DEVELOPMENT OF FUNCTIONAL NUTRIENT PRODUCTS FOR GESTATING AND LACTATING SOWS

NITHAT WICHASIT

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy in Animal Science - (Type 2.1)
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has been approved by the Graduate School as partial fulfillment of the requirements for the Doctor of Philosophy in Animal Science - (Type 2.1) of Naresuan University

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ABSTRACT

This thesis investigates the impact of functional nutrient supplementation (FNS) on the productivity and health of gestating and lactating sows, as well as their piglets. The studies aim to address the challenges faced by modern pig farming, including high neonatal mortality rates, insufficient milk production, and the growing demand for antibiotic-free animal products. Functional nutrients, including medium-chain fatty acids (MCFAs), dietary fiber (DF) in the form of lignocellulose, and heat-killed Lactobacillus plantarum L-137 (HK L-137), were selected for their potential to enhance sow productivity, improve colostrum quality, and boost piglet survival and growth. Five studies were conducted to explore different aspects of FNS in swine production, ranging from optimizing microencapsulation techniques to examining the physiological and biochemical responses of sows and piglets to these supplements.

The first study focused on optimizing the microencapsulation conditions of MCFAs using a spray drying technique combined with Response Surface Methodology (RSM). A Box-Behnken Design (BBD) was employed to study the effects of three variables: the ratio of sodium caseinate (NaCas) to maltodextrin (X1), the ratio of wall material to core material (X2), and homogenization speed (X3). A total of 17 runs were

conducted, and the optimal conditions were determined as a NaCas to maltodextrin ratio of 1:4.98, a wall material to core material ratio of 70:30, and a homogenization speed of 16,367 RPM. Under these conditions, the viscosity of the emulsion was 70.87 mPa.s, the total soluble solids were 27.67%, and emulsion stability at 1, 4, and 8 hours was 97.31%, 73.57%, and 38.20%, respectively. The microencapsulation process achieved an efficiency of 83.13% and a yield of 98.85%. The microcapsules displayed spherical shapes with multi-core structures and retained key fatty acids such as caprylic and lauric acids. This study demonstrated that optimizing microencapsulation conditions significantly enhances the stability and retention of MCFAs, making them suitable for functional nutrient supplementation in sows.

The second study evaluated the effects of microencapsulated medium-chain fatty acid (miMCFA) supplementation on sow performance, colostrum composition, and piglet growth. Thirty sows were divided into four groups: a control group and three treatment groups that received 25 g/day, 50 g/day, or 75 g/day of miMCFA from day 100 of gestation to day 7 of lactation. The results indicated that miMCFA supplementation significantly increased colostrum production in the 50 g/day and 75 g/day groups compared to the control group. Piglet birth weights and litter weights at day 7 were also higher in the miMCFA-supplemented groups, with the 50 g/day and 75 g/day groups showing the greatest improvements. Sows receiving higher levels of miMCFA also experienced less back-fat loss during lactation, suggesting improved body condition maintenance. Colostrum composition analysis revealed an increase in fat content in the supplemented groups, while protein, lactose, and IgG levels remained unchanged. These findings suggest that miMCFA supplementation enhances colostrum quality, supports better piglet growth, and helps maintain sow body condition during lactation.

The third study explored the combined effects of microencapsulated MCFAs, lignocellulose, and HK L-137 on sow performance, colostrum composition, and piglet immunity. Fifty sows were assigned to five treatment groups: a control group, a group supplemented with miMCFA, a group supplemented with miMCFA and lignocellulose, a group supplemented with miMCFA and HK L-137, and a group supplemented with all three components. The results showed that sows receiving miMCFA and the combination of all three components (miMCFA, lignocellulose, and

HK L-137) produced more live-born piglets and higher litter weights compared to the control group. Colostrum production was also significantly higher in these groups, and immunoglobulin G (IgG) levels were elevated in the groups receiving HK L-137. Colostrum fat content increased significantly in the groups supplemented with miMCFA and HK L-137. These results suggest that the combination of miMCFA, lignocellulose, and HK L-137 enhances colostrum quality, boosts piglet immunity, and improves sow performance, making it a promising nutritional strategy in modern swine production.

