Additionally, this improves the distribution and solubility while it reduces the volatility of EOs and the interaction with other feed additives during the feed manufacturing (Natrajan *et al.*, 2015).

The success of developed microcapsule containing specific compounds depends on several factors that influence the physicochemical characteristics, the swelling performance as well as the release efficiency. Various factors affecting microencapsulation are the selection of polymer material and cross-linking agents, method of encapsulation, the ratio of core to wall, economics and safety. These factors are essentially considered to suit in their applications. These are crucial steps of encapsulation, leading to high efficiency of encapsulated products.

There are different techniques available for the encapsulation of EOs. The techniques are divides into three types including chemical methods, physico-chemical conditions and physico-mechanical methods. Among these techniques, spray-drying is comparatively ease to scale up, cost effective, and less time consumption depending

on the characteristics of the core substance to be encapsulated (Jyothi *et al.*, 2010). However, the spray drying at the high inlet temperature (typically 200 ℃ - 250 ℃) can cause less encapsulation efficacy (%, EE), less stability, and change the active ingredient in the finished products (Fournaise *et al.*, 2020; Sun *et al.*, 2020).

The selection of microencapsulation processes depends upon %EE, the type of animal to be fed, the characteristics of the bioactive compound, choice of wall material, the target site to be release and cost (Lu *et al.*, 2021; Tolve *et al.*, 2021). Many studies have been performed to investigate microencapsulation of EOs using different wall materials as follows. In thermogravimetric analysis, encapsulated carvacrol in chitosan nanoparticle increased heat resistance compared to pure carvacrol (Hosseini *et al.*, 2013). The fabrication of holy basil essential oil (HBEO) using aluminum carboxymethyl cellulose-beeswax as protective wall could extend period of time in the simulated gastric fluid (SGF) and increased shelf life. Additionally, the holy basil oil entrapped in carboxymethyl cellulose with 1% stearic acid could maintain antioxidant ability during 3 months (Chitprasert and Ngamekaue, 2017).

In the poultry production, the fundamental research of encapsulated products is limited especially, in terms of formation process, stability including economic benefits. At present, commercial encapsulated products are not suitable for the livestock industry. However, this method was developed for food industry during the past few years.

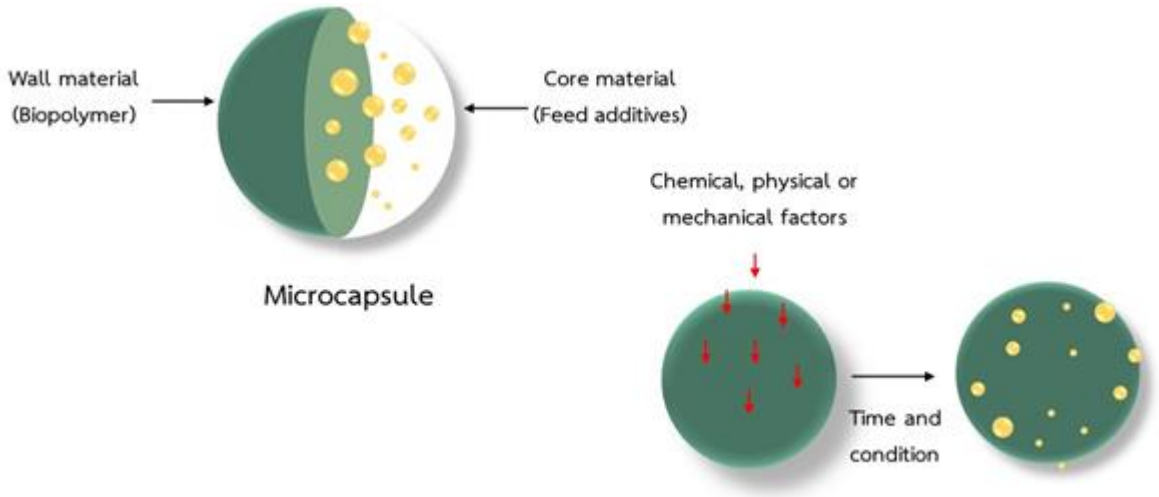

Figure 1 The basic structure of the microcapsules

8. Alginate and chitosan as coating materials

The crucial step in microencapsulation is the selection of appropriate wall material to increase the efficiency of feed additive. Their characteristics should be considered, including molecular weight, stability, toxicity and cost (Lu *et al.*, 2021). In this study, the wall materials would be focused on alginate and chitosan which can build barriers and prevent core material from its deterioration. Alginate (AG) is a naturally occurring polysaccharide with biocompatible, biodegradable, and non-toxic properties. AG consists of ß-(1, 4) linked D-mannuronic acid (M) and α-(1,4) linked L-guluronic acid (G). In general, AG is in the form of sodium alginate (SA) and it is an anionic polymer which can be fabricated in various forms such as gel, membranes, scaffolds including porous materials (Kang *et al.*, 2019). It is known that alginate is insoluble at low pH but soluble in alkaline condition. It has been applied to small intestine-specific delivery system in order to control release rate (Cuadros *et al.*, 2015). SA is able to form gel by inducing cross linking agents, such as Ca^{2}+ Sr^{2}+, and Cu^{2}. Clove oil entrapped in SA particles presented the outstanding inhibitory action of pathogenic bacteria growth (Radunz *et al.*, 2019). For long-term preservation, SA beads retained isoprenoids in wheat bran oil (Durante *et al.*, 2012). *In vitro* release test, encapsulated fish oil with SA had a lower secretion rate in the gastric phase than in

intestinal phase (Bannikova *et al.*, 2018). The essential oil-loaded $CaCl_2$ cross-liked SA beads exhibited higher antifungal activity than free oil after storage, resulting in reducing evaporation loss. Additionally, alginate nanocapsules containing turmeric oil showed good physical stability in long-term storage at 4 °C (Lertsutthiwong *et al.*, 2008). However, the long term holding capacity of SA porous structure is limited due to its open pore network (Peretz *et al.*, 2013). Therefore, the incorporation of other materials has a potential to produce synergistic effect. The single wall material may be dissatisfied when passing through desirable conditions (i.e., feed processing and GI tract).

In similar to SA, chitosan (CS) is the second most abundant polysaccharide with the promising characteristics such as biocompatibility, biodegradability, and non-toxicity. CS is composed of ß -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units derived from chitin. CS exhibits the solubility in acidic condition through the protonation of amino groups (Sabourian *et al.*, 2020). The entrapment of carvacrol into CS nanoparticles exhibited minimum inhibitory concentrations (MIC) as 0.257 mg/ml against *S. aureus, B. cereus* and *E. coli.* The cumulative release in low pH (pH 3) and neutral pH (pH 7) were 53% and 23%, respectively (Keawchaoon and Yoksan, 2011). CS nanoparticle cross-linked with sodium tripolyphosphate (TPP) remained the antimicrobial ability of cardomon essential oil (Jamil *et al.*, 2016). Garlic essential oil with CS as nanoencapsulation form was effective again *E.coil* compared to free form in addition to increased growth rate and gut health in broilers (Amiri *et al.*, 2021).

SA contains carboxylic acid groups, leading to a negatively charged polymer, whereas CS carries the amino groups to perform positively charged chains. Therefore, the combination of SA and CS resulted in the complementary charged biopolymers and potentially formed the polymer complexes (Keawchaoon and Yoksan, 2011; Cuadros *et al.*, 2015). Double type wall materials with/without crosslinkers could form the complex structure against the oxidative degradation (Lu *et al.*, 2021). SA and CS

have been widely used for intestinal delivery of active ingredients due to the unique pH responsiveness. It is known that SA is resistant to acidic gastric juice whereas CS is tolerable for intestinal juice (Ling *et al.*, 2019). Curcumin diethyl disuccinate encapsulated in CS/SA improved stability under UV, heat treatment and physiological environment (Sorasitthiyanukarn *et al.*, 2019). Gawad and Fellner (2019) demonstrated the foam prepared from the mixture of SA and CS was stable in the gastric condition to release glycerol in intestinal condition. Trabelsi *et al.* (2016) reported that the supplement of *Lactobacillus plantarum* TN8 encapsulated in SA and CS improved growth performance and reduced biochemical parameters in chicken blood.

CHAPTER 3

MATERIALS AND METHODS

1. MATERIALS

1.1 Chemicals

No	Chemical name	Company	Country	Grade
1	Acetone	Merck	Germany	ACS
2	Albumin, Bovine serum	Merck	Germany	ACS
3	Alginic acid sodium salt powder (sodium alginate)	Sigma-Aldrich	USA.	ACS
4	Bile extract porcine	Sigma-Aldrich	USA.	ACS
5	Boric acid (H_3BO_4)	Merck	Germany	ACS
6	Calcium chloride-dihydrate crystal ($CaCl_2$)	Merck	Germany	ACS
7	Chitosan	Sigma-Aldrich	USA.	ACS
8	Copper sulphate ($CuSO_4.5H_2O$)	Ajax finechem	New Zealand	ACS
9	Dichloromethane CH_2Cl_2	Merck	Germany	ACS
10	Diphenyl-1-picrylhydrazyl $C_{18}H1_2N_5O_7$	Sigma-Aldrich	USA.	ACS
11	Disodium ethylenediaminetetraacetate (EDTA) dehydrate	Merck	Germany	ACS
12	Disodium hydrogen phosphate anhydrous ($NaHPO_4$)	Merck	Germany	ACS
13	Di-sodium hydrogen phosphate heptahydrate	Merck	Germany	ACS
14	Ethanol C_2H_5OH	Merck	Germany	ACS
15	Hydrochloric acid fumingmin 37%	Merck	Germany	ACS
16	Lipase from porcine pancreas	Merck	Germany	ACS
17	Luminal (Chemiluminescence reagent) >98.0% ($C_8H_7N_3O_2$)	Tokyo chemical industry	Japan	ACS

18	Methanol CH_3OH	Merck	Germany	ACS
19	De Man Rogosa Sharpe MRS-BROTH	Merck	Germany	ACS
20	Mueller-hinton agar (MHA)	Sigma-Aldrich	USA.	ACS
21	Pepsin from porcine gastric mucosa	Sigma-Aldrich	USA.	ACS
22	Porcine bile extract	Merck	Germany	ACS
23	Potassium chloride GR KCl	Merck	Germany	ACS
24	Potassium peroxodisulfate	Merck	Germany	ACS
25	Potassium persulfate, >=99.0% $K_2O_8S_2$	Sigma-Aldrich	USA.	ACS
26	Sodium carbonate > 99.0% (Na_2CO_3)	Tokyo chemical industry	Japan	ACS
27	Sodium chloride NaCl	Merck	Germany	ACS
28	Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4\ H_2O$)	Merck	Germany	ACS
29	Sodium dodecyl-sulfate $CH_3(CH_2)_{11}OSO_3Na$	Merck	Germany	ACS
30	Sodium hydrogen carbonate $NaHCO_3$	Merck	Germany	ACS
31	Sodium hydroxide (NaOH)	Merck	Germany	ACS
32	Sodium triphosphate pentabasic ($Na_5O_{10}P_3$)	Merck	Germany	ACS
33	Sodium triphosphate pentabasic (TPP),	Sigma-Aldrich	USA.	ACS
34	Succinic acid ($C_4H_6O_4$)	Sigma-Aldrich	USA.	ACS
35	Sulfuric acid (H_2SO_4) 98%	Merck	Germany	ACS
36	2-Thiobarbituric acid ≥ 98%	Sigma-Aldrich	USA.	ACS
37	Trichloroacetic acid	Merck	Germany	ACS
38	Trolox	Merck	Germany	ACS
39	Trypsin from bovine pancreas	Sigma-Aldrich	USA.	ACS
40	Tween 80 (polysorbate 80)	Sigma-Aldrich	USA.	ACS
41	Xanthine Oxidase	Tokyo chemical industry	Japan	ACS

1.2 Instruments

No	Instruments	Model	Company	Country
1	Autopipette	B100-1	CAPP	Denmark
2	Balance	Practum-224-1S	Becthai Bangkok Equipment&Chemical	Thailand
3	Centrifuge	5811XK140939	Eppendorf	Germay
4	Fat analyzer	Soxtec 800, CA-1115	Foss, Eyela	Denmark
5	Fiber analyzer	FIWE 6	VELP Scientific	Italy
6	Filter papers NO 1	Whatman	GE healthcare	UK
7	Fourier transform infrared spectrometer (FTIR)	Alpha	Bruker	USA
8	Glass crucible	P2 02	VELP Scientific	Italy
9	Hot air oven	UF 110	Memmert	USA
10	Hot plate stirrer	IKA-13581200	IKA workscasia SDN BHD	Germany
11	Light microscope	BX5	Olympus	Japan
12	Muffle furnace	3-1750	Neytech vulcan	Germany
13	pH-metter	S220	Metter Toledo	Germany
14	Protein analyzer	8100, CA-1115, Scrubber 2508	Foss, Eyela	Denmark
15	Scanning electron microscopy	SEM-IT300	JEOL	Japan
16	Shaking water bath	WNB 14	Memmert	USA
17	Thermogravimetric analysis	TGA-Q50	TA Instruments	USA
18	Thermostat water bath vibrator	WNB 14	Memmert	USA
19	UV-vis spectrophotometer	Evolution 201	Thermo scientific	USA
20	Vacuum-freeze dryer	LL3000	Thermo scientific	USA

2. METHODS

In vitro study

2.1. Characterization of basil oil (BO) through gas chromatography-mass spectrometry (GC-MS) analysis

Steam-distillated basil oil from the leaves and flowers was obtained from Nano Artech Company Limited (Chon Buri, Thailand). It was used as a core material. The bioactive constituents of BO were analyzed using GC-MS. Basil oil was diluted in 1 mL of hexane. This solution (approximately 0.1 - 0.2 µl) was injected into the GC system at a temperature of 250 °C. The bioactive constituents were identified using their retention time and were expressed as percentage (%).

2.2 Preparation of BO encapsulated CS-SA microcapsules (BO/CS-SA) or microencapsulated basil oil (MBO)

SA (3 g) was dissolved in deionized water (DI) (100 mL) and tween 80 (2 mL). For the SA solution, $CaCl_2$ solution (0.5% w/v, 15 mL) in aqueous solution was gradually added and filled into a 96 well-plate (used as a template) before lyophilizing in a Thermo scientific power dry LL3000 vacuum-freeze dryer to obtain SA porous foam in tube form. The SA porous foam was manually cut into small circle pieces with a thickness of 2-3 mm to obtain SA microcapsules, abbreviated as SA. SA microcapsules were soaked in the pure BO (SA: BO for 1 g: 5 mL) for 30 min. Finally, the BO encapsulated SA microcapsules, namely BO/SA, were immersed in CS solution (1.5% w/v, 20 mL in succinic acid (1% w/v in aqueous solution)) for 2 h followed by immersing in TPP (1.0% w/v, 20 mL) in aqueous solution to obtain double-layered CS-SA microcapsules encapsulated with BO, abbreviated as BO/CS-SA. The BO/CS-SA was thoroughly washed with DI for three times before drying in a hot air oven at 30°C overnight. A schematic diagram of BO/CS-SA preparation is shown in Figure 2.

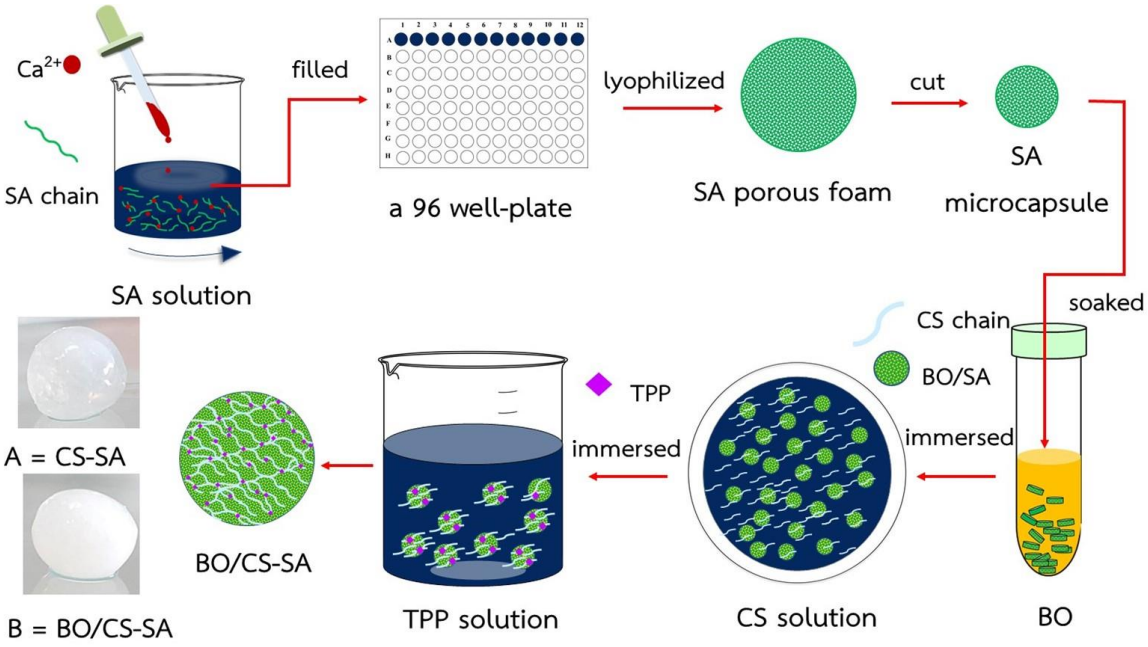

Figure 2 A schematic diagram of the preparation of BO-loaded CS-SA microcapsule

CS, chitosan; SA, sodium alginate; BO, basil oil; TPP, sodium tripolyphosphate, CS-SA; double-layered CS-SA microcapsules, BO/SA; BO encapsulated SA microcapsules, BO/CS-SA; double-layered CS-SA microcapsules encapsulated with BO

2.3 Determination of physico-chemical characteristics

2.3.1 Scanning electron microscopy (SEM) analysis

The shape and surface morphology of SA, BO/CS-SA were determined using a JEOL SEM-IT300 scanning electron microscopy. The samples were prepared to withstand the vacuum conditions and they were coated with gold metal under high vacuum and fixed directly on the specimen stage. The BO/CS-SA was cut across by a razor blade to observe the cross-section. The SEM photographs were recorded at different magnifications (50 - 200×).

2.3.2 Fourier transform infrared (FTIR) spectroscopy analysis

The chemical structures of microcapsules, i.e. SA, CS, BO and BO/CS-SA were identified by a Bruker Alpha Fourier transform infrared spectrometer using ATR technique in the range of 4000 cm^{-1} to 400 cm^{-1}. Each sample was measured three times.

2.3.3 Thermogravimetric analysis (TGA)

The thermal stability and components of microcapsules were validated by a TAQ50 thermogravimetric analyzer from 50℃ to 500 ℃ at 10℃ /min under nitrogen condition (as a carrier gas). The samples (~ 5 mg) were placed onto an alumina dish crucible. The flow rate of nitrogen was kept at 100 mL/min. The results were illustrated as TGA curve.

2.3.4 Encapsulation efficacy (EE, %) and BO content (%)

The finished microcapsule was measured % EE and BO content. Microcapsules (100 mg) were dissolved in 1 mL of dichloromethane and mixed vigorously on a vortex mixer for 2 min. The extracted solution was kept for 10 min without shaking. The mixture was filtered through filter paper (Whatman No:1). The supernatant containing the extracted oil was determined using a UV- vis spectrophotometer (Evolution 201

series, Thermo scientific) at 289 nm. Then, its concentration was calculated using the calibration curve (R^2 = 0.9956) of BO in dichloromethane. EE and BO content were calculated according to the following equation (1) and (2), respectively as described by Chitprasert and Sutaphanit (2014)

$$\text{Encapsulation efficacy, EE (\%)} = \frac{Wo}{Wi} \times 100 \quad (1)$$

where Wo is the mass of BO in the microcapsule, Wi is the initial mass of basil oil added for the encapsulation.

$$\text{BO content (\%)} = \frac{Wo}{Wm} \times 100 \quad (2)$$

where Wm is the mass of the microcapsule

2.4 Determination of BO content (%) in CS-SA microcapsule under temperature range of feed pelleting

BO contents were evaluated after heating at 75 ℃, 80 ℃, 85 ℃, 90 ℃, 95 ℃, and 100 ℃ for 3 min. The finished microcapsule was measured BO content after heating at 75 ℃, 80 ℃, 85℃, 90 ℃, 95 ℃ and 100 ℃ for 3 min (temperature range of feed pelleting). The data were calculated using a calibration curve of BO (R^2 = 0.9956) as described by Ngamekaue and Chitprasert (2019).

2.5 Determination of swelling percent (%) of CS-SA microcapsules under simulated gastrointestinal fluids

The swelling of BO/CS-SA was determined in simulated poultry digestive tract according to the protocol of Hezaveh and Muhamad (2013) with minor modifications. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were freshly prepared according to Chitprasert and Ngamekaue (2017).

For simulated gastric stage study, BO/CS-SA (1.0 g ± 0.05 g) was added into SGF (100 ml). The pH of the solution was adjusted to 6.0, 5.0, 3.0, and 2.0. The BO/CS-SA

was incubated at 39.5 ℃ ± 0.5 ℃ in a Memmert WNB 14 thermostat water bath for 5 min, 30 min, 120 min, and 180 min, respectively to represent the times in gastric stage. After incubation at each point, the BO/CS-SA was filtered and weighed whereas the SGF solution was collected for the use in the intestinal stage.

For simulated intestinal stage study, trypsin solution (2 mg/ml, 1 ml,), bile solution (40 mg/ml, 14 ml), pancreatic solution (3.2 mg/ml, 7.5 ml,), and SIF (7.5 ml) were mixed with the above SGF solution. The pH of SIF was adjusted to 5.5, and 7.0 whereas BO/CS-SA was incubated at 39.5 ℃ ± 0.5 ℃ in each pH for 220 min and 240 min, respectively, to represent the time in the intestinal stage. The treated BO/CS-SA was collected and weighed at each incubating time. The results were calculated by equation (1) to indicate the percentages of swelling.

$$\text{Swelling of the microcapsule (\%)} = \frac{Ms - Md}{Md} \times 100 \qquad (3)$$

Where Ms, and Md are the weight of the swelled and the dried BO/CS-SA.