The fourth study investigated the effects of functional nutrient supplementation (FNS) during gestation and lactation on sow and piglet performance, colostrum and milk composition, and IGF-1 gene expression. Sixty sows were assigned to three FNS levels—low (40g/d), mid (80g/d), and high (120g/d)—with a control group receiving no supplementation. The results showed that FNS had minimal effects on sow performance and piglet growth, with no significant differences in litter size, piglet birth weight, or weaning weight. However, FNS significantly influenced colostrum composition, with the low and mid supplementation groups showing higher fat content and increased IgG levels compared to the control and high groups. IGF-1 gene expression was significantly upregulated in the mid and high gestation groups, suggesting that FNS may enhance fetal growth through IGF-1 modulation. The study concludes that while FNS modestly impacts colostrum composition and immune function, its overall effects on sow and piglet performance are limited.

The fifth and final study focused on the metabolomic analysis of colostrum in sows supplemented with functional nutrients during gestation and lactation. Colostrum samples were analyzed using liquid chromatography-mass spectrometry (LC-MS) to identify key metabolites and determine their roles in piglet health and survival. The metabolomic analysis revealed distinct metabolic profiles between the control group and the FNS group, with upregulation of metabolites involved in lipid metabolism, immune function, and stress response in the FNS group. Pathway enrichment analysis indicated that carbohydrate metabolism and immune pathways were enhanced in the FNS group, while stress-related pathways were downregulated. These findings suggest that functional nutrient supplementation can influence the colostrum metabolome, potentially improving piglet health through enhanced bioactive

components in colostrum. However, the traditional productivity metrics, such as average daily feed intake, colostrum yield, and piglet survival rates, did not show significant differences between the control and FNS groups.

This thesis demonstrates the potential of functional nutrient supplementation to improve sow productivity, colostrum quality, and piglet immunity, while providing an alternative to antibiotics in modern swine production. The combined use of microencapsulated MCFAs, lignocellulose, and HK L-137 shows promise in enhancing the nutritional and immunological quality of colostrum, which is crucial for piglet health and survival. While FNS had a limited impact on traditional productivity metrics, its influence on the metabolome and immune-related parameters highlights its potential for improving piglet survival and growth in intensive pig farming systems. Further research is needed to optimize FNS strategies and explore their long-term effects on swine production outcomes.

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CHAPTER I

INTRODUCTION

Background of a study

Pigs are a crucial economic asset in Thailand, with the country producing 16.2 million live pigs in 2023, contributing over 130 billion baht to the economy. The modern pig farming industry relies heavily on breeding prolific sows, a practice that has led to significant genetic advancements. These improvements have resulted in contemporary sows producing over 20 piglets per litter, compared to the 10-12 piglets per litter typical of previous generations. However, this success has also introduced challenges that affect both the efficiency of pig farming and the welfare of the animals. One of the most pressing issues is the increase in neonatal mortality rates, particularly within the first week after birth. Studies indicate that the neonatal mortality rate in modern prolific sows is higher than in previous generations, with some farms experiencing rates as high as 20% during this critical first week. The causes of this increase in mortality are likely multifactorial, including insufficient milk production and health issues affecting both sows and piglets. These challenges can lead to slower growth rates and reduced pork quality.

Traditionally, antibiotics have been used to address some of these health issues in pig farming. However, the widespread use of antibiotics raises concerns about the potential for negative impacts on consumer health and the environment. Prolonged antibiotic use can lead to the development of antibiotic-resistant bacteria, which pose serious health risks to both animals and humans. These resistant bacteria can spread through the food chain, potentially affecting humans and leading to public health issues. Additionally, antibiotic resistance can impact the export of animal products, as many countries have strict regulations regarding the use of antibiotics in livestock. In response to these concerns, the livestock production industry has increasingly prioritized food safety and begun exploring ways to reduce or eliminate antibiotics in animal farming. This shift is driven by consumer demand for safer food and the need to address the environmental challenges associated with intensive pig farming. Raising prolific sows

contributes to environmental issues such as water and air pollution and greenhouse gas emissions, further emphasizing the need for alternative solutions. One promising alternative is the development of functional nutrient products for gestating and lactating sows. These products are designed to enhance health beyond basic nutrition, offering benefits that address the challenges associated with modern pig farming. Functional nutrients provide essential nutrients for the growth and development of both sows and piglets, contain antioxidants that boost the immune system, include beneficial probiotics for overall health, and supply sufficient energy for sows during gestation and lactation. Ensuring that sows have adequate energy during these periods is crucial, as it allows them to produce milk rich in immune-boosting properties for their piglets.

Medium Chain Fatty Acids (MCFAs) are one such functional nutrient, naturally occurring as medium-chain triglycerides in milk fat and various animal feed ingredients, particularly those derived from edible fats like coconut oil and palm kernel oil. The hydrolysis of medium-chain triglycerides occurs faster than that of long-chain triglycerides, and due to their increased water solubility, they do not require emulsification with bile. This makes MCFAs efficiently absorbed and digested, making them a valuable energy source for sows. Research has shown that supplementing sows with 10% coconut oil, a source of MCFAs, from day 84 of gestation until farrowing can increase the survival rate of newborn piglets. However, incorporating high levels of oil into pig diets can be challenging, leading to the development of powdered forms of oils or fats for supplements. Microencapsulation techniques have proven effective in improving the storage of bioactive compounds and allowing for their slow release in the animal's digestive system, a method widely adopted in the animal feed industry.

Dietary fiber (DF) is another critical component in sow diets, promoting gut health and immunity. DF refers to carbohydrate polymers that are partially or fully fermented in the large intestine after bypassing digestion in the small intestine. DF plays a crucial role in improving the gut microbiota of sows, enhancing digestion and immune function in pigs. Most research has focused on the effects of various DF diets on sow welfare, colostrum production, physiology, and performance. Additionally, studies have found that providing gestating sows with lignocellulose, a type of DF, can increase both the quantity and quality of milk. Lignocellulose enhances nutrient absorption, ensuring that embryos and piglets receive the nutrients necessary for proper growth. It also helps

prevent diarrhea and bladder stones in piglets. Incorporating lignocellulose into the diet of gestating sows, particularly in the days leading up to farrowing, is an effective strategy for improving piglet health, increasing milk production, reducing disease risk, and minimizing farm waste. However, more research is needed to fully understand the impact of DF on colostrum and milk quality, as well as piglet immunity.

Heat-killed Lactobacillus plantarum strain L-137 (HK L-137) is another promising functional ingredient. This lactic acid bacterium, known for its stability under high temperatures and pressures, has been shown to boost immunity, disease resistance, and stress tolerance in various animals. It has been widely used in mammals and aquatic animals such as mice, pigs, shrimp, and red sea bream. HK L-137 is recommended as an immune-boosting agent, playing a crucial role in innate immune responses, disease resistance, stress tolerance, and growth stimulation across species. However, research on its effects in sows and piglets is limited, and it remains unclear how long sows should receive HK L-137 supplementation or its impact on immune responses.

This research explores the impact of combining microencapsulated MCFAs, lignocellulose, and HK L-137 as a powdered functional nutrient supplement for sows. This supplement is designed for convenience, to be used during specific production phases, to stimulate feed intake, or to enhance immunity passed to piglets either in utero or through milk. Ultimately, this research aims to develop a prototype product that can be commercialized within the swine production industry, offering a natural, effective alternative to antibiotics.

Objective of the study

This research aims to address the problem of insufficient quantity and quality of milk produced by sows, which leads to piglets not reaching the standard weaning weight and experiencing high mortality rates due to malnutrition. Functional nutrient products are an innovative and promising alternative to reduce the use of antibiotics. These products can help enhance sow productivity, increase milk production, and improve the survival rates and body weight of piglets.

Scope of the study

This study tested indicators for the application of functional nutrient products as supplements for late-gestation and lactating sows. In Study 1, powdered fat was produced through microencapsulation, and the optimal conditions were determined using the response surface methodology. Study 2 focused on finding the most suitable formula for sows, measuring sow productivity, milk production, and piglet growth. Study 3 explored the appropriate timing and dosage levels, assessing sow productivity, milk production, piglet growth, and immune gene expression. Study 4 measured the relative quantities of metabolites that respond to the functional nutrient products.