2.6 Determination of BO release behavior under simulated gastrointestinal fluid

The released behavior was monitored using an evolution 201, thermo UV-Vis spectrophotometer. Procedure was performed as described in swelling section (Chitprasert and Ngamekaue, 2017). After incubation at each point, 1 mL of supernatant was withdrawn and the absorption at 289 nm was determined. The cumulative release percentage of BO was calculated using the following equation (4).

$$\text{Cumulative release percentage} = \sum_{t=0}^{t} \frac{M_t}{M_0} \times 100 \quad (4)$$

Where M_t is the cumulative amount of BO, M_0 is the initial amount of the BO encapsulated in BO/CS-SA.

2.7 Determination of antioxidant activity of BO and BO/CS-SA

Antioxidant activity of BO and BO/CS-SA was determined using DPPH radical assay according to the protocol of Brand-Williams *et al.* (1995) with some modifications. The BO/CS-SA (1 g) was prepared by extraction with dichloromethane (5 mL). Fifty microliters of samples were mixed with DPPH (0.2 mM, 1,000 µl) in methanol. Then, the mixture was shaken vigorously and kept at room temperature in the dark for 30 min. The change of the reaction solutions was monitored at 517 nm. The scavenging capacity and IC_{50} values were calculated with a calibration curve of trolox (R^2 = 0.9589) according to the following equation (5).

$$\text{DPPH radical scavenging activity (\%)} = 100 \times \left(\frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs blank}} \right) \quad \textbf{(5)}$$

Where Abs sample and Abs blank are the absorbances of the DPPH solution mixed with and without BO/CS-SA, respectively.

2.8 Determination of antimicrobial activity of BO and BO/CS-SA

Agar diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were performed in order to determine antimicrobial activity of BO and its particle. Three representative bacterial species including Escherichia coli ATCC *25922,* Staphylococcus aureus ATCC *25923* and *Salmonella* spp.

were selected in the current study and obtained from Food Risk Hub, Research Unit of Chulalongkorn University.

Agar diffusion method was determined according to the method described by Arfat *et al.* (2014). All pathogenic bacteria were cultivated on MHA. The colonies with a wire loop were transferred into NaCl solution (0.85% w/v, 5 ml). Each bacterial suspension was adjusted to a turbidity of 0.5 McFarland standards (1×10^{8} CFU/mL). A sterile cotton swab was dipped into the bacterial suspension and then streaked on agar. Then, sterile filter paper discs (diameter = 6 mm.) were dipped in extracted BO and placed on the agar surface. In order to encourage oil distribution, the plates were kept at refrigerator (4 ºC) for 30 min. The plates were then incubated at 37 ºC for 24 h. The data were interpreted based on the diameter of the inhibition zone (mm.).

MIC value was also tested using the macro dilution method according to the method of Radunz *et al.* (2019) with some modifications. The samples were diluted from 64 to 0.125 mg/mL with the solution of 0.85 % (w/v) NaCl containing 0.5 % (w/v) tween 80. Bacterial suspension was mixed with the different concentrations of samples in a sterile tube containing mueller hinton broth (MHB). The test tubes were vortexed and incubated at 37 ºC for 24 h. The solution was observed turbidity that indicated the growth of bacteria. Then, two tubes with no bacterial growth were used for determining MBC test. The MIC is defined as, the minimum concentration of oil to inhibit the bacterial growth whereas MBC is defined as the lowest bactericidal concentrations.

2.9 Determination of stability of BO and BO/CS-SA under storage

The storage stability of BO and BO/CS-SA was studied according to Chitprasert and Ngamekaue (2017). Briefly, BO (10 mL) and BO/CS-SA (10.0 g ± 0.5 g) were separately sealed in glass vials and wrapped with aluminum foil. The containers were

stored at room temperature for 90 days. Both samples were taken after 30 days, 60 days, and 90 days of storage to determine the antioxidant and antimicrobial activities.

2.10 Determination of tolerances of BO/CS-SA under acid, bile, trypsin and thermal treatment on antioxidant and antimicrobial properties.

Tolerant characteristics were conducted according to Liong and Shah (2005) with minor modifications. Acid tolerance was investigated by soaking BO/CS-SA (5.0 ± 0.5 g) into citrate phosphate buffer pH 2 (0.2 M, 20 mL). Bile salt tolerance was carried out by dissolving 3.0 g of porcine bile extract into DI (100 mL) with BO/CS-SA (5.0 ± 0.5 g). Trypsin tolerance was tested by preparing trypsin from bovine pancreas (1.0 g) in DI (100 mL) containing BO/CS-SA (5.0 ± 0.5 g). All tubes were incubated in a thermostat water bath vibrator at 39.5 ºC ± 0.5 ºC for 30 min. For thermal treatment, BO/CS-SA was tested at 85 ºC for 1 min (simulation of feed pelleting condition). The treated BO/CS-SA was immediately removed, and its antioxidant and antimicrobial activities were measured as in the previous procedures.

2.11 Determination of tolerances of BO/CS-SA under thermal treatment on bioactive constituents

Thermal treatment on bioactive constituents was conducted at 85 ° C for 1 min. The treated microcapsule was immediately removed and analyzed the bioactive constituents of BO using GC-MS. BO was diluted in 1 mL of hexane. The solution (approximately 0.1 - 0.2 µl) was injected into the GC system at a temperature of 250°C. The individual bioactive constituent was expressed as percentage (%).

In vivo **study**

2.12 Experimental design, diets, and husbandry

The experimental protocol was approved by the Animal Ethic Committee of Faculty of Veterinary Science, Chulalongkorn University (Protocol Review No. 1931092). A total of 288 one - day - old female broilers (Ross 308, initial weight = 47.0 ± 1.0 g) were provided by a commercial hatchery. Chicken was placed on floor pens (100 × 150 cm) with rice husk in a conventional house at the student training center, Department of Animal husbandry, Faculty of Veterinary Science, Chulalongkorn University, Nakhon Pathom, Thailand. The amount of allotted space per bird was surpassed the minimum space allowed for broilers up to 42 d age of 1 m^2. Broilers were randomly distributed among the 4 treatments. Each treatment was fed to 6 pens of 12 birds. Treatments consisted of a total of 4 corn-soybean meal based broiler diets formulated to meet or exceed the nutrient requirements for Ross 308 broiler chickens (Aviagen, 2009). Treatments were (i) basal diet without additives as a negative control (NC), (ii) basal diet with avilamycin at 10 ppm as a positive control (PC), (iii) basal diet with free basil oil (FBO) at 500 ppm, and (iv) basal diet with microencapsulated basil oil (MBO) at 500 ppm, respectively. FBO was prepared by spraying BO onto silicon dioxide (SiO_2) as a carrier. A feeding program consisted of 3 phases: a crumbled starter diet (days 1 - 21), a pelleted grower diet (days 22 - 35) and a pelleted finisher diet (days 36 - 42), respectively.

All diets were pelleted at a steam conditioning temperature of 80 ℃ for 40 seconds. The diet and clean tap water were provided ad libitum throughout the experiment. The feed ingredients and chemical compositions of the diets are shown in **Table 2**. A lighting program was 22 h light during the first 14 days under incandescent lamp and then 12 h light and 12 h darkness from d 15 to the end of the trial. The indoor temperature ranged from 25 to 28 ℃ and 33 to 35 ℃ in the morning and the

afternoon, respectively. Relative humidity (%, RH) was recorded in the range of 84 to 87%. Layout of experimental units for the study is shown in Figure 3.

Table 2 Ingredients and calculated composition of the basal diet (as-fed basis, g/kg)

Item	Feeding phase		
	Starter	Grower	Finisher
Ingredient			
Corn	560.00	613.20	635.90
Soybean meal	349.60	301.50	277.80
Soybean oil	46.00	45.00	48.60
MDCP [1]	18.10	15.80	14.90
Limestone	11.10	9.90	9.40
Salt	2.50	2.50	2.50
Vitamin and mineral premix [2]	2.00	2.00	2.00
DL-Methionine	2.80	2.60	2.50
L-Lysine HCl	1.70	1.60	1.60
L-Threonine	0.90	0.60	0.50
L-Isoleucine	0.20	0.10	0.00
Sodium bicarbonate	1.90	1.90	1.50
Choline Chloride 60%	0.70	0.80	0.80
Antimold (Propimpex)	2.00	2.00	2.00
Salinomycin (Sacox)	0.50	0.50	0.00
Total	1,000.00	1,000.00	1,000.00
Calculated nutrient composition			
Dry matter	879.30	878.10	878.00
Metabolizable energy (MJ/kg)	12.97	131.80	133.90
Crude protein	220.00	200.00	19.00
Ether extract	72.30	72.50	76.50
Crude fiber	29.50	28.30	27.60
Lysine	13.20	11.80	11.10

Methionine	6.20	5.70	5.50
Met + Cys	9.80	9.10	8.80
Calcium	9.00	8.00	7.60
Total phosphorus	7.70	7.00	6.70
Available phosphorus	4.50	4.00	3.80

[1] MDCP, mono-dicalcium phosphate

[2] Premix composition per kg of feed: Vitamin D = 2,400 IU, Vitamin E = 60.0 mg, Vitamin K = 3.0 mg, Vitamin B1 = 3.0 mg, Vitamin B2 = 8.0 mg, Vitamin B6 = 4.0 mg, Vitamin B12 = 0.02 mg, Niacin = 50.0 mg, Pantothenic acid = 15.0mg, Biotin = 0.40 mg, Folic acid = 2.0 mg, Cu = 15 mg, Fe =40 mg, Mn = 100 mg, Zn = 100 mg, I =1.0 mg, Se =1 mg.

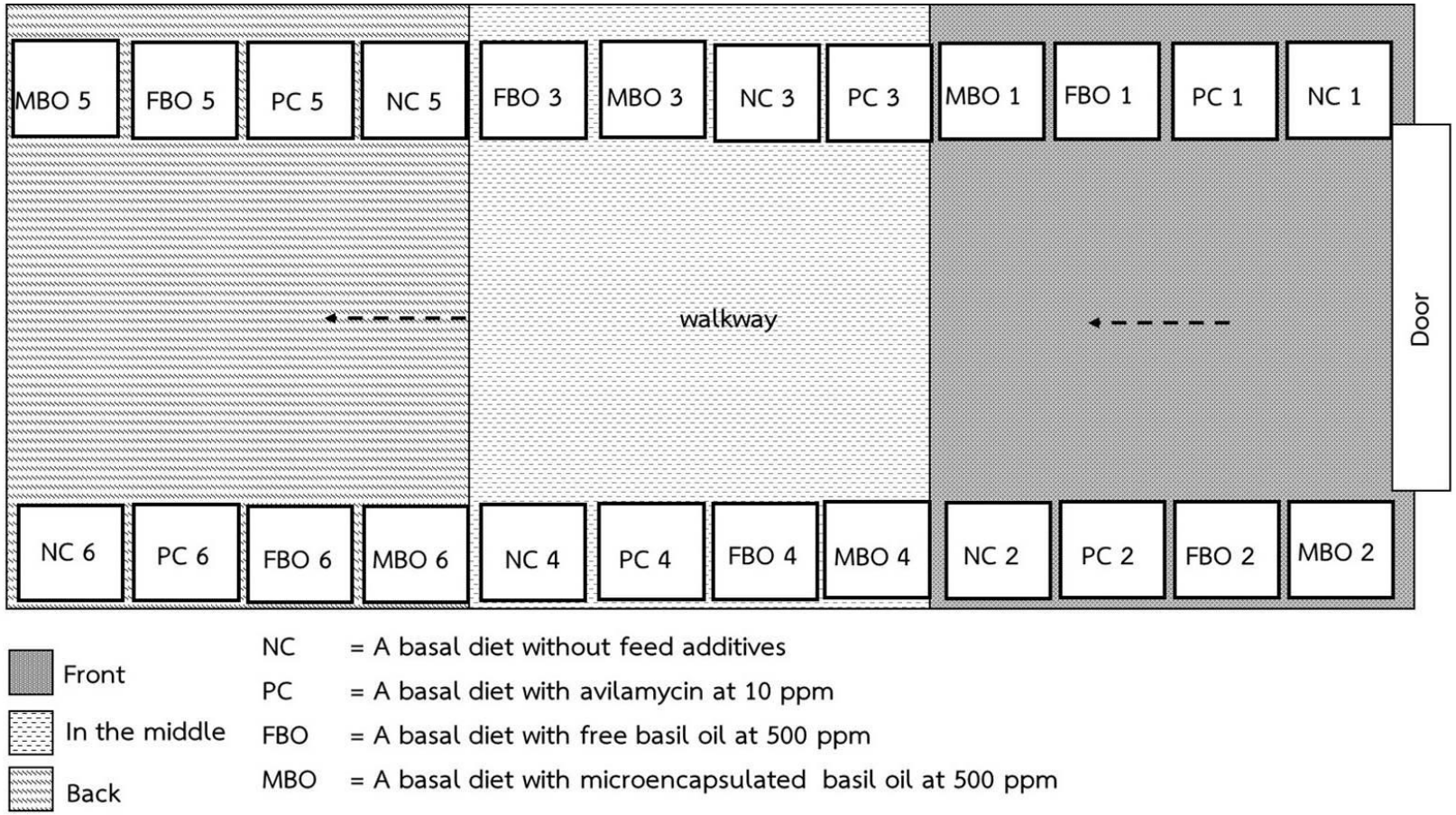

Figure 3 Layout of experimental units in a conventional house at the student training center, Department of Animal husbandry, Faculty of Veterinary Science, Chulalongkorn University, Nakhon Pathom.

2.12 Sample collection and analysis

2.12.1 Growth performance

Body weight (BW) and feed intake (FI) were measured at 1, 21, and 42 days of age in order to calculate average daily feed intake (ADFI), and average daily gain (ADG). The feed conversion ratio (FCR) was also calculated by dividing ADFI with respective ADG. Mortality rates were recorded and calculated throughout the experiment.

2.12.2 Apparent ileal digestibility (AID)

The broiler chickens (3 chicks from each replicate, 18 chicks per treatment) were randomly selected, carefully tagged using plastic band. An indigestible marker (20 g/kg diet of acid-insoluble ash, AIA, Celite™) was added in starter diet (days 18 to 21) and finisher diet (days 39 to 42) to calculate nutrient digestibility. At 21 and 42 days of age, these birds were euthanized by CO_2 asphyxiation. Then, ileal digesta samples (2-3 g) were collected and pooled for each replicate. Diets and ileal contents were dried to determine dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash and nitrogen free extract (NFE) according to the association of official analytical chemists (AOAC, 2006). Gross energy (GE) was analyzed using a bomb calorimeter with benzoic acid as a reference standard (Leco, Mode AC-500, United States). AIA was also determined using the method of Vogtmann *et al.* (1975). The apparent ileal digestibility (AID, %) was calculated by the following equation (6) (Angkanaporn *et al.*, 1996)

$$AID\ (\%) = 100 - [(AIA_{diet}/AIA_{digesta}) \times (Nutrient_{digesta}/Nutrient_{diet})] \times 100 \quad (6)$$

Where AIA_{diet} is the AIA concentration in the diet, $AIA_{digesta}$ is the AIA concentration in the ileal digesta, $Nutrient_{digest}$ is the nutrient concentration in the ileal digesta and $Nutrient_{diet}$ is the nutrient concentration in the diet.

2.12.3 Jejunal histomorphology

The segment of mid-jejunum (~ 2-3 cm) was taken from euthanized 3 birds per replicate on days 21 and 42. The whole jejunum was located between endpoint of the duodenal loop and Meckel's diverticulum. The intestinal tissue was flushed and immediately fixed in 10% formaldehyde solution, followed by tacking in paraffin wax. Afterward, fixed intestinal samples were placed on a glass slide and then stained using hematoxylin and eosin. Villus height (VH), villus width (VW) and crypt depth (CD) were measured using a light microscope at 100 × magnification (Olympus, Mode BX*5*, Japan). These data were calculated for the villus height to crypt depth (VH:CD) ratio and villus surface area (VSA). VSA was calculated by the following equation (7) (Baurhoo *et al.*, 2011)

Villus surface area (VSA, mm^2) = 2π× (Average villus width/2) × Villus height (7)

2.12.4 Microbial population

Enumeration of microbial populations was carried out by collecting fresh ceco-colon contents (1-2 g) from 3 birds per replicate on days 21 and 42. *Lactobacillus* spp. was determined by colony forming unit quantification technique. The samples were pooled, then diluted with 9 mL of sterile peptone water and mixed on a vortex stirrer. Then, each diluted sample was anaerobically cultured on De Man, Rogosa, sharpe agar (MRS agar) and incubated at 37 ºC for 48 h. The colonies of bacterial population are expressed as log_{10} colony forming units (CFU) per gram of fresh content. The enumeration of E. coli was determined by the most probable number (MPN) technique. Three series of dilutions contained nutrient broth and different amounts of sample (0.1, 1.0 and 10 mL). All tubes were incubated at 37 ºC for 24 h. Then, the positive tubes were used to confirm the growth of *E. coli.* The obtained results are expressed as MPN per gram of fresh content (Chandrapati and Williams, 2014).

2.12.5 Antioxidant capacity

Duodenal mucosa (~ 2-3 g) from 3 birds per replicate on days 21 and 42 was scrapped using a glass microscope slide, pooled and immediately stored at -20 °C. Then, the representative sample was homogenized with 0.05 M phosphate buffer solution at pH 7.4. The homogenates were centrifuged for 15 min at 1500 × g. (4 °C). The supernatant was then used for analyses of superoxide dismutase (SOD) and malondialdehyde (MDA) contents, which were used as indexes to monitor the antioxidant capacity.

The qualitative analysis of SOD was determined according to Eriksson and Borg (1991) with some modifications. The change of chemiluminescence of luminol which produced superoxide radical by the reaction of xanthine and xanthine oxidase was observed using a UV - vis spectrophotometer (Thermo scientific, Evolution 201 series, United States) at 560 nm. The reaction tube contained the supernatant of duodenal mucosa (50 μL), 0.25 mmol/l xanthine, 1.0 mmol/l luminol and 0.1 mmol/l EDTA in a 50 mmol/l carbonate buffer. The SOD activity was expressed as unit/mg protein of intestinal mucosa. The activity of SOD causing a 50% inhibition of the chemiluminescence was defined as 0.01 unit. The MDA concentration was measured by the thiobarbituric acid reactive substances (TBARS) method. One milliliter of supernatant was added and mixed with 1 mL of thiobarbituric acid (TBA). The mixture was heated in a water bath at 100 °C for 30 min and then cooled down. The absorbance was measured at 535 nm using a UV-visible spectrophotometer (Zeb and Ullah, 2016). Protein was determined using bovine serum albumin (BSA) as a standard (Bradford, 1976). All analyses were performed in triplicates.

2.13 Statistical analysis

In vitro study, storage stability and tolerance analysis were analyzed by one-way analysis of variance (ANOVA) in a completely randomized design. The mean ± standard deviation was further evaluated using Duncan's new multiple-range post-hoc test. The statistical significance was considered at $P<0.05$.

In vivo study, pen was the experimental unit for statistical analysis of all data. All data were statistically analyzed by analysis of variance (ANOVA) using SAS statistical software (1985). Differences between mean values of the treatments were determined using post-hoc Tukey's HSD test. The pen mean served as the experimental unit, according to the following model:

$$Yij = \mu + Ti + eij$$

where Yij is the dependent variable observation, μ is the overall mean, Ti is the effect of the treatment, and eij is the random error. A p-value ≤ 0.05 was considered as a significant effect of the treatment. Results are presented as mean and standard error of mean (SEM).

CHAPTER 4

RESULTS

1. Chemical composition of basil oil (*Ocimum basilicum* L.)

The BO from the leaves and flowers as extracted by steam-distillation was used in this study. As shown in **Table 3**, the presence of 19 active components in BO was identified by GC-MS analysis. Methyl chavicol or estragole (65.86%) and linalool (22.07%) are the dominant constituents in BO. Chemical structures of methyl chavicol and linalool are shown in Figure 4.

The remaining constituents are α-pinene (0.07%), β-pinene (0.05%), 6-methyl-5-hepten-2-one (0.09%), eucalyptol (0.15%), β-ocimene Y (0.13%), trans-linalool oxide (0.11%), cis-linalool oxide (0.17%), menthol (0.34%), Z-citral (1.50%), E-citral (1.53%), trans-caryophyllene (0.75%), α-bergamotene (1.39%), β-farnesene (0.87%), germacrene D (0.78%), β-bisabolene (0.24), δ-cadinene (0.08%) and cis-α-bisabolene (3.83 %).

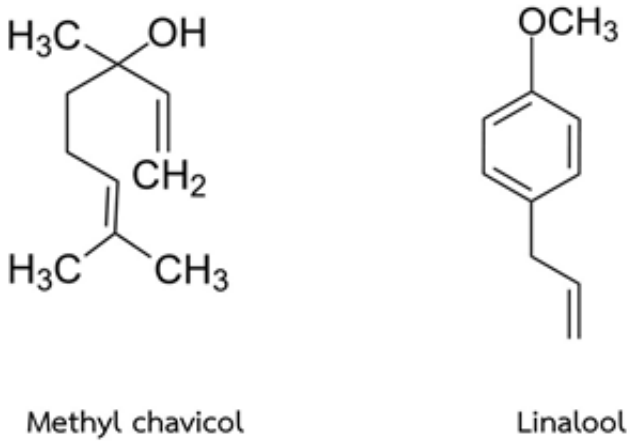

Figure 4 Chemical structures of methyl chavicol and linalool as major constituents of BO

Table 3 Chemical composition of basil oil (*Ocimum basilicum* Linn.)

No	Compound	R.I (s)	%
1	Alpha -pinene	396	0.07
2	Beta -pinene	471	0.05
3	6-Methyl-5-hepten-2-one	509	0.09
4	Eucalyptol	557	0.15
5	Beta-ocimene Y	591	0.13
6	trans-linalool oxide	625	0.11
7	Cis-linalool oxide	646	0.17
8	Linalool	685	22.07
9	Menthol	764	0.34
10	Methyl chavicol	790	65.86
11	Z-citral	814	1.50
12	E-citral	843	1.53
13	trans-caryophyllene	967	0.75
14	Alpha-bergamotene	977	1.39
15	Beta-farnesene	997	0.87
16	Germacrene D	1,021	0.78
17	Beta-bisabolene	1,040	0.24
18	Delta-cadinene	1,054	0.08
19	Cis-alpha-bisabolene	1,067	3.83
	Total		**100.00**

R.I. (s) = Retention time (unit: second).