Preliminary agreements

This research study was conducted using a randomized complete block design with the following preliminary agreements:

1. All sows in this study must be cared for and managed equally.
2. All sows must receive sufficient feed and water according to their age-related requirements.
3. All sows must undergo regular health checks.
4. All piglets must receive appropriate care.

These preliminary agreements are essential to ensure the reliability and accuracy of the study results.

Glossary of Terms

Medium-Chain Fatty Acids (MCFAs): Fatty acids with a carbon chain length of 6-12 atoms, commonly found in palm kernel oil, coconut oil, and olive oil. MCFAs are more easily absorbed by the body compared to long-chain fatty acids (LCFAs). They are an efficient energy source and contribute to overall health maintenance.

Lignocellulose: A complex bio composite consisting of lignin and cellulose. Lignocellulose is abundant in plants, bark, and wood fibers. It helps increase dietary fiber content in animal feed, benefiting the digestive health of pigs.

Probiotic Microorganisms: Beneficial microbes that support the immune system, reduce the risk of various diseases, and improve overall health in both humans and animals.

Colostrum: The first milk produced by sows within 24-72 hours after giving birth. Colostrum is a vital source of nutrition and immunity for newborn piglets, rich in proteins, fats, sugars, minerals, and antioxidants.

Neonatal Mortality Rate: The ratio of piglets that die within the first week after birth to the total number of piglets born in each litter.

Sow Health: Refers to the physical and mental well-being of sows, characterized by robust health, the absence of illness or pain, low stress levels, and the ability to efficiently nurture their piglets.

Research hypothesis

Supplementing sow diets with MCFAs, lignocellulose, and Probiotic L-1 3 7 can increase milk production, improve milk quality, reduce neonatal mortality rates in the first week after birth, and enhance sow health. These hypotheses are supported by previous research, which has shown that MCFAs, lignocellulose, and probiotics offer various benefits for pigs. MCFAs can enhance nutrient absorption, improve milk quality, and boost piglet immunity. Lignocellulose increases dietary fiber content in animal feed, positively impacting digestive health in pigs. Probiotic microorganisms strengthen the immune system, reduce disease risks, and improve overall health. However, this study represents the first experimental trial in Thailand to examine the effects of supplementing sow diets with MCFAs, lignocellulose, and Probiotic L-1 3 7 on sow productivity and colostrum quality. The findings from this research will provide valuable insights into the efficacy and safety of these dietary supplements for sows.

CHAPTER II

LITERATURE REVIEW

Functional Nutrition

Functional nutrition, also known as functional food, refers to foods consumed by animals that serve specific roles in enhancing overall health, beyond the basic nutrition provided by regular diets. Functional foods go beyond what is typically consumed in daily life, playing a significant role in strengthening the animal's health and boosting its immune system. The main components of food can be divided into two parts: nutrients and non-nutrients. Nutrients include proteins, carbohydrates, fats, vitamins, and minerals. Non-nutrients refer to naturally occurring chemical compounds that have physiological effects.

Commonly used functional nutrition includes:

1. Dietary Fiber: Helps reduce blood cholesterol levels and is commonly found in vegetables and fruits.

2. Oligosaccharides: Help regulate the digestive system by promoting the growth of beneficial bacteria in the gastrointestinal tract.

3. Dipeptides: Partially digested proteins that help reduce brain fatigue, anxiety, and stress, improve brain function, enhance mineral absorption, and boost the immune system.

4. Omega-3 Polyunsaturated Fatty Acids: Aid in brain and vision development and reduce the risk of ischemic heart disease.

5. Phytochemicals: A group of chemicals that act as antioxidants, such as polyphenols found in green and oolong tea, diallyl disulfides found in onions and garlic, and phytoestrogens found in soybeans and flaxseeds, which mimic female hormones.

6. Minerals and Vitamins: Act as antioxidants and help prevent conditions like ischemic heart disease and cataracts. Examples include beta-carotene, vitamin C, vitamin E, and selenium.