2. Characterization and performances of SA, and BO/CS-SA

Double-layered microencapsulation was carried out for site-specific delivery of BO to intestinal tract via the dual pH responsive CS-SA microcapsules. The characterization and performances of SA, and BO/CS-SA are studied. In the first step, SA microcapsules were prepared and lyophilized. Figure 5A and 5B show the significant porous networks on the SA surface. After SA microcapsules were immersed in BO and CS, the surface became smooth and BO/CS-SA was obtained (Figure 5C). The cross section of BO/CS-SA shows the porous structure (Figure 5D) indicating that SA porous networks were maintained. In fact, the cross section also shows the dense network especially on the surface which might belong to the CS chains coated on the surface. The size and surface morphology of the microcapsules were influenced by CS coating. The average diameter of BO/CS-SA was about 1,000 μm. The BO/CS-SA exhibited % EE as high as 81.1 ± 0.1 as a consequence of the high surface area.

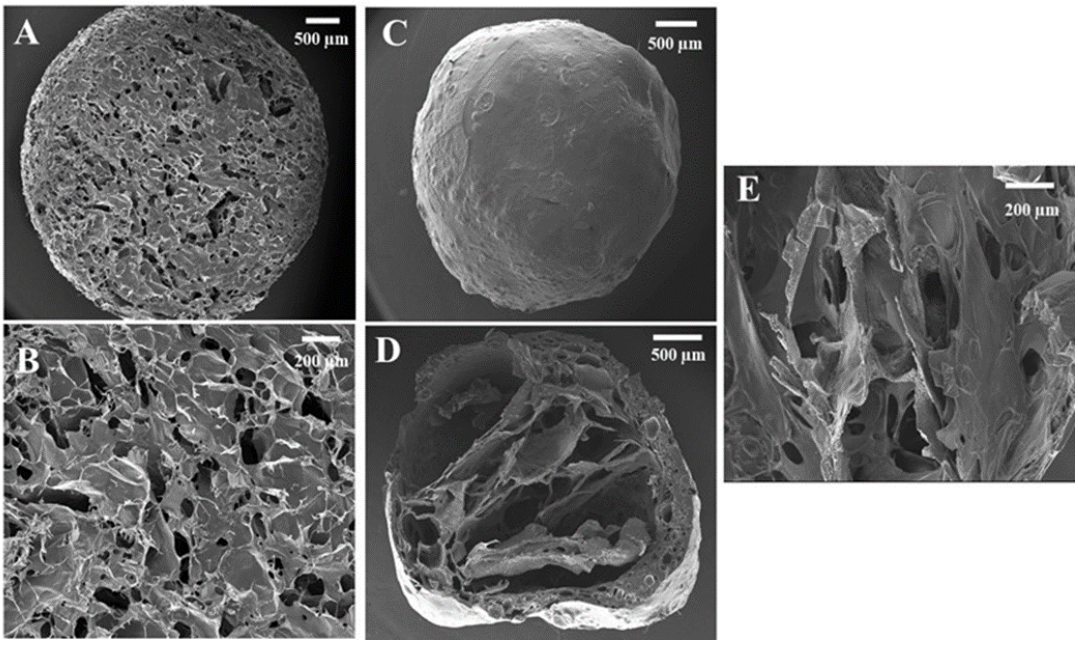

Figure 5 SEM micrographs of (A) SA, (B) SA surface, (C) BO/CS-SA, (D) BO/CS-SA cross section, and (E) CS-SA surface.

3. Fourier transform infrared (FTIR) analyses

The structural characterization by FTIR technique is given in Figure 6**.** SA shows the typical peaks at 3,400 cm^{-1} - 3,200 cm^{-1} and 2,924 cm^{-1} for O-H and C-H stretching, respectively. The carboxylate group (C=O), glycosidic linkage (C-O-C stretching (ß-(1-4)), and pyranose ring are confirmed at 1,592 cm^{-1}, 1,153 cm^{-1}, and 1,082^{-1} cm^{-1} and 1,033 cm^{-1}, respectively. In the case of CS, the unique peaks of amine and amide groups are confirmed at 1,652 cm^{-1} (amide I) and at 1,597 cm^{-1} (amide II).

It is known that the major constituents of BO are methyl chavicol (estragole) and linalool. Both contain aromatic ring which C-H bending can be confirmed at 808 cm^{-1} and 759 cm^{-1} (Figure 6C). The C-H stretching of methyl (CH_3) group belonging to linalool and methyl chavicol can be observed at 2,833 cm^{-1}, whereas the C=C bond is confirmed at 1,609 cm^{-1} and 1,509 cm^{-1}. In the case of BO/ CS-SA (Figure 6D), the major peaks represent BO, CS, and SA can be identified, suggesting the successful encapsulation of BO in CS-SA.

4. Thermal stability analysis

Thermal degradation of all microcapsules was studied to observe how double-layered CS-SA stabilized BO (Figure 7). The degradation of BO appeared from 50 ℃ as seen from the drastic weight loss. For SA and CS, the weight loss for moisture content about 10% at 80 ℃ followed by the degradation in steps from 250 ℃ to 400 ℃ can be identified. The char yields for SA and CS was about 35% which was typical of polysaccharides.

In the case of BO/CS-SA, the first weight loss from 100 ℃ to 149 ℃ for 40.3% was clarified. This might be because of corresponding to water and degradation of BO. The weight loss from 160 ℃ to 220 ℃ is due to the decomposition of CS and SA. The char yield for CS-SA polysaccharides was also identified (~13%)

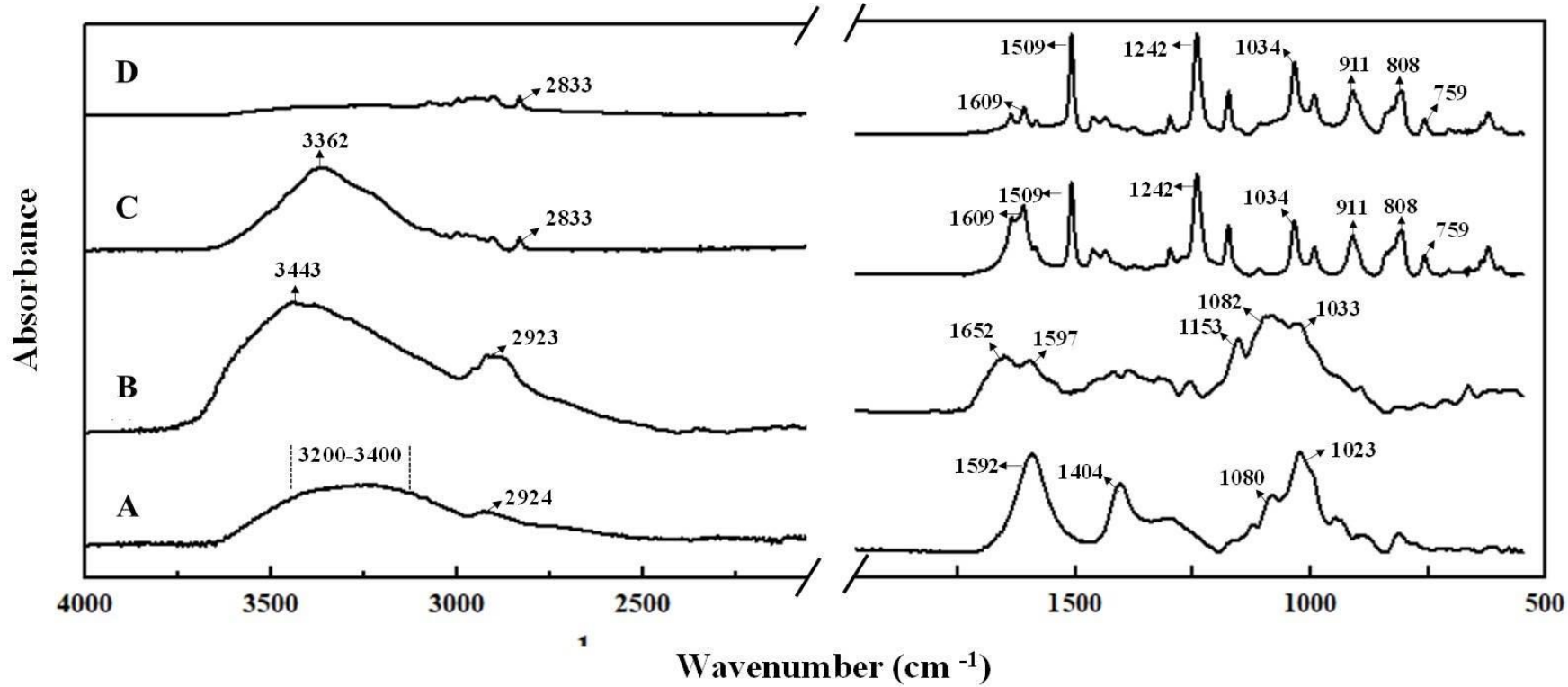

Figure 6 FTIR spectra of (A) SA, (B) CS, (C) BO, and (D) BO/ CS-SA.

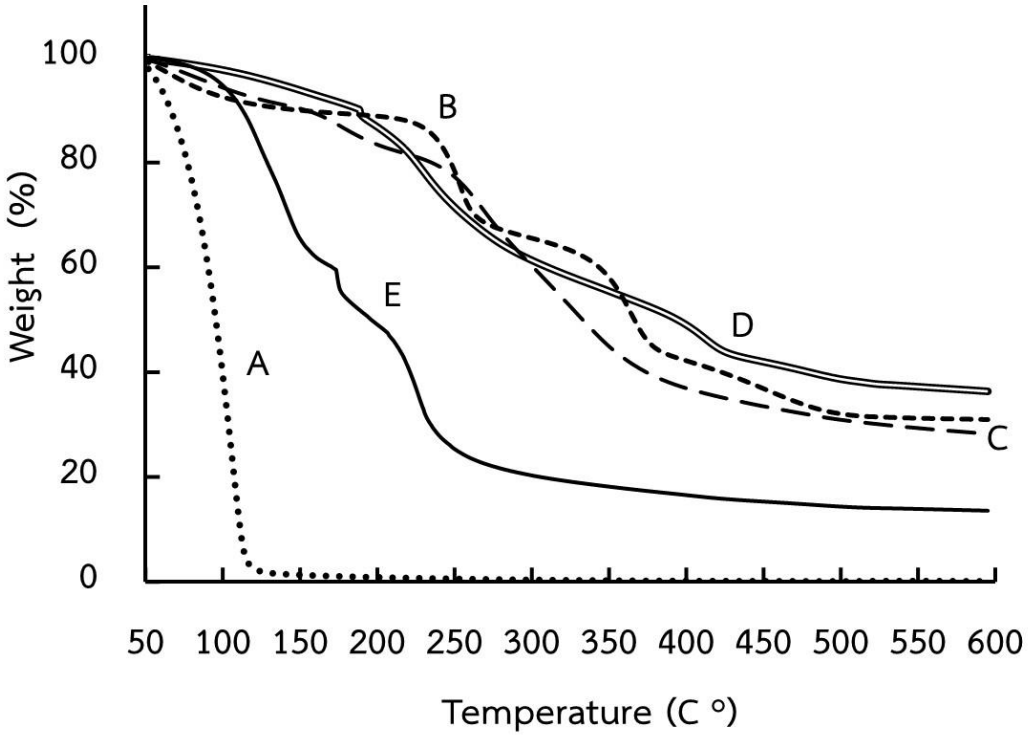

Figure 7 TGA thermograms of (A) BO, (B) SA, (C) CS, (D) CS-SA, and (E) BO/CS-SA.

5. BO content (%) in CS-SA microcapsule under temperature range of feed pelleting

BO content (%) in CS-SA microcapsule under temperature range of feed pelleting is shown **in Table 4.** Initially, the BO content was found to be 50.5% ± 0.3%. After isothermal treating at 75 ℃, 80 ℃ and 85 ℃ for 3 min, the BO content was maintained the same. The decrease of BO content was found at the first time at 90 ℃, representing 1.8% as compared with initial content ($P<0.05$). At 95 ℃ and 100 ℃, BO contents were significantly declined for 9.3%, and 9.5%, respectively.

Table 4 BO content in CS-SA microcapsule after heating at different temperatures for 3 min

Treatment	BO content (%)
before heating	50.5 ± 0.3 [a]
75 °C	50.5 ± 0.1 [a]
80 °C	50.3 ± 0.1 [a]
85 °C	50.4 ± 0.4 [a]
90 °C	49.6 ± 0.1 [b]
95 °C	45.8 ± 0.5 [c]
100 °C	45.7 ± 0.5 [c]

[a-c] superscript for significant differences ($P < 0.05$).

6 Dual pH responsive and the release performance under simulated poultry digestive tract.

Swelling and cumulative release studies were carried out to evaluate the release of BO in the animal gut. The weight change of BO/CS-SA weight was calculated the swelling percent (Figure 8A). In the first phase, the swellings are 13.9% ± 1.0%, 35.9% ± 1.7% and 55.5% ± 2.2% for the time intervals of 60 min, 120 min, and 180 min, respectively. The total weight of BO/CS-SA gradually increased to reach 2.5 times of the initial weight. After 180 min in SGF, the treatment was further treated in SIF. In the second phase, the swelling is about 28.0% within the first hour. At that time, the swelling is as high as 83.3% ± 3.3%.

The cumulative release of BO/CS-SA microcapsule is also plotted (Figure 8B). A good correlation between swelling of BO/CS-SA and release of BO can be clearly seen. Initially, the release was 28.3% ± 2.6% in the first hour. The initial burst release might be due to the BO on the CS-SA surface. The cumulative releases of BO are about 63.3% ± 4.6% and 86.8% ± 4.4% in SGF and SIF, respectively.

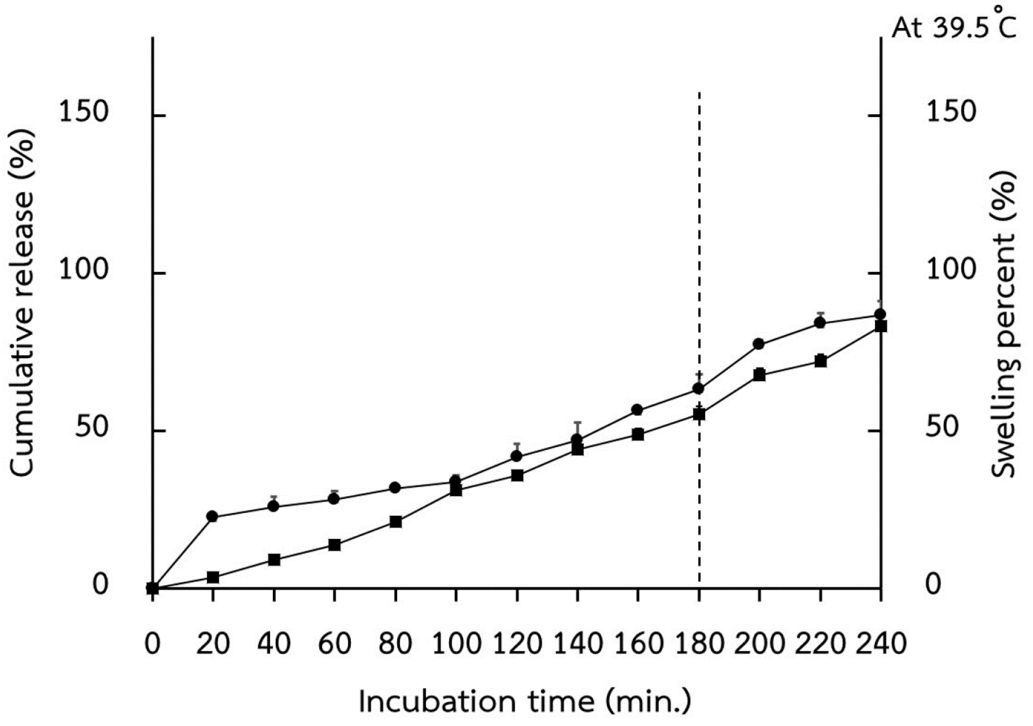

Figure 8 Performances of BO/CS-SA in terms of (A) swelling percent (%), and (B) cumulative release (%) in SGF and SIF at 39.5 ± 0.5 °C
Error bars present the standard deviation at each point.

7 Antioxidant activity

Table 5 shows the results of DPPH radical scavenging activity of BO and BO/CS-SA using dichloromethane extract. Initially, the scavenging activity of BO (100 mg/ml) was found to be 66.2% ± 0.8% and the IC_{50} was 72.8 µg/ml ± 1.8 µg/ml. For BO/CS-SA (100 mg/ml), the scavenging activity was 26.4% ± 2.9% with IC_{50} of 253.2 µg/ml ± 1.7 µg/ml.

Table 5 DPPH radical scavenging activity of BO and BO/CS-SA

Sample *	% DPPH radical scavenging	IC_{50} (mg/ml)
BO	66.2 ± 0.8	72.8 ± 1.8
BO/CS-SA	26.4 ± 2.9	253.2 ± 1.7
Trolox	71.4 ± 1.3	0.14 ± 0.0

* BO and BO/CS-SA = 100 mg/ml, Trolox =200 µg/ml

8. Antimicrobial activity

The studies on antimicrobial activities of BO and BO/CS-SA against gram-positive (*S. aureus* ATCC 25923) and gram-negative pathogenic bacteria (*E. coli* ATCC 25922, and *Salmonella* spp.) were carried out (**Table 6**). The antibacterial activity of BO (400 mg/ml) evaluated by the inhibition zones were 16.7 mm ± 1.5 mm, 11.0 mm ± 0.5 mm and 10.0 mm ± 1.0 mm for *S. aureus*, *E. coli*, and *Salmonella* spp., respectively**.** For BO/CS-SA (400 mg/ml), the antibacterial activity is as high as that of BO. The orders of antibacterial activity are *S. aureus* (inhibition zone of 15.0 mm ± 0.5 mm), followed by *E. coli* (10.0 mm ± 0.9 mm) and *Salmonella* spp. (9.2 mm ± 0.8 mm). The representative images of inhibition zone of BO and BO/CS-SA are presented in Figure 9.

Table 6 summarized the effect of BO and BO/CS-SA on the bacterial growth using MIC and MBC assays. The MIC values for BO were determined to be 5.0 mg/ml, 2.5 mg/ml, and 5.0 mg/ml, whereas, MBC values were 10 mg/ml, 5 mg/ml, 10 mg/ml for *E. coli*, *S. aureus*, and *Salmonella* spp., respectively. The MIC and MBC values of BO/CS-SA against all bacteria were the same as those of BO. Either BO or BO/CS-SA was more effective against *S. aureus* than *E. coli*, and *Salmonella* spp.

Table 6 Antimicrobial activity of BO and BO/CS-SA against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *Salmonella* spp.

Bacterial strains	Antimicrobial activity									
	Inhibition zone diameter (mm.)						MIC (mg/ml)		MBC (mg/ml)	
	BO			BO/CS-SA			BO	BO/CS-SA	BO	BO/CS-SA
E. coli	11.0	±	1.0	10.0	±	0.9	5.0	5.0	10.0	10.0
S. aureus	16.7	±	1.5	15.0	±	0.5	2.5	2.5	5.0	5.0
Salmonella spp.	10.0	±	1.0	9.2	±	0.8	5.0	5.0	10.0	10.0

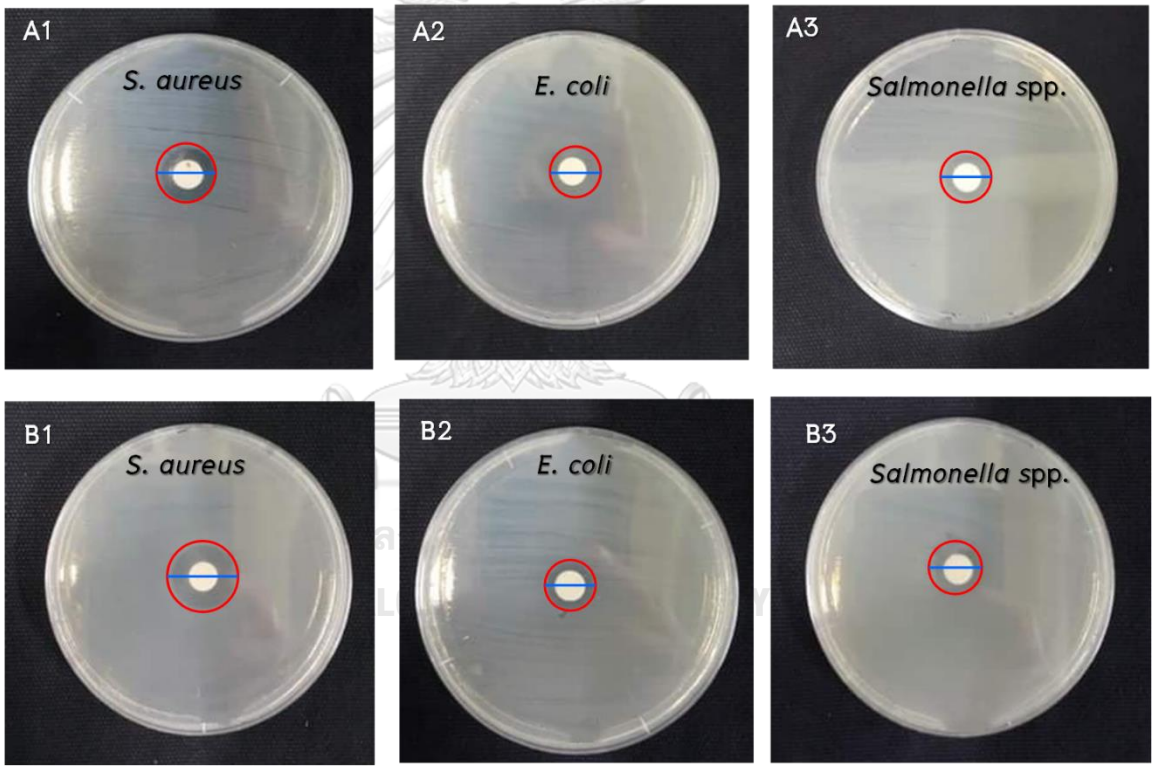

Figure 9 Antimicrobial activity of (A) BO and (B) BO/CS-SA against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *Salmonella* spp. as evaluated by inhibition zone

9. Impact of storage conditions on the antioxidant and antimicrobial stabilities of BO/CS-SA

Storage stability was monitored by measuring the antioxidant and antimicrobial activities during 90 days (**Table 7**). The inhibition of DPPH-free radical of BO was gradually decreased at 30 days and significantly declined for as much as 24% at 90 days ($P < 0.01$). For BO/CS-SA, the antioxidant activity shows almost no declined in any storage times ($P \sim 0.44$).

For antimicrobial stability, the studies were carried out against three pathogens, i.e. *E. coli*, *S. aureus*, and *Salmonella* spp. **Table 7** shows the decrease in inhibition zone diameter of all pathogens for BO within the storage time 30 days. However, the antimicrobial activity of BO/CS-SA was slightly decreased (for 8% -17%) when the storage time was as long as 90 days of the storage time.

Table 7 Antioxidant and antimicrobial stabilities of BO and BO/CS-SA under storage [1]

Sample	Days of storage	DPPH radical scavenging (%)	Inhibition zone diameter (mm.)		
			E. coil	*S. aureus*	*Salmonella* spp.
BO	0	66.2 ± 0.7 [a]	11.0 ± 1.0 [a]	16.7 ± 1.5 [a]	10.0 ± 1.0 [a]
	30	60.1 ± 1.3 [b]	9.7 ± 0.5 [b]	14.7 ± 0.4 [b]	9.3 ± 0.5 [b]
	60	56.1 ± 2.3 [c]	7.0 ± 0.0 [c]	12.5 ± 0.5 [c]	7.0 ± 0.0 [c]
	90	50.4 ± 1.2 [d]	7.0 ± 0.0 [c]	7.0 ± 0.0 [d]	7.0 ± 0.0 [c]
BO/CS-SA	0	26.9 ± 1.4	10.0 ± 0.8	15.0 ±0.5 [a]	9.2 ± 0.7
	30	25.7 ± 1.4	10.0 ± 1.0	14.5 ±0.5 [a]	9.7 ± 0.5
	60	24.7 ± 1.7	10.9 ± 1.2	14.5 ± 0.5 [a]	9.9 ± 0.2
	90	25.4 ± 2.0	8.3 ± 0.5	13.3 ± 0.5 [b]	8.4 ± 0.6

[a-d] superscripts for significant differences in the column at $P < 0.05$

[1] Data are the means ± SD from three investigations.

10 Effect of acid, bile, trypsin, and thermal treatment on the antioxidant and antimicrobial activities of BO/CS-SA

For *in vivo* delivery, BO has to be in the several conditions such as acid, bile, trypsin. The role of CS-SA to maintain BO antioxidant was studied based on DPPH radical scavenging ability (Figure 10). Initially, the scavenging activity of BO/CS-SA was 26.9% ±1.4%. When BO/CS-SA was incubated with acid, bile, trypsin, including thermal treatment (at 85 ℃ for 1 min), the activities were remained at 22.8% ± 1.8%, 22.6% ± 2.0%, 21.8% ± 1.8% and 22.0% ± 2.8%, respectively.

The antimicrobial activities of BO/CS-SA were studied and the evaluation was based on the clear zone diameter. Figure 11 shows that the BO/CS-SA before incubation is in the range of 10 mm-15 mm depending on the type of pathogens. After incubating with acid, bile, trypsin conditions, including the thermal treatment, the changes of inhibition are still maintained in the same level.

The comparison study on bioactive constituents in BO/CS-SA under thermal treatment is summarized in **table 8**. Methyl chavicol and linalool of BO/CS-SA before thermal treatment were 65.86% and 22.07%. After thermal treatment, the methyl chavicol and linalool were decreased by 0.57% and 0.66%, respectively. The rest of compounds was determined in a reduction < 1%.

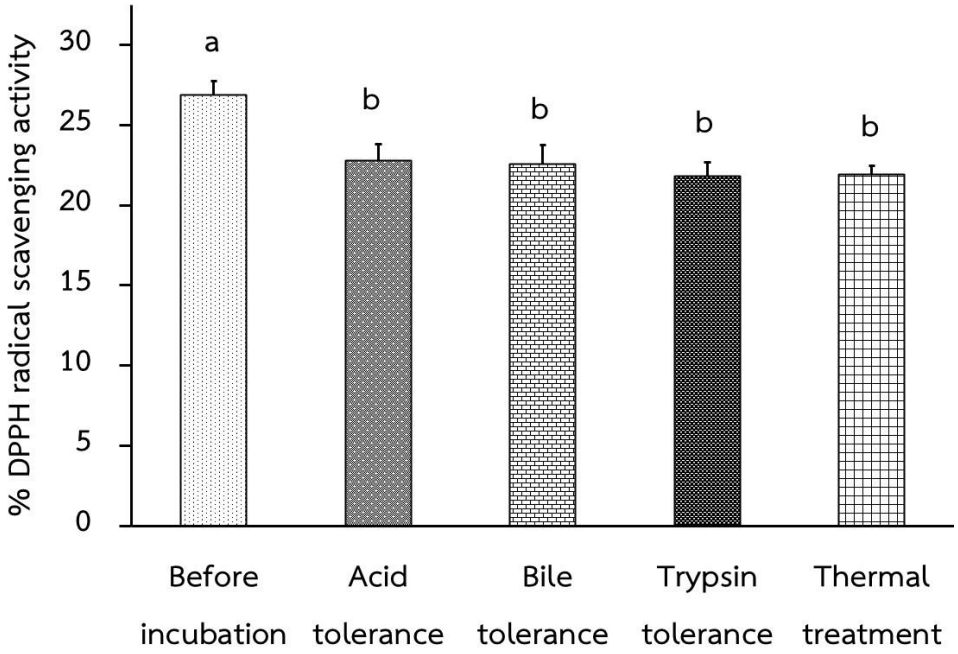

Figure 10 Antioxidation ability of BO/CS-SA under acid, bile, trypsin, and thermal treatment as evaluated by DPPH radical scavenging.

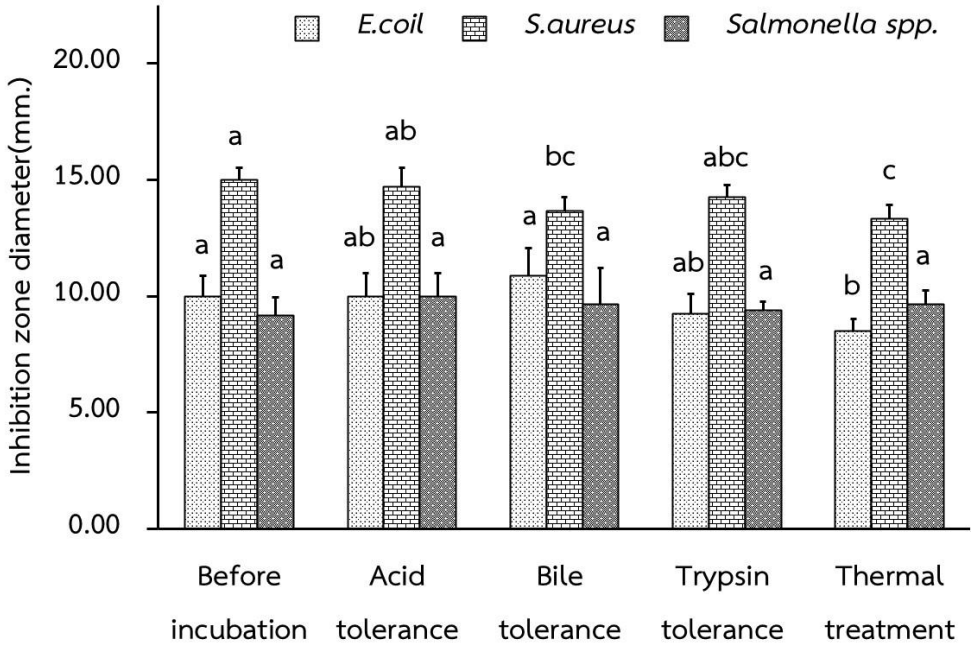

Figure 11 Antimicrobial ability of BO/CS-SA under acid, bile, trypsin, and thermal treatment as evaluated by inhibition zone
(Black for *E. coli*, white for *S. aureus*, and gray for *Salmonella* spp.).

Table 8 Thermal treatment of BO/CS-SA on chemical composition of BO

No.	Compound	Before heating		After heating	
		R.I (s)	%	R.I (s)	%
1	Alpha-pinene	396.00	0.07	396.00	0.06
2	Beta-pinene	471.00	0.05	469.20	0.05
3	6-methyl-5-hepten-2-one	508.80	0.09	508.80	0.09
4	Eucalyptol	556.80	0.15	554.40	0.19
5	Beta-Ocimene Y	591.00	0.13	591.00	0.13
6	Trans-linalool oxide	625.20	0.11	625.20	0.11
7	Cis-linalool oxide	645.60	0.17	645.60	0.16
8	**Linalool**	**684.60**	**22.07**	**684.00**	**21.41**
9	Menthol	764.40	0.34	764.40	0.34
10	**Methyl chavicol**	**790.20**	**65.86**	**789.60**	**66.43**
11	Z-citral	814.20	1.50	813.60	1.52
12	E-citral	843.00	1.53	843.00	1.57
13	Trans-caryophyllene	967.20	0.75	967.80	0.71
14	Alpha-bergamotene	976.80	1.39	976.80	1.57
15	Beta-farnesene	996.60	0.87	996.60	0.85
16	Germacrene D	1,021.20	0.78	1,021.20	0.76
17	Beta-bisabolene	1,040.40	0.24	1,040.40	0.23
18	Delta-cadinene	1,054.20	0.08	0.00	0.00
19	Cis-alpha-bisabolene	1,066.80	3.83	1066.80	3.82
	Total		100.00		100.00

R.I. (s) = Retention time (unit: second).

11. Growth performance

The influences of experimental diets on growth performance of broiler chickens are summarized in **Table 9**. Final body weight (FBW) of broilers in NC was significantly lower than that in other groups ($P < 0.01$). Experimental diets had no significant effect on ADFI throughout the study. During d 1 - 21, the supplementation of FBO and MBO at 500 ppm and PC tended to increase ADG ($P = 0.059$), but no differences were found during d 22 - 42 ($P = 0.131$). Overall, ADG in broilers fed with PC, BO and BO/CS-SA was improved compared with that in NC ($P < 0.01$), but there were no significant differences among 3 treatments. Broilers fed with PC, FBO and MBO diets had a lower FCR than those fed with the NC diet ($P < 0.01$) during days 1 - 21 and tended to improve FCR than those fed with NC diet ($P = 0.058$) during days 22 - 42. Overall, broilers receiving MBO and PC diets exhibited a lower FCR than those receiving NC ($P = 0.008$), but there were no significant differences between NC and FBO diets. The overall mortality rate was not affected by treatments ($P = 0.862$).

Table 9 Growth performance of broiler chickens on d 21 and d 42 [1]

Item	Dietary treatment [2]				SEM	*P*-value
	NC	PC	FBO	MBO		
Initial body weight (g)	47.36	46.81	46.67	47.08	0.154	0.710
Final body weight (g)	1,722.78 [b]	1,896.67 [a]	1,831.11 [a]	1,880.56 [a]	39.229	< 0.010
Average daily feed intake (g/d)						
d 1 - 21	49.58	51.02	50.60	51.17	0.360	0.738
d 22 - 42	111.18	107.78	108.89	109.54	0.711	0.762
d 1 - 42	80.38	79.40	79.74	80.36	0.240	0.944
Average daily gain (g/d)						
d 1 - 21	31.52	34.76	33.43	34.10	0.699	0.059
d 22 - 42	52.25	57.74	55.79	57.57	1.274	0.131
d 1 - 42	41.89 [b]	46.25 [a]	44.61 [a]	45.84 [a]	0.983	< 0.010
Feed conversion ratio						
d 1 - 21	1.57 [a]	1.47 [b]	1.51 [b]	1.50 [b]	0.022	< 0.010
d 22 - 42	2.16	1.87	1.95	1.91	0.063	0.058
d 1 - 42	1.92 [a]	1.72 [b]	1.79 [ab]	1.75 [b]	0.045	0.008
Mortality rate (%)	4.17	2.78	2.78	2.78	0.0347	0.862

Different subscript letters indicate significant differences in the rows (P < 0.05).

[1] Data represent the mean of 6 pens (3 broilers/pen) per treatment.

[2] NC, broilers fed a basal diet without additives; PC, broilers fed a basal diet with avilamycin at 10 ppm; FBO and MBO broilers fed a basal diet with unencapsulated basil oil and micro-encapsulated at 500 ppm. SEM = standard error of the mean.

12 Apparent ileal digestibility (AID)

The influence of BO/CS-SA supplementation on AID is shown in **Table 10** On day 21, PC, FBO and MBO exhibited a significant positive effect on the AID of DM and NFE ($P < 0.05$) compared to NC. The AID of CP was increased by PC and MBO diets than NC diet ($P = 0.002$), but there were no significant differences between NC and FBO diets. Broilers fed with MBO diet improved the AID of EE ($p = 0.032$) and GE ($P < 0.01$) than those fed with NC diet. The AID of CF and ash was not influenced by experimental diets on day 21, but the digestibility of CF was greater in PC, FBO and MBO diets than that in NC diet ($P < 0.05$) on day 42. The AID of CP and EE, and CF with MBO supplementation was significantly increased ($P < 0.05$) compared to that of NC group on day 42. However, there was no significant difference among diets supplemented with additives. The digestibility of DM, ash and NFE was not influenced by experimental diets on day 42. Broilers fed with MBO had greater AID of CP than those fed with FBO on day 21 ($P < 0.05$), whereas MBO supplementation had more beneficial effect on GE digestibility ($P < 0.05$) than NC and PC groups on day 42.

Table 10 Apparent ileal digestibility (AID) of broiler chickens on d 21 and d 42 [1]

Item	Dietary treatment [2]				SEM	P-value
	NC	PC	FBO	MBO		
d 21						
Dry matter (DM)	0.61 [b]	0.66 [a]	0.66 [a]	0.67 [a]	0.015	0.003
Crude protein (CP)	0.71 [c]	0.76 [ab]	0.74 [bc]	0.78 [a]	0.015	0.002
Ether extract (EE)	0.79 [b]	0.83 [ab]	0.83 [ab]	0.85 [a]	0.011	0.032
Crude fiber (CF)	0.59	0.65	0.61	0.66	0.015	0.171
Ash	0.31	0.35	0.35	0.34	0.009	0.677
Gross energy (GE, MJ)	0.65 [c]	0.67 [bc]	0.69 [ab]	0.71 [a]	0.012	<0.001
Nitrogen free extract (NFE)	0.58 [b]	0.66 [a]	0.65 [a]	0.66 [a]	0.019	0.006
d 42						0
Dry matter (DM)	0.62	0.66	0.65	0.66	0.010	0.055
Crude protein (CP)	0.69 [b]	0.76 [a]	0.73 [ab]	0.76 [a]	0.016	0.028
Ether extract (EE)	0.80 [b]	0.85 [a]	0.83 [a]	0.85 [a]	0.013	0.003
Crude fiber (CF)	0.59 [b]	0.66 [a]	0.65 [a]	0.65 [a]	0.016	0.002
Ash	0.28	0.35	0.30	0.31	0.014	0.107
Gross energy (GE, MJ)	0.67 [c]	0.70 [bc]	0.70 [ab]	0.73 [a]	0.012	0.002
Nitrogen free extract (NFE)	0.62	0.65	0.64	0.66	0.008	0.313

Different subscript letters indicate significant differences in the rows ($P < 0.05$).

[1] Data represent the mean of 6 pens (3 broilers/pen) per treatment.

[2] NC, broilers fed a basal diet without additives; PC, broilers fed a basal diet with avilamycin at 10 ppm; FBO and MBO broilers fed a basal diet with unencapsulated basil oil and micro-encapsulated at 500 ppm. SEM = standard error of the mean.

13. Jejunal histomorphology

The morphological changes of the mid - jejunum in broilers are presented in **Table 11**. Jejunal VH was improved by MBO in comparison to NC and PC treatments on day 21 (P = 0.036). However, there was no statistical difference between FBO and MBO diets. On day 42, FBO and MBO groups had higher VH than NC and PC groups ($P < 0.01$). Jejunal CD and VW were not significantly affected by dietary treatments. On day 21, broilers fed with MBO diet had greater VH: CD value than those fed with NC, PC and FBO diets (P = 0.012). Moreover, it had a similar trend (P=0.022) on day 42, except that there were no significant differences between MBO and FBO treatments. The MBO diet increased VSA of jejunum compared with the NC and PC diets ($P < 0.05$) on day 21 and day 42. VSA in FBO and MBO groups did not differ significantly on day 21, but the difference was observed on day 42 ($P < 0.01$). The representative images of mid-jejunal of broilers chickens are shown in Figure 12**.**

Table 11 Jejunal histomorphology of broiler chickens on d 21 and d 42 [1]

Item	Dietary treatment [2]				SEM	*P*-value
	NC	PC	FBO	MBO		
d 21						
Villus height (VH, μm)	703.64 [b]	713.69 [b]	743.94 [ab]	812.75 [a]	24.62	0.036
Crypt depth (CD, μm)	117.28	114.63	112.61	110.58	1.43	0.469
Villus width (VW, μm)	32.83	34.95	35.22	33.09	0.62	0.350
Villus height to crypt depth ratio (VH:CD)	6.17 [b]	6.31 [b]	6.77 [b]	7.77 [a]	0.36	0.012
Villus surface area (VSA, mm^2)	0.72 [c]	0.77 [b]	0.79 [ab]	0.83 [a]	0.02	< 0.010
d 42						
Villus height (VH, μm)	913.44 [b]	923.09 [b]	1,050.08 [a]	1,126.78 [a]	51.59	< 0.010
Crypt depth (CD, μm)	140.40	141.67	147.51	149.55	2.22	0.197
Villus width (VW, μm)	30.48	32.76	29.77	30.18	0.67	0.194
Villus height to crypt depth ratio (VH:CD)	6.86 [b]	6.67 [b]	7.28 [ab]	7.68 [a]	0.22	0.028
Villus surface area (VSA, mm^2)	0.86 [c]	0.92 [b]	0.97 [b]	1.06 [a]	0.04	< 0.010

Different subscript letters indicate significant differences in the rows (P < 0.05).

[1] Data represent the mean of 6 pens (3 broilers/pen) per treatment.

[2] NC, broilers fed a basal diet without additives; PC, broilers fed a basal diet with avilamycin at 10 ppm; FBO and MBO, broilers fed a basal diet with unencapsulated basil oil and micro-encapsulated at 500 ppm. SEM = standard error of the mean.

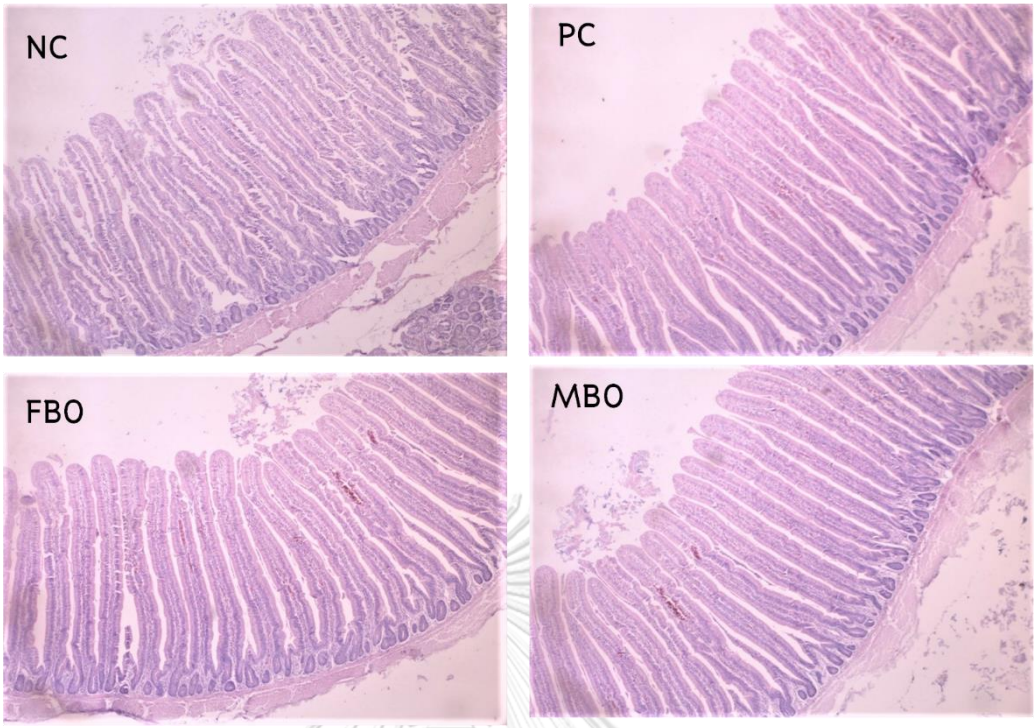

Figure 12 Representative images of mid-jejunal of broilers chickens
NC, broilers fed a basal diet without additives; PC, broilers fed a basal diet with avilamycin at 10 ppm; FBO and MBO, broilers fed a basal diet with free basil oil and microencapsulated at 500 ppm.

14. Bacterial population

The effects of dietary MBO on bacterial population in broilers are shown in **Table 12**. Experimental diets affected neither the population of *E. coli* nor *Lactobacillus* spp. in ceco-colon in any phases.

Table 12 *Lactobacillus* spp. and *E. coli* populations in ceco-colon of broiler chickens on d 21 and d 42 [1]

Item	Dietary treatment [2]				SEM	*P*-value
	NC	PC	FBO	MBO		
d 21						
Lactobacillus spp., $\log_{10}$ cfu/g	6.00	6.22	4.20	5.83	0.462	0.960
E. coli, MPN/g	9.27	11.00	11.00	9.93	0.426	0.236
d 42						
Lactobacillus spp., $\log_{10}$ cfu/g	5.60	7.13	4.43	5.28	0.564	0.415
E. coli, MPN/g	5.47	3.14	2.01	3.62	0.721	0.471

[1] Data represent the mean of 6 pens (3 broilers /pen) per treatment.