Therefore, functional supplements for sows during lactation and nursing are extremely important. Sows need sufficient energy and immunity to support their piglets, especially during the rapid growth phase at the end of gestation and during lactation.

Nutrition Influencing Productivity and Lactation in Sows

1. The Importance of Colostrum

Colostrum is a vital source of nutrients that piglets must receive. Its production is regulated by hormones produced by the endocrine glands, with prolactin being the key hormone necessary for milk secretion. Generally, the concentration of immune antibodies such as IgG, IgA, and IgM in sow colostrum varies with sow parity. Klobasa and Butler (1987) reported that the concentrations of IgG and IgA in sow colostrum increase significantly by the fourth pregnancy and tend to rise progressively thereafter. Colostrum also contains vitamins and minerals that piglets receive from the sow, particularly vitamin E, which is stored in the sow's fat tissue. Increasing the concentration of vitamin E in the diet during gestation allows this nutrient to be passed through the placenta or stored in colostrum. Additionally, vitamin A and D are often supplemented in the sow's diet to improve the IgG levels in piglets, which is crucial for their survival. While these vitamins do not alter the composition of colostrum, they enhance the absorption of IgG in piglets, providing them with better disease resistance after birth.

When piglets are born, they instinctively seek out the udder to consume colostrum. However, as the number of piglets born increases, competition for access to the udder intensifies, leading to smaller piglets receiving less or insufficient colostrum. This results in weaker immune protection for those piglets. To address this issue, products have been developed to help smaller piglets receive the energy and immunity they need at birth. These products include oils enriched with vitamins A, E, and D, along with specific amino acids, to enhance the immune system of newborn piglets and provide them with an initial energy boost.

2. Medium-Chain Fatty Acids

Medium-chain fatty acids (MCFAs) are fatty acids with carbon chains of 6-12 atoms. These fatty acids are present in edible medium-chain triglyceride oils such as coconut oil, palm kernel oil, and milk fats. Studies in animal models have shown that

MCFAs can serve as an immediate energy source for animals because they are absorbed directly into the portal system. In addition to being rapidly absorbed, MCFAs are also quickly oxidized. Over 75% of a pig's liver energy is derived from fatty acid oxidation (Odle et al., 1991), which helps improve piglet growth performance (Odle et al., 1989, 1991) and increases the digestibility of feed. MCFAs in feed can inhibit intestinal microorganisms and improve feed efficiency. Furthermore, they are an ideal alternative to antibiotics due to their antibacterial properties, which help prevent diarrhea.

3. Dietary Fiber

Dietary fiber is the part of the plant cell wall that cannot be digested by the enzymes in the gastrointestinal tract of monogastric animals. Certain microbes in the large intestine can digest some components of dietary fiber. Although dietary fiber is not a nutrient and does not provide energy to the body, it plays a crucial role in the nutrition and health of monogastric animals. It helps maintain normal digestive and excretory functions. The main components of dietary fiber are complex sugars known as polysaccharides (Ivarsson et al., 2011), which include complex carbohydrates that are not starch, such as cellulose, hemicellulose, pectin, and mucilage, as well as non-carbohydrate compounds like lignin. Dietary fibers are categorized into two types based on their solubility in water: soluble dietary fiber and insoluble dietary fiber. The sum of both types is referred to as total dietary fiber (Zhang et al., 2013).

1. **Insoluble Dietary Fiber:** These are complex carbohydrates that are mostly found in the tough structure of plant cell walls. They are difficult to break down and absorb water well, swelling like a sponge when in contact with water, which increases the volume of food residue and softens it, making it easier to excrete. Examples include cellulose, hemicellulose, lignin, cutin, and wax, which are typically found in vegetables and grains. Cellulose content can reach 20-50% of the dry weight of plants, while hemicellulose, cutin, and wax are found in smaller amounts in the plant cell walls. Lignin is found in hardwoods.

2. **Soluble Dietary Fiber:** This type of fiber is found in plant cells, usually mixed with plant starch. It can be partially digested and has the property of forming viscosity when combined with water, creating a dense structure that may form a gel. This gel can slow the absorption of charged substances such as sugars and certain minerals. Examples of soluble fibers include pectin, beta-glucans, and various gums,

which can be found in citrus fruits, barley, and oats. The chemical structure of soluble dietary fibers differs from that of insoluble fibers.