[2] NC, broilers fed a basal diet without additives; PC, broilers fed a basal diet with avilamycin at 10 ppm; FBO and MBO broilers fed a basal diet with unencapsulated basil oil and micro-encapsulated at 500 ppm. SEM = standard error of the mean

15. Antioxidant capacity

The results of antioxidant capacity are illustrated in Figure 13. On day 21, broilers in MBO group significantly increased SOD of duodenal mucosa compared with those in NC and PC groups ($P < 0.01$). However, there was no significant difference in SOD of duodenal mucosa between FBO and MBO groups. The NC group has the least activity of SOD. On day 42, broilers in MBO group showed the highest SOD activity (220.12 U/mg protein) in comparison with other groups ($P < 0.01$). The highest value of MDA was found in broilers receiving NC diet for days 21 and 42. On the other hand, a significant reduction ($P < 0.01$) in MDA level were found in duodenal mucosa of birds MBO diet throughout the experiment.

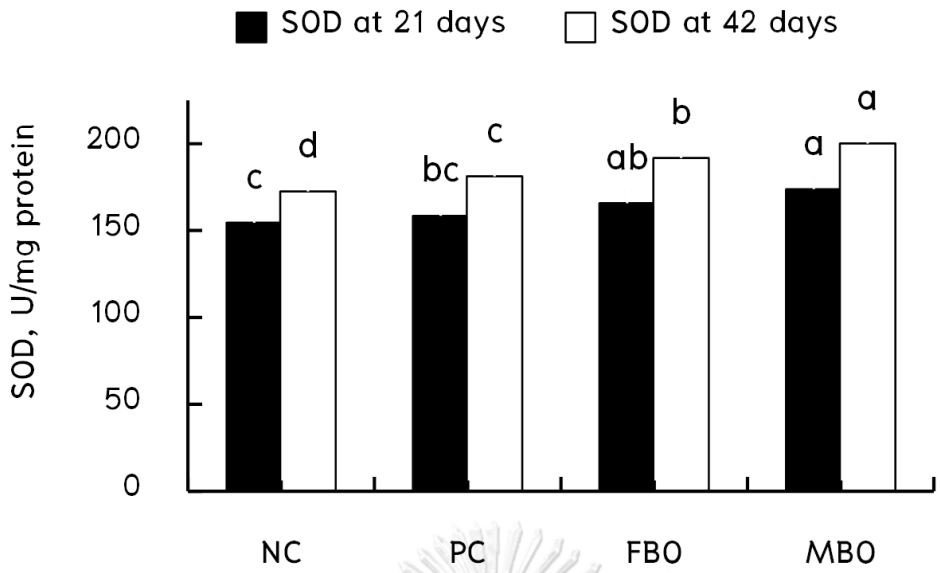

Figure 13 Superoxide dismutase (SOD) activity in duodenal mucosa on d 21 and d 42. Error bars present the standard deviation at each point.

[a-c] Different letters in the bars indicate a significant difference between treatments ($P < 0.05$).

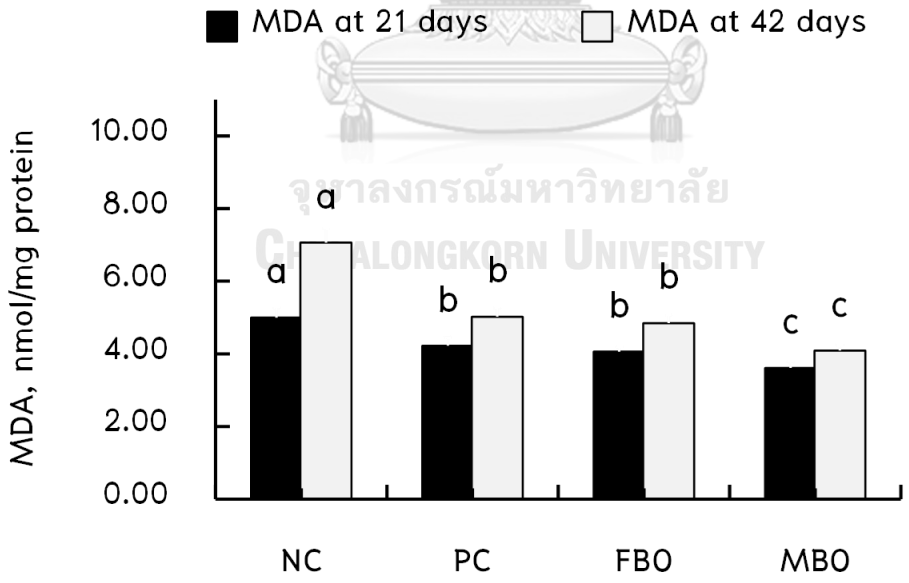

Figure 14 Malondialdehyde (MDA) concentration in duodenal mucosa on d 21 and d 42. Error bars present the standard deviation at each point.

[a-c] Different letters in the bars indicate a significant difference between treatments ($P < 0.05$).

CHAPTER 5

DISCUSIONS

1. Chemical composition of basil oil (*Ocimum basilicum* L.)

EOs contains various compounds, including monoterpene derivatives (camphor, limonene, linalool, 1,8-cineole, citral, geraniol), phenylpropanoid derivatives (eugenol, methyl eugenol, chavicol, methyl chavicol, methyl-cinnamate) and others. In this study, BO consisting of methyl chavicol and linalool as main constituents were identified by GC-MS. Our results are in agreement with the previous results reported by Padalia *et al.* (2017) and Ahmed *et al.* (2019) that methyl chavicol (56.1–89.7%) and linalool (1.0-33.7%) are the major constituents of *Ocimum basilicum* L. Both compounds showed antimicrobial and antioxidant activities based on *in vitro* study. However, EOs are complex mixtures containing numerous constituents. A number of factors related to climate, habitat, agriculture, environment and storage time may lead to these different compositions and properties of EOs.

2. Characterization and performances of SA, and BO/CS-SA

The present work demonstrated the application of the complementary polysaccharides to fabricate a dual pH responsive microporous structure to encapsulate the active compound and maintain the activity through the acid and base conditions so that the release at the targeted organ is possible. The use of polysaccharide SA to preform SA microcapsules followed by encapsulating the active model compound i.e., BO before covering with the complementary polysaccharide i.e., CS leading to successful BO/CS-SA microcapsules.

The SEM micrographs clearly shows the characterization of SA, and BO/CS-SA. SA microcapsules were relatively cylindrical shape with large pores on the top surface and honeycomb-like appearance. The diameters of SA pore are in the range of 53 μm to 325 μm which can be termed as macropores. $CaCl_2$ provides ionic gelation with alginate, resulting in the interconnected networks. The trapped water was removed by the lyophilization (Glenn *et al.*, 2010). The porous structure may lead to a great attribute not only to protect but also to diffuse active ingredient. (Faidi *et al.*, 2019). CS-SA microcapsules were in spherical shape with the surface smooth. The average diameters were 1,000 μm with a range from 800.02 to 1,645.40 μm. The BO/CS-SA can be classified into matrix microcapsule, which the core material is incorporated within the matrix material. This might be not only encouraged the controlled release but also acted as barriers. The range size of BO/CS-SA is similar to that of the feed ingredients (approximately, 500 μm - 2,000 μm) (Cuadros *et al.*, 2015). Their shape, size and surface characteristics can affect other properties, especially % EE, solubility, fluidity as well as distribution during feed mixing process (Campelo-Felix *et al.*, 2017).

The CS-SA presented the high % EE of BO in its structure. This can be explained by double-layered microencapsulation that was highly effective for encapsulation. This directly connected with high surface area and pore size, leading to the high absorption. There were contrast to findings of EE in previous investigations. For instance, the clove loaded-microcapsule using alginate as a wall material showed a poor EE rate of 24.77 ± 7.47% (Faidi *et al.*, 2019), while polylactic acid microcapsules of linalool showed a high EE rate of 81.10 ± 10.0% (Dusankova *et al.*, 2019).

To confirm successful coating of BO within CS-SA materials, the structural characterization was carried out by FTIR technique. The major peaks of BO/CS-SA can be identified BO, CS, and SA indicating the successful encapsulation of BO in CS-SA.

Considering the TGA results, thermal degradation of BO/CS-SA was observed how double-layered CS-SA could stabilize BO. The degradation of BO appeared from

50 ℃ as the drastic weight loss. The degradation rate was 95.62%, while the temperature of maximum degradation was 104.60 ℃. The result clearly indicated that degradation behavior of BO exhibited a weak thermal stability. This result is in agreement with the study of Garcia-Sotelo *et al.* (2019), who identified the evaporation temperature of BO ranged from 45.00 to 164.20 ℃. The degradation of BO/CS-SA appeared from 100 ℃ to 149 ℃ for 40.3%. Also, BO/CS-SA represented a delay in the decomposition rate as compared to BO. This implied that CS-SA network with high resistance to thermal degradation improved thermal stability of BO.

As the feed pelleting process involves with the temperature at around 75 ℃ – 85 ℃, it comes to the question whether CS-SA can maintain BO during thermal treatment or not. The results illustrated that BO content remained constant in CS-SA microcapsule after heating at 75 ℃, 80 ℃ and 85 ℃ for 3 min. Nonetheless, the decrease of BO content was found for the first time when the microcapsules were thermally treated at above 90 ℃. The possibility is that the crosslink network of CS with TTP may be initially decomposed at this point, resulting in the evaporation of BO. This is an important point as practical in the commercial process. It was clarified the resistance to decomposition of CS-SA network during high-temperature process.

3 The release performance under simulated poultry digestive tract.

Due to the complexity of the animal gut, swelling and cumulative release studies are useful tools to predict the release behavior of BO. The swelling reflects the CS-SA responsive to the digestive fluid. During SGF treatment, it is expected that gastric acidic pH (pH 2.0) induces the protonation of amine group of CS to allow water penetration resulting in swelling. The treatment in SIF is rather neutral (pH 6.5) but its pKa is higher than pKa of SA (~3.6) resulting in deprotonation of SA to continue swelling. A good correlation between swelling of BO/CS-SA and release of BO can be clearly demonstrated.

It was reported that the controlled release of core substance was dependent on the type of polymer and its characteristic i.e., cross-linking degree, pH medium environment, mechanical forces, interaction with biological compound or biological responses, and incubation time (Bruneau *et al.*, 2019; Kleemann *et al.*, 2020). In this study, the crosslink networks allow the release. The rapid release (approximately 28%) in the first hour. This noted that burst release might be due to the BO on the CS-SA surface. Initially, the destruction of TPP cross-linker was disturbed due to the ion-exchange between phosphate and potassium in SGF. At the same time, the acidity of SGF initiated the CS protonation. The erosion of CS facilitated the penetration of SGF into SA micropores. However, the carboxylate groups of SA chains and their networks in SGF were still maintained to assist BO and withstand the pH variations including endogenous enzymes in the gut if it is in *vivo* system. In the final step, the crosslink network of SA was damaged because SA started to deprotonate in the SIF, resulting in the diffusion of BO. In fact, Azad *et al.* (2020) demonstrated the swelling of SA while the ion-exchange between calcium ion and phosphate ion in SIF occurred. During the destruction of calcium ion crosslink network, BO was gradually released. Based on the release mechanism above, it is expected that the dual pH responsive of CS and SA leads to a successful release of BO at the lower part of intestine.

4. Antioxidant and antimicrobial activities of BO/CS-SA

It comes to the question whether BO released from BO/CS-SA maintains its antioxidant activities or not. Based on their chemical composition, a good relationship between the relative percentage of methyl chavicol and the antioxidant activity was observed. The present study revealed that BO and BO/CS-SA exhibited a great scavenging activity on DPPH radicals. This result is in agreement with Bunrathep *et al.* (2007) who clarified that BO containing 92.48 % of methyl chavicol had an excellent DPPH radical scavenging activity. This is related to the presence of methyl chavicol (phenylpropene), which is a major component of BO. In fact, methyl chavicol is an excellent hydrogen donating molecule to inactivate DPPH radicals (Bozin *et al.*, 2006).

The evaluation of antimicrobial activity was executed using three methodologies. The results demonstrated that BO and BO/CS-SA showed the moderate antimicrobial activity against gram-positive pathogenic bacteria (*S. aureus* ATCC 25923) and gram-negative pathogenic bacteria (*E. coli* ATCC 25922, and *Salmonella* spp.). Presence of linalool in BO is responsible for antimicrobial properties. Linalool, 3,7-dimethyl-1,6-octadien-3-ol, is a monoterpene found in aromatic structures. The extraction of *O. basilicum* leaves containing linalool (55.2%) had a strong antimicrobial activity against both *S. aureus* and *Pseudomonas aeruginosa.* (Silva *et al.*, 2015). Mechanisms of linalool have high permeability to the outer and inner mitochondrial membranes, leading to the damage of bacteria and then cell death (Arfat *et al.*, 2014). The MIC results of both BO and BO/CS-SA were more effective against *S. aureus* than *E. coli* and *Salmonella* spp. These results are in agreement with the finding of Koroch *et al.* (2017) who reported that BO exhibited a higher inhibiting potential against gram-positive bacteria than gram-negative bacteria due to the integrity of the cell membrane. These results confirmed that the double - layered microencapsulation process was efficient for retaining the bioactive properties of BO. According to our work, BO/CS-SA had the same efficiency as BO, demonstrating that this process was not harmful to their abilities.

5. Impact of storage times on the antioxidant and antimicrobial stabilities of BO/CS-SA

In the practical applications, it is possible that the microcapsules are stored for a certain period before use. During that time, the active compounds might lose their activity through the oxidation. The role of microcapsules is to maintain the antioxidant and antimicrobial activities. The changes of the antioxidant and antimicrobial activities under storage conditions were studied. In fact, the stability of BO was reported to be under several factors especially, oxygen, light, moisture, temperature, and storage time (Tavares *et al.*, 2020). These factors induced the oxidation process, leading to loss of performance and shelf life (Zeng *et al.*, 2015). It was found that the scavenging radical

activity of BO was significantly declined with increasing storage time, reflecting its instability. In contrast, treated BO/CS-SA reserved its antioxidant capacity until the end of storage. These findings are in agreement with a previous report of Ngamekaue and Chitprasert (2019), who demonstrated that holy basil oil decreased antioxidant activity during storage times, whereas its microcapsule successfully retained antioxidant activity at 30 °C for 3 months.

For the antimicrobial stability, it was found that inhibition effect of BO against three pathogens was instable during the storage. The reduction of the antimicrobial activity might be due to the oxidation and the degradation of BO. BO/CS-SA showed the great effectiveness throughout storage. Nevertheless, slight impact of storage on *S. aureus* appeared in the third month. It is possible that storage time after 60 days may influence on the deterioration of microcapsule. This needed to be further investigated. CS-SA microcapsule was effective in stabilization of BO. While SA provides micropores as the container of BO, the CS and SA networks play the role of the barriers to protect the oxidation and degradation. The results clearly demonstrated that CS-SA microcapsule maintained the antioxidant and antimicrobial activities throughout the storage period. Additionally, our results found that bioactive compounds in BO regrading antimicrobials had less stability than its antioxidants under influence of storage. Similarly, Ali *et al.* (2018) stated that chavicol structure of dried piper beetle extracts exhibited the good stability under storage condition.

6. Tolerances of BO/CS-SA under acid, bile, trypsin conditions and thermal treatment on antioxidant and antimicrobial properties.

After exposure to undesirable conditions, antioxidant activity was a slight decrease in the range of 4 to 5% but it was not different for all conditions. CS-SA microcapsule in acid and trypsin maintained the antimicrobial activity of BO against the three pathogens. In bile condition, the CS-SA microcapsule potentially sustained

the inhibition of *E. coli* and *Salmonella* spp., except *S. aureus.* Under thermal treatment, BO/CS-SA had poor antimicrobial activity against *E. coli* and *S. aureus*. Nevertheless, the inhibition effect depended upon many factors i.e. bacteria strain and concentration of active compounds (Turek and Stintzing, 2013). The loss of some activity might be due to the decomposition of CS-SA microcapsule, leading to leakage of some active constituents in digestive solution. The decomposition mechanism of CS-SA microcapsule in a gastric condition is explained in swelling and *in vitro* release sections. The interaction between polysaccharide microcapsule and either bile or trypsin is still unclear. The most susceptibility of BO/CS-SA appeared in heat treatment when compared with other conditions. Furthermore, the remaining activities > 90% was achieved.

In fact, volatile substances lose their biological activities during passing through pellet machine when exposed to steam pressure and conditioning temperature. Methyl chavicol and linalool in BO/CS-SA are stable under thermal treatment. These results reflect how the double layered structure formed by SA and CS enhances the resistance to the harsh environment conditions and still retains the bioactive constituent, antioxidant and antimicrobial stabilities of BO.

7. BO encapsulated CS-SA microcapsules (BO/CS-SA) enhances growth performance, apparent ileal digestibility, intestinal morphology, microbial population, and antioxidant capacity of broiler chickens

This experiment was performed in a conventional house during the summer period. When broilers are raised under high temperature environment, they usually undergo chronic heat stress (over 30°C for 10 h/day and for 20 consecutive days), leading to lower performance (Attia *et al.*, 2018). In this study, the resistance to chronic heat stress in broilers via the development of natural antioxidants with microencapsulation was examined. Microencapsulation has been known as a promising

process for encouraging the performance of additives in a wide range of industries. This technique can be used to improve thermostability and oxidative stability, to extend shelf life, to cover undesirable tastes and to reduce the irritation on users in addition to improve the release properties (Peanparkdee *et al.*, 2016). In this section, we focused on the biological properties of BO/CS-SA as to be applied practically in commercial poultry. The CS-SA microcapsules are beneficial for the delivery system of BO into animal body with a minimum damage.

In general, the small intestine is a target site for the evaluation of EOs properties. The changes in intestinal physiology are the main criteria for the consideration of gut morphological development and function.

The result indicated that broilers in NC had the shortest VH and the lowest VH:CD and VSA, whereas a positive impact represented in MBO group. This evidence indicates the vital role of MBO to improve the intestinal morphology. Consistent with our results, the inclusion of EOs (basil, caraway, laurel, lemon, oregano, sage, tea, and thyme) increased the length of villi and their absorptive surface areas of broilers, thus increasing BWG (Khattak *et al.*, 2014). MBO had slightly more positive effects on VH, VH:CD and VSA of jejunum than FBO. Previous results indicated that there was no effect in histomorphology and microbiota in broilers between free and encapsulated forms of garlic and *Phyllanthus niruri* L extract (Natsir *et al.*, 2013).

The improvement of jejunal histomorphology in this study could be elucidated by the action of methyl chavicol (phenylpropene) which is the dominant constituent of BO. It acts as free radical scavenger by donating its phenolic OH groups to reactive oxygen species (ROS) including superoxide anion radical ($O_2^{\bullet}-$), hydroxyl radical ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2) thereby potentially reducing oxidative stress, tissue damage, and lipid peroxidation (Santos *et al.*, 2018). BO loaded with nystatin represented a high ability to get rid of free radicals (Badea *et al.*, 2015). As it is known, the increase in villi height is associated with large absorptive area and nutrient uptake

efficiency, leading to BWG increase (Ghazanfari *et al.*, 2015). Likewise, the improvement of VH:CD involves the turnover rate of villi which reduced energy for maintaining the gut function and increased energy reserves (Peng *et al.*, 2016). In addition, the integrity of intestinal tissue has been reported to be directly linked with the improvement in brush border enzyme expression and nutrient transport systems (Oso *et al.*, 2019; Obajuluwa *et al.*, 2020).

The positive effect of MBO on VH possibly reflected the more concentration of BO in the jejunum. As a consequence, the VH: CD and VSA in MBO were also increased. In the same way, the supplementation of microencapsulated phytogenic at 100 mg/kg with pure curcumin at 50 mg/kg increased VH:CD ration in broilers (Galli *et al.*, 2020).

The exposure of broilers to high ambient temperature may trigger over ROS production, causing oxidative stress. As mentioned, animals lost balance of antioxidant defense system.

SOD is a key enzyme in the antioxidant defense system and MDA is a marker of lipid peroxidation (Mohammadi *et al.*, 2019). Our study demonstrated a strong positive impact of MBO on SOD activity and MDA content in duodenal mucosa, whereas an opposite effect was observed in birds fed the control diet. The current findings were comparable with the study of Hashemipour *et al.* (2013) who described that thymol and carvacrol exhibited greater activity of antioxidant enzyme in thigh muscle, serum, and liver of broilers. Another study demonstrated that star anise essential oil increased the SOD in the serum of broilers (Ding *et al.*, 2017). In the grower-finisher period, the difference of SOD activity among treatments was clearly observed. This might be due to an increase in metabolic rate and high environmental temperature. Excessive ROS may cause impaired defense mechanism such as inactivation of antioxidant enzyme. Besides, plant derivatives may enhance antioxidant enzymes via the regulation of gene expression (Zhao *et al.*, 2017). *In vitro* study, the FBO and MBO had strong antioxidant activity in inhibiting DPPH radicals. Also, the less amount of MDA was found under *in*

vivo studies. The positive effect on MDA content is related to the mechanism of methyl chavicol as previously described in intestinal parameter. It was reported that ginger extract decreased MDA in broilers exposed to heat stress (Wen *et al.*, 2020). Alleviation of intestinal inflammation by increasing antioxidant capacity has been reported previously (Broderick *et al.*, 2020). As compared with FBO group, the MBO with SA and CS decreased the MDA levels and increased SOD activity, especially in the last period of age. Microencapsulation could be functioned as a protective means in antioxidant activity of BO. Similarly, CS particles sustained the antioxidant activity of clove essential oil better than its free form (Hadidi *et al.*, 2020).

Antimicrobial activity of EOs is linked to their functional groups of bioactive constituents. EOs could destroy bacterial cell wall as the main target site, leading to damaging bacteria and eventually the bacterial death. Methyl chavicol and linalool showed strong antibacterial activity based on *in vitro* study (Moghaddam *et al.*, 2014) but no significant effect was found in this experiment, which is consistent with the results of Riyazi *et al.* (2015), El-Ashram and Abdelhafez (2020) and Hong *et al.* (2012). The study of Wang *et al.* (2019) suggested that a combination of encapsulated thymol (10%) and sorbic acid (20%) plus fumaric acid (20%) increased the population of *Bifidobacterium* in laying hens. Broilers in this experiment had no response on bacterial population possibly due to relatively low percentage of linalool, absence of eugenol and synergistic action between them as compared to previous report by Pandey *et al.* (2014). Koroch *et al.* (2017) found that sweet basil with linalool (54%) - eugenol (19%) chemotype showed the high antimicrobial activity. However, the mode of action of those constituents is still not fully understood and the studies of their effects in poultry production are limited. It is difficult to directly compare these results with those previous reports because of high variation of bioactive compounds and optimal dosage in each product, bacterial profile and physiological functions of the host animals (Turek and Stintzing, 2013). BO with high methyl chavicol *in vitro* study had great antioxidant

activity but weak antibacterial activity that was in line with Bozin *et al.* (2006). Due to the property of BO, the combination of BO and other antimicrobial additives such as organic acids may enhance highly potential growth performance in broilers. A blend of EO (4% thyme and 4% carvacrol) and mixed organic acid (0.5% hexanocia acid, 3.5% benzonic acid and 0.5% butyric acid) in Ca-alginate and whey protein modulated the gut microbiota community in necrotic enteritis-infected broiler chickens (Pham *et al.*, 2020).

The improvement in above-mentioned parameters associated with nutrient digestibility and growth performance. In comparison to FBO, BO/CS-SA excerted greater AID of CP and GE by 5.01% and 2.13% for 21 day and by 3.17% and 3.83% for day 42, respectively. Similarly, the addition of thyme and star anise encouraged apparent total tract digestibility (ATTD) of GE and nutrient in broilers orally challenged with *Clostridium perfringens* (Cho *et al.*, 2014). Carvacrol, thymol, and limonene at 100 ppm in encapsulated form improved AID of nutrients and growth performance in broilers (Hafeez *et al.*, 2016). This is possibly due to the stimulated secretion of bile, mucus, and endogenous enzymes (trypsin, chymotrypsin, lipase, and amylase) in pancreas and intestinal wall (Jamroz *et al.*, 2005; Obajuluwa *et al.*, 2020). The high CP digestibility in MBO group may enhance bioavailable amino acids and cell proliferation, resulting in increased intestinal integrity. The AID result was positively linked to growth response.

Under high ambient temperature, broilers had lower FI and BW than the standard performance of Ross 308. However, MBO was effective in enhancing ADG and FCR by 9.43% and 8.85%, respectively compared to NC in overall period. These findings are in agreement with the result of Mountzouris *et al.* (2011) and Li *et al.* (2015) who reported that the supplementation of a mixed essential oil resulted in an increase BWG with reduction in FCR. In case of encapsulated forms, turmeric extract nanoparticle had a significant influence on BWG. Hosseini and Meimandipour (2018) also showed that birds receiving thyme essential oil in chitosan nanoparticles improved

BWG and FCR compared with those in the control. On the contrary, it had no significant influence on FI and FCR in broilers (Sundari *et al.*, 2014). Cumin essential oil in chitosan particles also improved the growth performance in broilers (Amiri *et al.*, 2020). Nevertheless, the different outputs of growth performance may be due to variable compositions and dosages of active ingredients, basal diets, animal condition, and experimental condition (Peng *et al.*, 2016).

The bioavailability of FBO and MBO after passing through feed processing and the digestive system was described as follows. The FBO partially lost in feed processing due to evaporation and degradation during hydrothermal process whereas the unique characteristic of CS-SA matrix improved the stability of MBO. In another study, Locali-Pereira *et al.* (2020) investigated that double-layer microcapsules preserved the composition of pink pepper essential oil. The FBO entering the proventriculus may stimulate the secretion of hydrochloric acid (HCl) and pepsin, leading to increased digestibility. However, the movement of FBO into the intestine was relatively limited because of its poor solubility in water.

Morphological changes of MBO were found in proventriculus as follows. The outer layer of MBO (CS) was swelled because of the gastric pH environment. It might cause the protonation of amine groups of CS, resulting in the chain expansion and absorption of a large amount of water. As a result, CS was gradually solubilized. The inner layer of MBO (SA) maintained in acidic pH and assisted BO to withstand endogenous enzymes. The SA facilitated the delivery of BO as a result of the hydrophilic properties. The remaining FBO was formed as micelles by bile salts thereby enhancing their properties at the end of duodenum and jejunum. Similar to FBO, the SA of MBO was initially dissolved at pH > 5 due to the protonation of the ionic carboxylate groups, leading to the diffusion of BO into lower part of intestine. Consequently, MBO may maintain the most possible amount of bioactive compound,

thus facilitating intestinal function. According to Omonijo *et al.* (2018a), alginate microcapsules of thymol showed a delayed release in stomach and subsequently functioned in intestine. The release mechanism, as previously described, was dependent on type of polymer and its characteristic, cross-linking degree, environmental conditions, and incubation time (Kleemann *et al.*, 2020). Nevertheless, there are both advantages and disadvantages of two forms at different parts of GI tract. As a result, MBO and FBO did not have any difference in growth performance. Another possibility is that the optimal efficiency of BO on overall responses may be less than 500 ppm. Future work should consider the dose reduction of MBO to prove the proposed objectives and the maximum economic benefit. Additionally, methyl chavicol and linalool are a relatively new group of feed additives and their applications with microencapsulation should be still investigated. The potential of BO in this study on growth performance was found to be equally effective in dietary avilamycin.

CONCLUSION AND SUGGESTION

The present work demonstrated the application of the complementary polysaccharides to fabricate doubled-layer microcapsule to encapsulate the active compound in BO and maintain the activity through the acid and base conditions so that the release at the targeted organ is possible.

1. BO/CS-SA was successfully prepared via double-layered microencapsulation as proved by FTIR.
2. CS-SA microcapsule increased the thermal stability of BO as proved by TGA
3. The release studies under simulated poultry digestive tract confirmed that CS-SA microcapsule displayed the controlled release of BO in the intestinal stage.
4. The double layered CS-SA also played a major role in maintaining the antimicrobial and antioxidant properties under the severe environment conditions such as acid, bile, trypsin, and heat so that BO remained active.
5. The double layered CS-SA maintained the amount of BO in the microcapsule even after treated at high temperature (75°C - 90°C).
6. Dietary experiment with MBO improved ADG and FCR in broilers
7. The broilers fed diet with MBO exhibited a greater the AID of CP and GE compared with those in other groups.
8. Microbial populations were not affected by feeding dietary treatments.
9. Either FBO or MBO groups had positive effects on broilers in terms of VH, VH:CD ratio and VSA of broilers in comparison to NC and PC ($P < 0.05$).
10. SOD activity in the duodenal mucosa was significantly increased in response to dietary supplementation with MBO diet, whereas MDA level was significantly decreased.

11. The MBO is better than FBO, especially in promoting VH: CD and VSA, encouraging CP and GE digestibility as well as modulating antioxidant capacity.
12. Both MBO and FBO groups exhibited the beneficial effect on overall performance as same as avilamycin supplementation thereby considering as alternative to AGPs.
13. The microencapsulation could be considered a promising driver of BO efficacy, consequently MBO could be potentially used as a feed additive for improvement of intestinal integrity and nutrient utilization, leading to a better performance in broiler chickens.

Appendix A

The preparation of standard curve, media, buffers and other solutions

1 Standard curve

1.1. Basil oil (BO) standard

The BO (100%) was diluted ranging from 50% to 0.0 % with dichloromethane. Then, the mixture was be shaken vigorously and kept at room temperature in the dark room. The UV-vis spectrophotometer was used for monitoring the change in reaction solutions at 289 nm. All samples were analyzed in triplicate. Absorbance was plotted against various concentrations of standards (Figure 15).

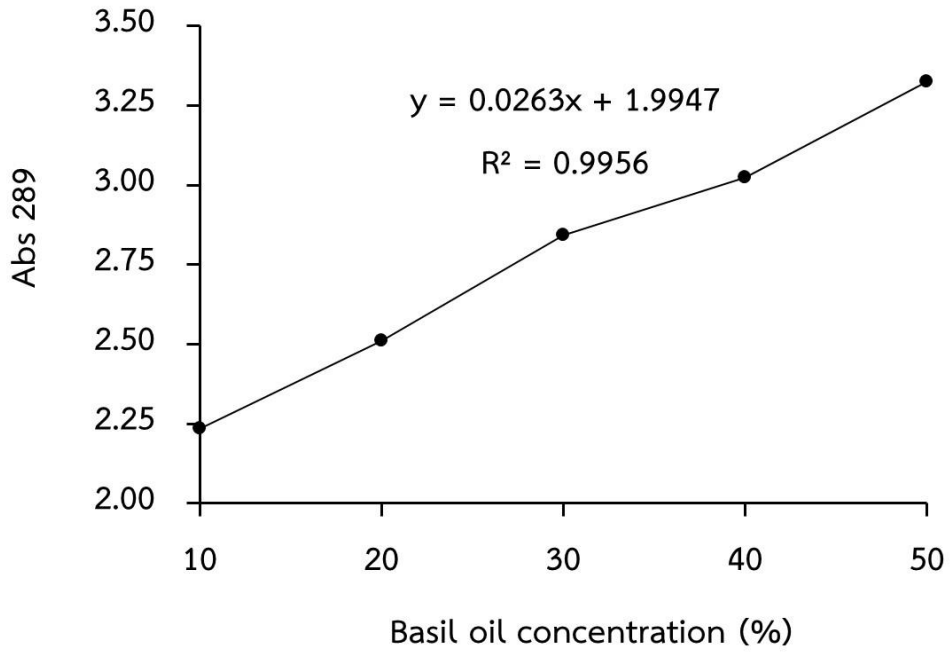

Figure 15 Standard curve of basil oil (BO)

1.2. Trolox standard

Trolox [6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid], a water-soluble vitamin E analog, serves as a positive control. A standard curve with 8 points (ranging from 25 to 200 µmole was prepared using a solution of Trolox 1500 µM, 400 µL of each point was withdrawn and mixed with 2 mL of DPPH solution. Then, the mixture was be shaken vigorously and kept at room temperature for 30 min in the dark room. The results were read at 517 nm wavelength using the UV-vis spectrophotometer (Figure 16).

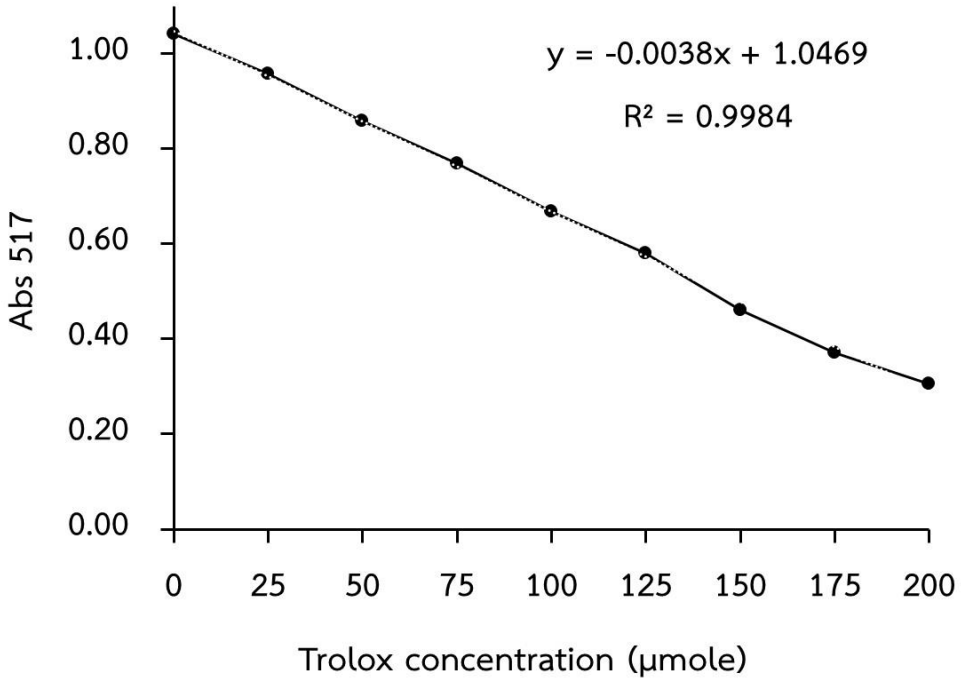

Figure 16 Standard curve of Trolox

1.3. Bovine serum albumin (BSA) standard

BSA (0.1 g) was dissolved in distilled water (10 mL) at room temperature. The stock BSA solution was diluted ranging from 250-2,000 µg/mL. Each concentration (60 µL) was mixed with 940 µL of Bradford reagent. Then, the mixture was be shaken vigorously and kept at room temperature. All samples were analyzed in triplicate. The samples were allowed to incubated at room temperature for 10 min. Absorbance was plotted against various concentrations of standards (Figure 17).

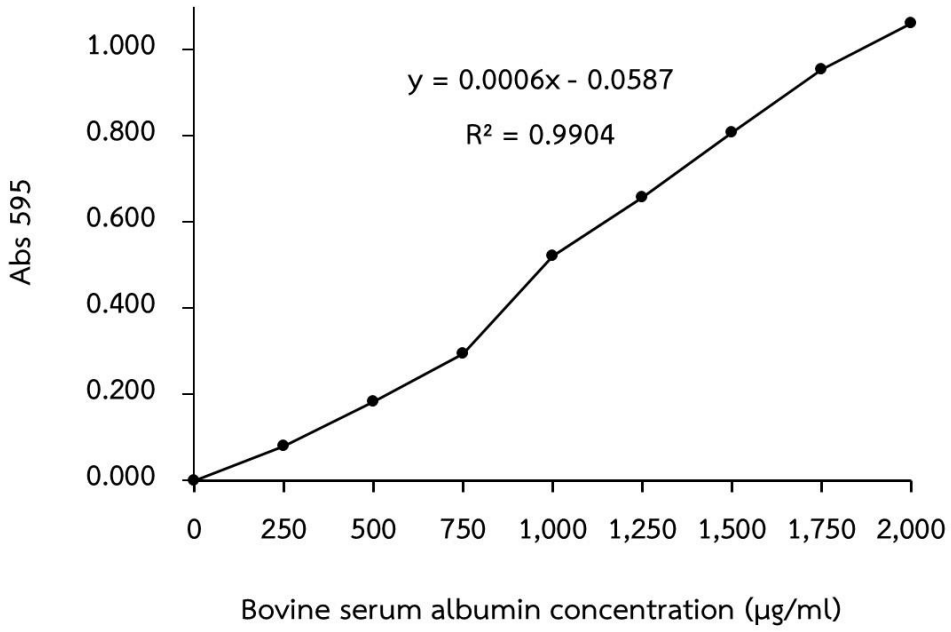

Figure 17 Standard curve of Bovine serum albumin

2. Media

2.1. Nutrient broth (NB)

This medium was used for growth and maintenance of bacteria.

It contains:	Bacto peptone	3.0 g
	Yeast extract	5.0 g
	Distilled water	1,000.0 mL

The media were autoclaved at 121°C for 15 min.

2.2. Nutrient agar (NA)

This medium was prepared by adding 15 g of agar to NB.

2.3. Mueller-Hinton agar (MHA)

This medium was used for growth and maintenance of bacteria.

It contains:	Beef extract	2.0 g
	Casein acid	17.5 g
	Starch	1.5 g
	Agar	17.0 g

The MHA was prepared by adding 38 g of MHA in 1,000 mL of distilled water. The mixture was heated and stirred to completely dissolve the medium. Then, the medium was autoclaved at 121°C for 15 min.

2.4. De Man, Rogosa, sharpe agar (MRS agar)

This medium was used for growth and maintenance of bacteria.

It contains:	Proteose peptone	10.0 g
	Beef extract	10.0 g
	Yeast extract	5.0 g
	Dextrose (Glucose)	20.0 g
	Tween 80	1.0 g
	Ammonium citrate	2.0 g
	Sodium acetate	5.0 g
	Magnesium sulphate	0.10 g
	Manganese sulphate	0.05 g
	Dipotassium hydrogen phosphate	2.0 g
	Agar	12.0 g

The MRS was prepared by adding 66.73 g of MRS in 1,000 mL of distilled water. The mixture was heated and stirred to completely dissolve the medium. Then, the medium was autoclaved at 121°C for 15 min.

3. Buffer solution

3.1. Preparation of 0.05 M phosphate buffer at pH 7.4.

Sodium di-hydrogen orthophosphate (NaH_2PO_4 $7H_2O$, 10.107 g) and di-sodium hydrogen phosphate (Na_2HPO_4 H_2O, 1.697 g) were dissolved in distilled water (800 mL). The mixture was stirred constantly with a magnetic stirrer and adjusted to the require pH with 1.0 N HCl or 1.0 N NaOH. The mixture was made up to 1 liter in volumetric flas

3.2. Preparation of 0.05 M carbonate bicarbonate buffer at pH 10.1

Sodium bicarbonate ($NaHCO_3$, 1.703 g) and sodium carbonate (Na_2CO_3, 3.151g) were dissolved in distilled water (800 mL). The mixture was stirred constantly with a magnetic stirrer and adjusted to the require pH with 1.0 N HCl or 1.0 N NaOH. The mixture was made up to 1 liter in volumetric flask.

3.3. Preparation of 1.0 mmol/l luminol

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, 0.035 g) was dissolved in the 0.25 mmol/l carbonate buffer (200 mL). The mixture was stirred constantly with a magnetic stirrer. The mixture was made up to 1 liter in volumetric flask.

3.4. Preparation of 50 mmol/l carbonate buffer

Sodium bicarbonate ($NaHCO_3$, 2.9 g) and sodium carbonate (Na_2CO_3, 1.6 g) were dissolved of in deionized water (800 mL). The mixture was stirred constantly with a magnetic stirrer and adjusted to the require pH with 1.0 N HCl or 1.0 N NaOH. The mixture was made up to 1 liter in volumetric flask. Final buffer contains 15 mM $Na2CO_3$ and 35 mM $NaHCO_3$.

3.5. Preparation of 0.1 mmol/l Ethylenediaminetetraacetic acid (EDTA)

EDTA (292.24 mg) was dissolved in distilled water (5 mL). The solution was stirred with a magnetic stirrer and adjusted to the require pH 8.0 with NaOH. The resulting solution should be clear and colorless. Finally, the solution was made up volume to 10 mL to get 0.1M EDTA. The solution is stable for 1 year when stored at 4°C.

3.6. Preparation of 0.375% w/v thiobarbituric acid (TBA)

TBA (0.375 g) was mixed 0.25N hydrochloric acid (100 mL) in a volumetric flask. The solution was stirred with a magnetic stirrer.

3.7. Preparation of 15% w/v trichloroacetic acid (TCA)

TCA (165.198 g) was added in distilled water (800 mL) in a suitable container. The solution was stirred with a magnetic stirrer. Finally, the solution was added distilled water until the volume is 1 L. The resulting solution is 15% w/v TCA.

3.8. Preparation of 0.25 N hydrochloric acid (HCl).

HCl (37.5%, 20.44 mL) was added in deionized water (800 mL) in a volumetric flask (1,000 mL) using a pipette. The temperature of the solution was rise rapidly. The container was put in an ice bucket while stirring continuously. Then, the solution was made up to 1 liter in volumetric flask.

4 Proximate analysis

4.1 Dry matter (DM)

Dry matter is residue remaining after drying. Moisture is evaporated from the sample by drying.

A dried crucible was weighed (W1). The 2 g of sample were added and weighed (WS) in a dried crucible. Then, the sample was dried at 105°C for 24 h. in a hot air oven. After drying, the crucible was placed in a desiccator to cool. The cool sample was weighed (W2). Dry matter (%, w/w) was calculated as follows:

$$\%DM = (W2-W1)/WS \times 100$$

4.2 Crude protein (CP)

Reagents:

Sulfuric acid (H_2SO_4), selenium reagent mixture, sodium hydroxide (NaCl), 4% boric acid (H_3BO_3), and 0.1 N hydrochloric acid (HCl) standard solution.

Procedure:

In digestion step, the sample (2 g) and selenium reagent mixture as catalyst (0.25 g) were added into a Kjeldahl tube. H_2SO_4 (20 mL) was added and rinse sample into tube. The sample was digested at 420 °C for 2 h. The digested sample should be clear and free of undigested material. The sample was cooled down to room temperature.

In distillation step, the previous digestion tube was placed to distillation unit. H_3BO_3 (4% w/v, 25 mL) with indicator was added to Erlenmeyer flask (250 mL). At the end of distillation, the distilled solution should be light green solution.

In titration step, distilled solution was titrated with standard 0.1 N HCl solution. The color of distilled solution was change from light green to pink.

In calculation step, Total nitrogen (%) was calculated as follows

Total nitrogen, % = 1.40 × (ml HCl, sample -ml HCl, blank) ×normality HCl / g sample

%CP = Total nitrogen % × 6.25

4.3 Crude fiber (CF)

Reagents:3.125% H_2SO_4 and 3.125% NaOH

Procedure:

The sample (1 g, WS) was added in a filter crucible. It was put in the crude fiber apparatus and added H_2SO_4 (3.125%, 60 mL) to boil for 20 min. The sample was rinsed with deionized water 2 times (100 mL pre times), then adding NaOH (3.125%, 60 mL) and boiling for 20 min. The sample was rinsed with deionized water 2 times (100 mL pre times). The filter crucible was transfer and dried in a hot air oven for at 105°C for 6 h. The sample was cooled down in a desiccator and weighed the sample after cooling (W1). Next, the sample was ignited in a muffle furnace at 550°C for 5 h. The cool sample was reweighed after cooling (W2). Crude fiber content was calculated as follows:

% Crude fiber = W1-W2/WS × 100

4.4 Ether extract (EE)

Reagents: petroleum ether

Procedure:

The alumina cup was dried at 105°C for 6 h and then weighted (W1). The sample (1 g, WS) was added in alumina cup and placed in crude fat analyzer. The sample with petroleum ether (60 mL) was extracted in analyzer for 2 h. The ending of extraction, alumina cup with residue sample was removed and dried at 105°C for 5 h in an air oven and then weighed (W2). Ether extract content was calculated as follows.

%EE =W2-W1/WS× 100

4.5 Ash

Procedure

The pre-ignited crucible was weighed (W1). One gram of sample was weighed (WS) and added to the dried crucible. The sample was ignited in a muffle furnace at 550°C for 5 h. Then, the ignited crucible was cooled down in a desiccator and then weighed after reaching room temperature (WS). Ash content was calculated as follows:

% Ash =W2-W1/WS×100

4.6 Nitrogen free extract (NFE)

NFE was calculated using the following equation:

% NFE = 100 % – (% EE + % CP + % Ash + % CF).

APPENDIX B

Statistical analysis

ANOVA

Table 13 BO content in CS-SA microcapsule after heating at 75℃, 80℃, 85℃, 90℃, 95℃ and 100 ℃ for 3 min

Parameter	Source	SS	df	MS	F	Sig.
BO content	Treatment	179.75	6	29.96	222.22	<.001
	Error	4.72	35	0.13		
	Total	184.47	41			

SS; Sum of Squares, MS; Mean Square

Table 14 Antioxidation ability of BO/CS-SA under acid tolerance, bile tolerance, trypsin tolerance, and thermal treatment as evaluated by DPPH radical scavenging

Parameter	Source	SS	df	MS	F	Sig.
DPPH radical scavenging activity	Treatment	52.60	4	13.15	3.2	0.06
	Error	41.09	10	4.11		
	Total	93.70	14			

SS; Sum of Squares, MS; Mean Square

Table 15 Antimicrobial ability of BO/CS-SA under acid tolerance, bile tolerance, trypsin tolerance, and thermal treatment as evaluated by inhibition zone

Parameter	Source	SS	df	MS	F	Sig.
E. coli	Treatment	9.59	4	2.40	2.84	0.08
	Error	8.43	10	0.84		
	Total	18.02	14			
S. aureus	Treatment	5.79	4	1.45	3.93	0.04
	Error	3.68	10	0.37		
	Total	9.47	14			
Salmonella spp.	Treatment	1.184	4	0.3	0.34	0.85
	Error	8.76	10	0.88		
	Total	9.94	14			

SS; Sum of Squares, MS; Mean Square

Table 16 Antioxidant stability of BO under storage

Parameter	Source	SS	df	MS	F	Sig.
BO	Treatment	399.85	3	133.28	56.02	<.0001
	Error	19.03	8	2.38		
	Total	418.88	11			

SS; Sum of Squares, MS; Mean Square

Table 17 Antioxidant stability of BO/CS-SA under storage

Parameter	Source	SS	df	MS	F	Sig.
BO/CS-SA	Treatment	7.46	3	2.49	1.00	0.44
	Error	19.79	8	2.47		
	Total	27.25	11			

SS; Sum of Squares, MS; Mean Square

Table 18 Antimicrobial stability of BO under storage

Parameter	Source	SS	df	MS	F	Sig.
E. coil	Treatment	36.000	3	12.000	36.000	<.0001
	Error	2.667	8	0.333		
	Total	38.667	11			
S. aureus	Treatment	156.396	3	52.132	75.640	<.0001
	Error	5.513	8	0.689		
	Total	161.909	11			
Salmonella spp.	Treatment	22.000	3	7.333	22.000	<.0003
	Error	2.667	8	0.333		
	Total	24.667	11			

SS; Sum of Squares, MS; Mean Square

Table 19 Antimicrobial stability of BO under storage

Parameter	Source	SS	df	MS	F	Sig.
E. coil	Treatment	10.107	3	3.369	3.810	0.058
	Error	7.073	8	0.884		
	Total	17.180	11			
S. aureus	Treatment	4.500	3	1.500	5.540	0.024
	Error	2.167	8	0.271		
	Total	6.667	11			
Salmonella spp.	Treatment	3.843	3	1.281	3.530	0.068
	Error	2.900	8	0.363		
	Total	6.743	11			

SS; Sum of Squares, MS; Mean Square

Table 20 Growth performance of broiler chickens on day 21

Parameter	Source	SS	df	MS	F	Sig.
Initial weight	Treatment	1.698	3	0.566	0.460	0.711
	Error	24.393	20	1.220		
	Total	26.090	23			
Final body weight	Treatment	13847.729	3	4615.910	2.840	0.064
	Error	32562.006	20	1628.100		
	Total	46409.735	23			
Average daily feed intake	Treatment	9.318	3	3.106	0.420	0.738
	Error	146.395	20	7.320		
	Total	155.713	23			
Average daily gain	Treatment	35.131	3	11.710	2.920	0.059
	Error	80.181	20	4.009		
	Total	115.312	23			
Feed conversion ratio	Treatment	0.035	3	0.012	10.200	0.000
	Error	0.023	20	0.001		
	Total	0.058	23			

SS; Sum of Squares, MS; Mean Square

Table 21 Growth performance of broiler chickens on day 42

Parameter	Source	SS	df	MS	F	Sig.
Initial weight	Treatment	13847.729	3	4615.910	2.840	0.064
	Error	32562.006	20	1628.100		
	Total	46409.735	23			
Final body weight	Treatment	110802.641	3	36934.214	9.970	0.000
	Error	74076.956	20	3703.848		
	Total	184879.596	23			
Average daily feed intake	Treatment	36.466	3	12.155	0.390	0.762
	Error	624.814	20	31.241		
	Total	661.280	23			
Average daily gain	Treatment	116.918	3	38.973	2.110	0.131
	Error	369.216	20	18.461		
	Total	486.134	23			
Feed conversion ratio	Treatment	0.289	3	0.096	2.940	0.058
	Error	0.656	20	0.033		
	Total	0.946	23			

SS; Sum of Squares, MS; Mean Square

Table 22 Growth performance of broiler chickens on d 1-42

Parameter	Source	SS	df	MS	F	Sig.
Initial weight	Treatment	1.698	3	0.566	0.460	0.711
	Error	24.393	20	1.220		
	Total	26.090	23			
Final body weight	Treatment	110802.641	3	36934.214	9.970	0.000
	Error	74076.956	20	3703.848		
	Total	184879.596	23			
Average daily feed intake	Treatment	4.128	3	1.376	0.120	0.945
	Error	221.474	20	11.074		
	Total	225.602	23			
Average daily gain	Treatment	69.570	3	23.190	9.970	0.000
	Error	46.535	20	2.327		
	Total	116.104	23			
Feed conversion ratio	Treatment	0.149	3	0.050	5.200	0.008
	Error	0.191	20	0.010		
	Total	0.340	23			

SS; Sum of Squares, MS; Mean Square

Table 23 Apparent ileal digestibility (AID) of broiler chickens on d 21

Parameter	Source	SS	df	MS	F	Sig.
Dry matter	Treatment	83.461	3	27.820	11.460	0.003
	Error	19.418	8	2.427		
	Total	102.878	11			
Crude protein	Treatment	82.662	3	27.554	12.490	0.002
	Error	17.644	8	2.205		
	Total	100.306	11			
Ether extract	Treatment	46.970	3	15.657	4.900	0.032
	Error	25.544	8	3.193		
	Total	72.514	11			
Crude fiber	Treatment	84.931	3	28.310	2.160	0.171
	Error	105.049	8	13.131		
	Total	189.980	11			
Ash	Treatment	13.749	3	4.583	0.520	0.677
	Error	96.861	8	8.806		
	Total	110.610	11			
Gross energy	Treatment	47.779	3	15.926	15.950	0.001
	Error	7.989	8	0.999		
	Total	55.769	11			
Nitrogen free extract	Treatment	138.648	3	46.216	9.110	0.006
	Error	40.586	8	5.073		
	Total	179.233	11			

SS; Sum of Squares, MS; Mean Square

Table 24 Apparent ileal digestibility (AID) of broiler chickens on d 42

Parameter	Source	SS	df	MS	F	Sig.
Dry matter	Treatment	37.953	3	12.651	3.880	0.055
	Error	26.057	8	3.257		
	Total	64.009	11			
Crude protein	Treatment	97.230	3	32.410	5.210	0.028
	Error	49.741	8	6.218		
	Total	146.971	11			
Ether extract	Treatment	56.793	3	18.931	12.040	0.003
	Error	12.575	8	1.572		
	Total	69.369	11			
Crude fiber	Treatment	95.061	3	31.687	13.330	0.002
	Error	19.023	8	2.378		
	Total	114.084	11			
Ash	Treatment	67.808	3	22.603	2.820	0.107
	Error	64.039	8	8.005		
	Total	131.847	11			
Gross energy	Treatment	49.622	3	16.541	14.130	0.002
	Error	9.368	8	1.171		
	Total	58.990	11			
Nitrogen free extract	Treatment	27.394	3	9.131	1.400	0.313
	Error	52.269	8	6.534		
	Total	79.663	11			

SS; Sum of Squares, MS; Mean Square

Table 25 Jejunal morphology of broiler chickens on d 21

Parameter	Source	SS	df	MS	F	Sig.
Villus height	Treatment	43636.306	3	14545.436	3.440	0.0364
	Error	84545.705	20	4227.285		
	Total	128182.011	23			
Crypt depth	Treatment	147.156	3	49.052	0.880	0.469
	Error	1117.010	20	55.850		
	Total	1264.166	23			
Villus width	Treatment	27.454	3	9.151	1.160	0.3495
	Error	157.720	20	7.886		
	Total	185.174	23			
Villus height to crypt depth ratio	Treatment	9.422	3	3.141	4.710	0.0121
	Error	13.347	20	0.667		
	Total	22.769	23			
Villus surface area	Treatment	0.041	3	0.014	11.340	0.000
	Error	0.024	20	0.001		
	Total	0.065	23			

SS; Sum of Squares, MS; Mean Square

Table 26 Jejunal morphology of broiler chickens on d 42

Parameter	Source	SS	df	MS	F	Sig.
Villus height	Treatment	191662.881	3	63887.627	12.730	<.0001
	Error	100398.456	20	5019.923		
	Total	292061.337	23			
Crypt depth	Treatment	354.440	3	118.147	1.710	0.197
	Error	1381.834	20	69.092		
	Total	1736.274	23			
Villus width	Treatment	32.330	3	10.777	1.730	0.194
	Error	124.919	20	6.246		
	Total	157.249	23			
Villus height to crypt depth ratio	Treatment	3.621	3	1.207	4.020	0.022
	Error	6.011	20	0.301		
	Total	9.632	23			
Villus surface area	Treatment	0.119	3	0.040	47.230	<.0001
	Error	0.017	20	0.001		
	Total	0.136	23			

SS; Sum of Squares, MS; Mean Square

Table 27 Bacterial populations of broiler chickens on d 21

Parameter	Source	SS	df	MS	F	Sig.
E. coli	Treatment	13.093	3	4.364	1.430	0.264
	Error	61.067	20	3.053		
	Total	74.160	23			
Lactobacillus spp.	Treatment	27.906	3	9.302	0.100	0.960
	Error	1899.273	20	94.964		
	Total	1927.179	23			

SS; Sum of Squares, MS; Mean Square

Table 28 Bacterial populations of broiler chickens on d 42

Parameter	Source	SS	df	MS	F	Sig.
E. coli	Treatment	37.462	3	12.487	0.870	0.471
	Error	286.005	20	14.300		
	Total	323.467	23			
Lactobacillus spp.	Treatment	22.895	3	7.632	1.000	0.415
	Error	153.364	20	7.668		
	Total	176.259	23			

SS; Sum of Squares, MS; Mean Square

Table 29 Antioxidant capacity of broiler chickens on d 21

Parameter	Source	SS	df	MS	F	Sig.
Malondialdehyde	Treatment	6.021	3	2.007	47.600	<.0001
	Error	0.843	20	0.042		
	Total	6.864	23			
Superoxide dismutase	Treatment	1295.722	3	431.907	8.940	0.001
	Error	966.416	20	48.321		
	Total	2262.138	23			

SS; Sum of Squares, MS; Mean Square

Table 30 Antioxidant capacity of broiler chickens on d 42

Parameter	Source	SS	df	MS	F	Sig.
Malondialdehyde	Treatment	29.099	3	9.700	141.920	<.0001
	Error	1.367	20	0.068		
	Total	30.466	23			
Superoxide dismutase	Treatment	2618.574	3	872.858	33.470	<.0001
	Error	521.601	20	26.080		
	Total	3140.176	23			

SS; Sum of Squares, MS; Mean Square

REFERENCES

Abd El-Hack ME, El-Saadony MT, Saad AM, Salem HM, Ashry NM, Abo Ghanima MM, Shukry M, Swelum AA, Taha AE, El-Tahan AM, AbuQamar SF and El-Tarabily KA. 2022. Essential oils and their nanoemulsions as green alternatives to antibiotics in poultry nutrition: a comprehensive review. Poult. Sci. 101 (2): 101584.

Abers M, Schroeder S, Goelz L, Sulser A, St Rose T, Puchalski K and Langland J. 2021. Antimicrobial activity of the volatile substances from essential oils. BMC Complement Med. Ther. 21 (1): 124.

Abolfathi ME, Tabeidian SA, Foroozandeh Shahraki AD, Tabatabaei SN and Habibian M. 2019. Effects of ethanol extract of Elecampane (*Inula helenium* L.) rhizome on growth performance, diet digestibility, gut health, and antioxidant status in broiler chickens. Livest. Sci. 223: 68-75.

Ahmed AF, Attiac FAK, Liua Z, Lia C, Weia J and Kanga W. 2019. Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum* L.) plants. Food Sci. Hum. Wellness. 8: 299-305.

Al-Kasmi B, Alsirawan MB, Bashimam M and El-Zein H. 2017. Mechanical microencapsulation: The best technique in taste masking for the manufacturing scale-Effect of polymer encapsulation on drug targeting. J. Essent. Oil Bear. Pl. 260: 134-141.

Ali A, Chong CH, Mah SH, Abdullah LC, Choong TSY and Chua BL. 2018. Impact of storage conditions on the stability of predominant phenolic constituents and antioxidant activity of dried Piper betle extracts. Molecules. 23 (2): 484.

Amiri N, Afsharmanesh M, Salarmoini M, Meimandipour A, Hosseini S and Ebrahimnejad H. 2020. Effects of nanoencapsulated cumin essential oil as an alternative to the antibiotic growth promoter in broiler diets. J. Appl. Poult. Res. 29 (4): 875-885.

Amiri N, Afsharmanesh M, Salarmoini M, Meimandipour A, Hosseini SA and Ebrahimnejad H. 2021. Nanoencapsulation (*in vitro* and *in vivo*) as an efficient technology to boost the potential of garlic essential oil as alternatives for antibiotics in broiler nutrition. Animal. 15 (1): 100022.

Angkanaporn K, Ravindran V and Bryden WL. 1996. Additivity of apparent and true ileal amino acid digestibilities in soybean meal, sunflower meal, and meat and bone meal for broilers. Poult. Sci. 75 (9): 1098-1103.

Arfat YA, Benjakul S, Prodpran T, Sumpavapol P and Songtipya P. 2014. Properties and antimicrobial activity of fish protein isolate/fish skin gelatin film containing basil leaf essential oil and zinc oxide nanoparticles. Food Hydrocolloids. 41: 265-273.

Attia YA, Al-Harthi MA and Sh. Elnaggar A. 2018. Productive, physiological and immunological responses of two broiler strains fed different dietary regimens and exposed to heat stress. Ital. J. Anim. Sci. 17 (3): 686-697.

Attia YA, Bakhashwain AA and Bertu NK. 2017. Thyme oil (*Thyme vulgaris* L.) as a natural growth promoter for broiler chickens reared under hot climate. Ital J Anim Sci. 16 (2): 275-282.

Aviagen. 2009. Ross broiler management manual. Scotland, UK: www. aviagen. pp. 1-114.

Azad AK, Al-Mahmood SMA, Chatterjee B, Wan Sulaiman WMA, Elsayed TM and Doolaanea AA. 2020. Encapsulation of black seed oil in alginate beads as a pH-sensitive carrier for intestine-targeted drug delivery: *in vitro*, *in vivo* and ex *vivo* study. Pharmaceutics. 12 (3): 219.

Badea G, Bors AG, Lacatusu I, Oprea O, Ungureanu C, Stan R and Meghea A. 2015. Influence of basil oil extract on the antioxidant and antifungal activities of nanostructured carriers loaded with nystatin. Cr. Chim. 18 (6): 668-677.

Bannikova A, Evteev A, Pankin K, Evdokimov I and Kasapis S. 2018. Microencapsulation of fish oil with alginate: *In-vitro* evaluation and controlled release. LWT-Food Sci. Technol. 90: 310-315.

Baurhoo N, Baurhoo B, Mustafa AF and Zhao X. 2011. Comparison of corn-based and Canadian pearl millet-based diets on performance, digestibility, villus morphology, and digestive microbial populations in broiler chickens. Poult. Sci. 90 (3): 579-586.

Beier RC, Byrd JA, 2nd, Kubena LF, Hume ME, McReynolds JL, Anderson RC and Nisbet DJ. 2014. Evaluation of linalool, a natural antimicrobial and insecticidal essential oil from basil: effects on poultry. Poult. Sci. 93 (2): 267-272.

Betancourt L, Hume M, Rodriguez F, Nisbet D, Sohail MU and Afanador-Tellez G. 2019. Effects of Colombian oregano essential oil (*Lippia origanoides* Kunth) and Eimeria species on broiler production and cecal microbiota. Poult. Sci. 98 (10): 4777-4786.

Bozin B, Mimica-Dukic N, Simin N and Anackov G. 2006. Characterization of the volatile composition of essential oils of some *Lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils. J. Agric. Food Chem. 54 (5): 1822-1828.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Brand-Williams W, Cuvelier M-E and Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28 (1): 25-30.

Broderick T, Gutierrez O, Lee J and Duong T. 2020. Evaluation of functional feed additive administration in broiler chickens to 21 days. J. Appl. Poult. Res.: 1-32.

Bruneau M, Bennici S, Brendle J, Dutournie P, Limousy L and Pluchon S. 2019. Systems for stimuli-controlled release: Materials and applications. J. Control. Release. 294: 355-371.

Bunrathep S, Palanuvej C and Ruangrungsi N. 2007. Chemical compositions and antioxidative activities of essential oils from four *Ocimum* species endemic to Thailand. J. Health Res. 21 (3): 201-206.

Campelo-Felix PH, Souza HJ, Figueiredo JA, Fernandes RV, Botrel DA, de Oliveira CR, Yoshida MI and Borges SV. 2017. Prebiotic carbohydrates: Effect on reconstitution, storage, release, and antioxidant properties of Lime essential oil microparticles. J. Agric. Food Chem. 65 (2): 445-453.

Celebioglu A and Uyar T. 2019. Encapsulation and stabilization of alpha-lipoic acid in cyclodextrin inclusion complex electrospun nanofibers: antioxidant and fast-dissolving alpha-lipoic acid/cyclodextrin nanofibrous webs. J. Agric. Food Chem. 67 (47): 13093-13107.

Celi P, Verlhac V, Pérez Calvo E, Schmeisser J and Kluenter A-M. 2019. Biomarkers of gastrointestinal functionality in animal nutrition and health. Anim. Feed Sci. Technol. 250: 9-31.

Chandrapati S and Williams MG. 2014. Total viable conunts | Most Probable Number (MPN). In: Encyclopedia of Food Microbiology. ed. 3M Company, St. Paul, MN, USA: 621-624.

Chitprasert P and Ngamekaue N. 2017. Stability enhancement of *Ocimum sanctum* Linn. essential oils using stearic acid in aluminum carboxymethyl cellulose film-coated gelatin microcapsules. J. Food Sci. 82 (6): 1310-1318.

Chitprasert P and Sutaphanit P. 2014. Holy basil (*Ocimum sanctum* Linn.) essential oil delivery to swine gastrointestinal tract using gelatin microcapsules coated with aluminum carboxymethyl cellulose and beeswax. J. Agric. Food Chem. 62 (52): 12641-12648.

Cho JH, Kim HJ and Kim IH. 2014. Effects of phytogenic feed additive on growth performance, digestibility, blood metabolites, intestinal microbiota, meat color and relative organ weight after oral challenge with *Clostridium perfringens* in broilers. Livest. Sci. 160: 82-88.

Citarasu T. 2009. Herbal biomedicines: a new opportunity for aquaculture industry. Aquacult. Int. 18 (3): 403-414.

Cuadros TR, Erices AA and Aguilera JM. 2015. Porous matrix of calcium alginate/gelatin with enhanced properties as scaffold for cell culture. J. Mech. Behav. Biomed. Mater. 46: 331-342.

Delshadi R, Bahrami A, Tafti AG, Barba FJ and Williams LL. 2020. Micro and nano-encapsulation of vegetable and essential oils to develop functional food products with improved nutritional profiles. Trends Food Sci. Tech. 104: 72-83.

Ding X, Yang CW and Yang ZB. 2017. Effects of star anise (*Illicium verum* Hook.f.), essential oil, and leavings on growth performance, serum, and liver antioxidant status of broiler chickens. J. Appl. Poult. Res. 26 (4): 459-466.

Durante M, Lenucci MS, Laddomada B, Mita G and Caretto S. 2012. Effects of Sodium Alginate Bead Encapsulation on the Storage Stability of Durum Wheat (*Triticum Durum* Desf.) Bran Oil Extracted by Supercritical CO_2. J. Agr. Food Chem. 60 (42): 10689-10695.

Dusankova M, Pummerova M and Sedlarik V. 2019. Microspheres of essential oil in polylactic acid and poly (methyl methacrylate) matrices and their blends. J. Microencapsul. 36 (3): 305-316.

El-Ashram S and Abdelhafez GA. 2020. Effects of phytogenic supplementation on productive performance of broiler chickens. J. Appl. Poult. Res. 29 (4): 852-862.

El-Dakar AY, Shalaby SM, Nemetallah BR, Saleh NE, Sakr EM and Toutou MM. 2015. Possibility of using basil (*Ocimum basilicum*) supplementation in Gilthead sea bream (*Sparus aurata*) diet. Egypt. J. Aquat. Res. 41 (2): 203-210.

Eriksson UJ and Borg LAH. 1991. Protection by free oxygen radical scavenging enzymes against glucose-induced embryonic malformations in vitro*. Diabetologia. 34: 325-331.

Faidi A, Lassoued MA, Becheikh MEH, Touati M, Stumbe JF and Farhat F. 2019. Application of sodium alginate extracted from a Tunisian brown algae Padina pavonica for essential oil encapsulation: Microspheres preparation, characterization and *in vitro* release study. Int. J. Biol. Macromol. 136: 386-394.

Fournaise T, Burgain J, Perroud C, Scher J, Gaiani C and Petit J. 2020. Impact of formulation on reconstitution and flowability of spray-dried milk powders. Powder Technol. 372: 107-116.

Galli GM, Gerbet RR, Griss LG, Fortuoso BF, Petrolli TG, Boiago MM, Souza CF, Baldissera MD, Mesadri J, Wagner R, da Rosa G, Mendes RE, Gris A and Da Silva AS. 2020. Combination

of herbal components (curcumin, carvacrol, thymol, cinnamaldehyde) in broiler chicken feed: Impacts on response parameters, performance, fatty acid profiles, meat quality and control of coccidia and bacteria. Microb. Pathog. 139: 1-11.

Garcia-Sotelo D, Silva-Espinoza B, Perez-Tello M, Olivas I, Alvarez-Parrilla E, González-Aguilar GA and Ayala-Zavala JF. 2019. Antimicrobial activity and thermal stability of rosemary essential oil:β–cyclodextrin capsules applied in tomato juice. LWT - Food Sci. Technol. 111: 837-845.

Gawad R and Fellner V. 2019. Evaluation of glycerol encapsulated with alginate and alginate-chitosan polymers in gut environment and its resistance to rumen microbial degradation. Asian-Australas. J. Anim. Sci. 32 (1): 72-81.

Geravand M, Sharifi SD, Yaghobfar A, Mohammadi A, Hosseini SA and Ghazanfari S. 2021. Growth performance, ascites sensitivity, and ileal microbiota as affected by licorice essential oil in broiler chicken diets. Livest. Sci. 251: 104670.

Ghazanfari S, Mohammadi Z and Adib Moradi M. 2015. Effects of Coriander essential oil on the performance, blood characteristics, intestinal microbiota and histological of broilers. Rev. Bras. Cienc. Avic. 17 (4): 419-426.

Gholami-Ahangaran M, Ahmadi-Dastgerdi A, Azizi S, Basiratpour A, Zokaei M and Derakhshan M. 2022. Thymol and carvacrol supplementation in poultry health and performance. Vet. Med. Sci. 8 (1): 267-288.

Glenn GM, Klamczynski AP, Woods DF, Chiou B, Orts WJ and Imam SH. 2010. Encapsulation of plant oils in porous starch microspheres. J. Agric. Food Chem. 58 (7): 4180-4184.

Hadidi M, Pouramin S, Adinepour F, Haghani S and Jafari SM. 2020. Chitosan nanoparticles loaded with clove essential oil: Characterization, antioxidant and antibacterial activities. Carbohydr. Polym. 236: 1-8.

Hafeez A, Manner K, Schieder C and Zentek J. 2016. Effect of supplementation of phytogenic feed additives (powdered vs. encapsulated) on performance and nutrient digestibility in broiler chickens. Poult. Sci. 95 (3): 622-629.

Hashemipour H, Kermanshahi H, Golian A and Veldkamp T. 2013. Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. Poult. Sci. 92 (8): 2059-2069.

Hezaveh H and Muhamad II. 2013. Controlled drug release via minimization of burst release in pH-response kappa-carrageenan/polyvinyl alcohol hydrogels. Chem. Eng. Res. Des. 91 (3): 508-519.

Hinneburg I, Damien Dorman HJ and Hiltunen R. 2006. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem. 97 (1): 122-129.

Hong J-C, Steiner T, Aufy A and Lien T-F. 2012. Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. Livest. Sci. 144 (3): 253-262.

Horky P, Skalickova S, Smerkova K and Skladanka J. 2019. Essential oils as a feed additives: pharmacokinetics and potential toxicity in monogastric animals. Animals. 9 (6): 352.

Hosseini SA and Meimandipour A. 2018. Feeding broilers with thyme essential oil loaded in chitosan nanoparticles: an efficient strategy for successful delivery. Br. Poult. Sci. 59 (6): 669-678.

Hosseini SF, Zandi M, Rezaei M and Farahmandghavi F. 2013. Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and *in vitro* release study. Carbohydr. Polym. 95 (1): 50-56.

Jamil B, Abbasi R, Abbasi S, Imran M, Khan SU, Ihsan A, Javed S, Bokhari H and Imran M. 2016. Encapsulation of Cardamom essential oil in chitosan nano-composites: *In vitro* efficacy on antibiotic-resistant bacterial pathogens and cytotoxicity studies. Front. Microbiol. 7: 1-10.

Jamroz D, Wiliczkiewicz A, Wertelecki T, Orda J and Skorupinska J. 2005. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. Br. Poult. Sci. 46 (4): 485-493.

Jamroz JO, C. Kamel2, A. Wiliczkiewicz, T. Wertelecki and J. Skorupinska. 2003. The Influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. J. Anim. Feed Sci. 12: 583-596.

Jugreet BS, Suroowan S, Rengasamy RRK and Mahomoodally MF. 2020. Chemistry, bioactivities, mode of action and industrial applications of essential oils. Trends Food Sci. Tech. 101: 89-105.

Jyothi NV, Prasanna PM, Sakarkar SN, Prabha KS, Ramaiah PS and Srawan GY. 2010. Microencapsulation techniques, factors influencing encapsulation efficiency. J. Microencapsul. 27 (3): 187-197.

Kang ZL, Wang TT, Li YP, Li K and Ma HJ. 2019. Effect of sodium alginate on physical-chemical, protein conformation and sensory of low-fat frankfurters. Meat Sci. 162: 1-7.

Keawchaoon L and Yoksan R. 2011. Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. Colloids Surf. B Biointerfaces. 84 (1): 163-171.

Khattak F, Ronchi A, Castelli P and Sparks N. 2014. Effects of natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. Poult. Sci. 93 (1): 132-137.

Kleemann C, Schuster R, Rosenecker E, Selmer I, Smirnova I and Kulozik U. 2020. *In-vitro*-digestion and swelling kinetics of whey protein, egg white protein and sodium caseinate aerogels. Food Hydrocolloids. 101: 1-9.

Kohlert C, Van Rensen I, März R, Schindler G, Graefe E and Veit M. 2000. Bioavailability and pharmacokinetics of natural volatile terpenes in animals and humans. Planta medica. 66 (6): 495-505.

Koroch AR, Simon JE and Juliani HR. 2017. Essential oil composition of purple basils, their reverted green varieties (*Ocimum basilicum*) and their associated biological activity. Ind. Crop. Prod. 107: 526-530.

Kumari M, Agnihotri D, Chanotiya CS, Mathur AK, Lal RK and Mathur A. 2017. Chemical and genetic stability of methyl chavicol-rich Indian basil (*Ocimum basilicum* var. CIM-Saumya) micropropagated *in vitro*. S. Afr. J. Bot. 113: 186-191.

Lertsutthiwong P, Noomun K, Jongaroonngamsang N, Rojsitthisak P and Nimmannit U. 2008. Preparation of alginate nanocapsules containing turmeric oil. Carbohyd. Polym. 74 (2): 209-214.

Li HL, Zhao PY, Lei Y, Hossain MM and Kim IH. 2015. Phytoncide, phytogenic feed additive as an alternative to conventional antibiotics, improved growth performance and decreased excreta gas emission without adverse effect on meat quality in broiler chickens. Livest. Sci. 181: 1-6.

Li P, Piao X, Ru Y, Han X, Xue L and Zhang H. 2012. Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian-Aust J Anim. Sci. 25 (11): 1617-1626.

Ling K, Wu H, Neish AS and Champion JA. 2019. Alginate/chitosan microparticles for gastric passage and intestinal release of therapeutic protein nanoparticles. J. Control. Release. 295: 174-186.

Liong M and Shah N. 2005. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. J. Dairy Sci. 88 (1): 55-66.

Locali-Pereira AR, Lopes NA, Menis-Henrique MEC, Janzantti NS and Nicoletti VR. 2020. Modulation of volatile release and antimicrobial properties of pink pepper essential oil by microencapsulation in single- and double-layer structured matrices. Int. J. Food Microbiol. 335: 108890.

Lu W, Yang X, Shen J, Li Z, Tan S, Liu W and Cheng Z. 2021. Choosing the appropriate wall materials for spray-drying microencapsulation of natural bioactive ingredients: Taking phenolic compounds as examples. Powder Technol. 394: 562-574.

Masyita A, Mustika Sari R, Dwi Astuti A, Yasir B, Rahma Rumata N, Emran TB, Nainu F and Simal-Gandara J. 2022. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chemistry: X. 13.

Moghaddam M, Alymanesh MR, Mehdizadeh L, Mirzaei H and Ghasemi Pirbalouti A. 2014. Chemical composition and antibacterial activity of essential oil of *Ocimum ciliatum*, as a new source of methyl chavicol, against ten phytopathogens. Ind. Crop. Prod. 59: 144-148.

Mohammadi A, Ghazanfari S and Sharifi SD. 2019. Comparative effects of dietary organic, inorganic, and nano-selenium complexes and rosemary essential oil on performance, meat quality and selenium deposition in muscles of broiler chickens. Livest. Sci. 226: 21-30.

Mountzouris KC, Paraskevas V, Tsirtsikos P, Palamidi I, Steiner T, Schatzmayr G and Fegeros K. 2011. Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. Anim. Feed Sci. Technol. 168 (3-4): 223-231.

Nadeem F, Hanif MA, Bhatti IA, Jilani MI and Al-Yahyai R. 2020. Basil. In: Medicinal Plants of South Asia. ed. 47-62.

Naidu JR, Ismail R and Sasidharan S. 2016. Chemical profiling and antioxidant activity of Thai basil (*Ocimum basilicum*). J Essent Oil Bear Pl. 19 (3): 750-755.

Natrajan D, Srinivasan S, Sundar K and Ravindran A. 2015. Formulation of essential oil-loaded chitosan-alginate nanocapsules. J. Food Drug. Anal. 23 (3): 560-568.

Natsir MH, Hartutik O, Sjofjan and Widodo E. 2013. Effect of either powder or encapsulated form of garlic and *Phyllanthus niruri* L mixture on broiler performances, intestinal characteristics and intestinal microflora. Int. J. Poult. Sci. 12 (11): 676-680.

Ngamekaue N and Chitprasert P. 2019. Effects of beeswax-carboxymethyl cellulose composite coating on shelf-life stability and intestinal delivery of holy basil essential oil-loaded gelatin microcapsules. Int. J. Biol. Macromol. 135: 1088-1097.

Obajuluwa OV, Sanwo KA, Egbeyale LT and Fafiolu AO. 2020. Performance, blood profile and gut morphometry of broiler chickens fed diets supplemented with Yohimbe (*Pausynistalia yohimbe*) and Larvacide. Vet. Anim. Sci. 10: 1-9.

Omonijo FA, Kim S, Guo T, Wang Q, Gong J, Lahaye L, Bodin JC, Nyachoti M, Liu S and Yang C. 2018a. Development of novel microparticles for effective delivery of thymol and lauric acid to pig intestinal tract. J. Agric. Food Chem. 66 (37): 9608-9615.

Omonijo FA, Ni L, Gong J, Wang Q, Lahaye L and Yang C. 2018b. Essential oils as alternatives to antibiotics in swine production. Anim Nutr. 4 (2): 126-136.

Oso AO, Suganthi RU, Reddy GBM, Malik PK, Thirumalaisamy G, Awachat VB, Selvaraju S, Arangasamy A and Bhatta R. 2019. Effect of dietary supplementation with phytogenic blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. Poult. Sci. 98 (10): 4755-4766.

Padalia RC, Verma RS, Upadhyay RK, Chauhan A and Singh VR. 2017. Productivity and essential oil quality assessment of promising accessions of *Ocimum basilicum* L. from north India. Ind. Crop. Prod. 97: 79-86.

Pandey AK, Singh P and Tripathi NN. 2014. Chemistry and bioactivities of essential oils of some *Ocimum* Species: An Overview. Asian Pac. J. Trop. Biomed. 4 (9): 682-694.

Peanparkdee M, Iwamoto S and Yamauchi R. 2016. Microencapsulation: A review of applications in the food and pharmaceutical industries. Rev. Agric. Sci. 4: 56-65.

Peng QY, Li JD, Li Z, Duan ZY and Wu YP. 2016. Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits and jejunal morphology in broiler chickens. Anim. Feed. Sci. Technol. 214: 148-153.

Peretz S, Florea-Spiroiu M, Anghel D-F, Bala D, Stoian C and Zgherea G. 2013. Preparation of porous calcium alginate beads and their use for adsorption of O-nitrophenol from aqueous solutions. Microfluidics and Nanoengineering. 22: 123-136.

Pham VH, Kan L, Huang J, Geng Y, Zhen W, Guo Y, Abbas W and Wang Z. 2020. Dietary encapsulated essential oils and organic acids mixture improves gut health in broiler chickens challenged with necrotic enteritis. J. Anim. Sci. Biotechnol. 11: 1-18.

Poonkodi K. 2016. Chemical Composition of Essential Oil of *Ocimum Basilicum* L. (Basil) and Biological Activities-An Overview. J. Crit. Rev. 3 (3): 56-62.

Pu J, Chen D, Tian G, He J, Zheng P, Mao X, Yu J, Huang Z, Luo J, Luo Y and Yu B. 2020. Effects of benzoic acid, *Bacillus* coagulans and oregano oil combined supplementation on growth performance, immune status and intestinal barrier integrity of weaned piglets. Anim Nutr. 6 (2): 152-159.

Radunz M, da Trindade MLM, Camargo TM, Radunz AL, Borges CD, Gandra EA and Helbig E. 2019. Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove *(Syzygium aromaticum,* L.) essential oil. Food Chem. 276: 180-186.

Rattanachaikunsopon P and Phumkhachorn P. 2010. Antimicrobial Activity of Basil (*Ocimum basilicum*) Oil Against *Salmonella Enteritidis In Vitro* and in Food. Biosci. Biotechnol. Biochem. 74 (6): 1200-1204.

Rawat R, Tiwari V, Singh R and Bisht IS. 2017. Assessment of the essential oil composition in *Ocimum* species of Uttarakhand. J. Essent. Oil Bear. Pl. 20 (5): 1331-1341.

Riyazi SR, Ebrahimnezhad Y, Hosseini SA, Meimandipour A and Ghorbani A. 2015. Effects of antibiotic growth promoter, probiotic and basil essential oil supplementation on the intestinal microflora of broiler chickens. J. Anim. Sci. Biotechnol. 4 (2): 185-189.

Sabourian P, Tavakolian M, Yazdani H, Frounchi M, van de Ven TG, Maysinger D and Kakkar A. 2020. Stimuli-responsive chitosan as an advantageous platform for efficient delivery of bioactive agents. J. Control. Release. 317: 216-231.

Saini RK, AE AB, Roohinejad S, Rengasamy KRR and Keum YS. 2020. Chemical stability of lycopene in processed products: A review of the effects of processing methods and modern preservation strategies. J. Agr. Food Chem. 68 (3): 712-726.

Santos BCS, Pires AS, Yamamoto CH, Couri MRC, Taranto AG, Alves MS, Araujo A and de Sousa OV. 2018. Methyl Chavicol and its synthetic analogue as possible antioxidant and antilipase agents based on the in vitro and in silico assays. Oxid. Med. Cell. Longev. 2018: 1-12.

SASInstitute. 1985. SAS user's guide: Statistics, version 5 ed. In: SAS Institute Inc., Cary, North Car-olina. ed.

Silva V, Sousa J, Guerra F, Pessôa H, Frietas A, Alves L and Lima E. 2015. Antibacterial activity of *Ocimum basilicum* essential oil and linalool on bacterial isolates of clinical importance. Int. J. Pharmacogn. Phytochem. Res. 7 (6): 1066-1071.

Sorasitthiyanukarn FN, Ratnatilaka Na Bhuket P, Muangnoi C, Rojsitthisak P and Rojsitthisak P. 2019. Chitosan/Alginate Nanoparticles as a Promising Carrier of Novel Curcumin Diethyl Diglutarate. Int. J. Biol. Macromol. 131: 1125-1136.

Stevanovic ZD, Bosnjak-Neumuller J, Pajic-Lijakovic I, Raj J and Vasiljevic M. 2018. Essential Oils as Feed Additives-Future Perspectives. Molecules. 23 (7): 1-20.

Su G, Wang L, Zhou X, Wu X, Chen D, Yu B, Huang Z, Luo Y, Mao X, Zheng P, Yu J, Luo J and He J. 2021. Effects of essential oil on growth performance, digestibility, immunity, and intestinal health in broilers. Poult. Sci. 100 (8): 101242.

Sun X, Cameron RG and Bai J. 2020. Effect of spray-drying temperature on physicochemical, antioxidant and antimicrobial properties of pectin/sodium alginate microencapsulated carvacrol. Food Hydrocolloids. 100: 1-9.

Sundari Z, Yuwanta T and Martien R. 2014. Effect of nanocapsule level on broiler performance and fat deposition. Int. J. Poult. Sci. 13 (1): 31-35.

Tanhaeian A, Sekhavati MH and Moghaddam M. 2020. Antimicrobial activity of some plant essential oils and an antimicrobial-peptide against some clinically isolated pathogens. Chem. biol. technol. 7 (1): 1-11.

Tavares IMC, Sumere BR, Gomez-Alonso S, Gomes E, Hermosin-Gutierrez I, Da-Silva R and Lago-Vanzela ES. 2020. Storage stability of the phenolic compounds, color and antioxidant activity of jambolan juice powder obtained by foam mat drying. Food Res. Int. 128: 1-9.

Tekce E, Çınar K, Bayraktar B, Takma Ç and Gül M. 2020. Effects of an essential oil mixture added to drinking water for temperature-stressed broilers: performance, meat quality, and thiobarbituric acid-reactive substances. J. Appl. Poultry Res. 29 (1): 77-84.

Temiz U and Öztürk E. 2018. Encapsulation methods and use in animal nutrition. Selcuk J. Agr. Food Sci. 32 (3): 624-631.

Tolve R, Tchuenbou-Magaia F, Di Cairano M, Caruso MC, Scarpa T and Galgano F. 2021. Encapsulation of bioactive compounds for the formulation of functional animal feeds: The biofortification of derivate foods. Anim. Feed Sci Tech. 279: 115036.

Trabelsi I, Ktari N, Ben Slima S, Bouchaala K and Ben Salah R. 2016. Effects of supplementation with *L. plantarum* TN8 encapsulated in alginate-chitosan in broiler chickens. Int. J. Biol. Macromol. 89: 677-681.

Turek C and Stintzing FC. 2013. Stability of essential oils: A review. Compr. Rev. Food Sci. Food Saf. 12 (1): 40-53.

Vogtmann H, Pfirter H and Prabucki A. 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Br. Poult. Sci. 16: 531-534.

Wadhwa G, Kumar S, Mittal V and Rao R. 2019. Encapsulation of babchi essential oil into microsponges: physicochemical properties, cytotoxic evaluation and anti-microbial activity. J. Food Drug Anal. 27 (1): 60-70.

Wang H, Liang S, Li X, Yang X, Long F and Yang X. 2019. Effects of encapsulated essential oils and organic acids on laying performance, egg quality, intestinal morphology, barrier function, and microflora count of hens during the early laying period. Poult. Sci. 98 (12): 6751-6760.

Wen C, Liu Y, Ye Y, Tao Z, Cheng Z, Wang T and Zhou Y. 2020. Effects of gingerols-rich extract of ginger on growth performance, serum metabolites, meat quality and antioxidant activity of heat-stressed broilers. J. Therm. Biol. 89: 1-6.

Yarmohammadi BS, Jazi V, Mohebodini H, Ashayerizadeh A, Shabani A and Toghyani M. 2020. Effects of dietary lavender essential oil on growth performance, intestinal function, and antioxidant status of broiler chickens. Livest. Sci. 233: 1-7.

Ydjedd S, Bouriche S, RubénLópez-Nicolás, Sánchez-Moya T, Frontela-Saseta C, Ros-Berruezo G, Rezgui F, Louaileche H and Kat D-E. 2017. Effect of *in vitro* gastrointestinal digestion on encapsulated and nonencapsulated phenolic compounds of Carob (*Ceratonia siliqua* L.) pulp extracts and their antioxidant capacity. J. Agric. Food Chem. 65: 827-832.

Zeb A and Ullah F. 2016. A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. J. Anal. Methods Chem. 2016: 1-5.

Zeng Z, Zhang S, Wang H and Piao X. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: A Review. J. Anim. Sci. Biotechnol. 6 (7): 1-10.

Zhao P, Piao X, Pan L, Zeng Z, Li Q, Xu X and Wang H. 2017. Forsythia suspensa extract attenuates lipopolysaccharide-induced inflammatory liver injury in rats via promoting antioxidant defense mechanisms. Anim. Sci. J. 88 (6): 873-881.

VITA

NAME	Sureerat Thuekeaw
DATE OF BIRTH	19 August 1988
PLACE OF BIRTH	Denchai District Phrae Province Thailand
INSTITUTIONS ATTENDED	Bachelor of science (B.S.) Agriculture (Animal Science) Chiangmai University, Thailand Masters of Science (M.S.) Agriculture (Animal Science) Chiangmai University, Thailand
HOME ADDRESS	House No. 181/1 Moo 7 Pangpawan Sub-district Denchai District Phrae Province Thailand Postal Code 54110
PUBLICATION	Thuekeaw, S., Tapingkae, W. and Yachai. M. 2013. Yield performance and changes in fibrolytic enzymes of Pleurotus cultivated on corn by-products. Agricultural Sci. J. 44: 1 (Suppl.): 51-54. Tapingkae, W., Thuekaew, S. and Yachai. M. 2014. Effects of crude fibrolytic enzymes fro, Pleurotus ostreatus spent mushroom substrate on the in vitro digestibility and nutrient utilization of broiler diet. J. Agriculture. 30 (2): 171-180. Thuekeaw, S. 2016. Effects of spent mushroom substrate extract on in vitro digestibility of broiler diet. Khon Kaen Agr. J. 44 Suppl. 2: 673-680. Thuekeaw, S., Buwjoom, T and Maneewan, B. 2017. Chemical composition of oil palm fruit meal and the using of oil palm fruit meal in Thai Native chicken diet. Maejo annual conference. Thuekeaw, S., Angkanaporn, K., Chirachanchai, S., Nuengjamnong, C., 2021. Dual pH responsive via double - layered microencapsulation for controlled release of active ingredients in simulated gastrointestinal tract: A model case of chitosan-alginate microcapsules containing basil oil (Ocimum

basilicum Linn.). Polym. Degrad. Stabil. 191.
Thuekeaw, S., Angkanaporn, K., Chirachanchai, S., Nuengjamnong, C., 2022. Microencapsulated basil oil (Ocimum basilicum Linn.) enhances growth performance, ontestinal morphology, and antioxidant capacity of broiler chickens in the tropics. Anim. Biosci. 35:752-762.