Dietary fiber in food affects not only the digestive system but also bowel function, depending on the level and type of dietary fiber and its physical and chemical properties, such as solubility and viscosity. Soluble dietary fiber increases the viscosity of food, which affects food and enzyme interactions, creating areas in the intestinal lining where water can be easily absorbed. This slows down digestion and nutrient absorption. Insoluble dietary fiber, on the other hand, resists microbial degradation, increases fiber content in feces, and facilitates excretion while reducing nitrogen, depending on the type of fiber. In summary, dietary fiber impacts nutrient and energy digestibility, varying according to the amount and type of fiber as well as its physical and chemical properties. The differences in dietary fibers also affect bowel function (Jha et al., 2010, cited by Jha and Berrocoso, 2015). The particle size of dietary fiber can also influence digestive and enzymatic functions. Lindberg's (2014) study found that reducing dietary fiber particle size improved feed efficiency and nutrient digestibility in weaned pigs. This effect may be explained by the development of digestive and absorptive capabilities in the small intestine and increased carbohydrate digestion in the large intestine as the animal ages.

4. Dead Probiotic Cells and Probiotic Cell Components

Dead microorganisms play a significant role in the food and pharmaceutical industries, promoting health similarly or even better than live microorganisms. Studies have shown that dead bacterial cells or their components can enhance immune balance. Internationally, dead cells of lactic acid bacteria, particularly various strains of Lactobacillus, are produced using different methods, such as heat-killing, UV radiation, and sonication. When consumed, these dead cells have been found to promote health similarly to live cells, enhancing non-specific immune responses, preventing gastrointestinal infections, reducing blood cholesterol levels, inhibiting cancer-causing metabolites, and inducing macrophages to engulf pathogens. Research has shown that dead cells or components of various Lactobacillus species, such as Lactobacillus plantarum and Lactobacillus gasseri, can stimulate the production of interleukin-12 (IL-12), which enhances the body's immune system. Heat-killed Lactobacillus plantarum strain L-137 can induce macrophage activity and stimulate spleen cells to produce IL-

12 and interferon-gamma to destroy foreign invaders or viruses in the body (Murosaki et al., 1998). Additionally, it can induce the immune system to produce interferon-beta, which helps protect against influenza infections. Dead bacteria also help reduce or prevent the production of immunoglobulin E (IgE), an antibody that responds to allergens like dust mites, pollen, dander, and certain foods. Normally, the body produces immunoglobulin G (IgG) to respond to immune challenges, helping to destroy bacteria, viruses, fungi, and toxins. However, in allergic conditions, IgE is produced in response to allergens, leading to allergic diseases and related health issues.

Beyond their health benefits, dead probiotics also enhance the quality of food and pharmaceutical products by extending shelf life, making storage and transportation easier, and facilitating consumption. With the increasing diversity of probiotic products and the growing popularity of flavor and aroma enhancements, using dead probiotic cells or components can improve product stability, reduce the risk of changes in products supplemented with live probiotics, and provide another way to benefit from microorganisms. In some countries, dead probiotics and their components are included in probiotic products alongside live microorganisms.

5. Immunobiotics or Heat-Killed Bacteria Strain L-137 (HK L-137)

Immunobiotics are natural immune-boosting substances produced from the bacterium *Lactobacillus plantarum* strain L-137 (HK L-137), a lactic acid bacterium commonly found in fermented foods. This beneficial probiotic is rendered non-viable through heat treatment, meaning it cannot multiply, but its cell wall and membrane structures remain intact and can still stimulate the immune system. This microorganism is beneficial for both humans and animals, capable of enhancing immunity in chickens and helping to prevent diseases that reduce growth performance.

Currently, the use of dead microorganisms, or immunobiotics, has seen significant success in aquaculture by stimulating non-specific immune responses, making it an increasingly popular method. The use of immunobiotics promotes the immune system, enhancing immune reactions, increasing resistance to pathogens, and improving survival rates after infection (Sato et al., 2009). This approach is considered a safe alternative to antibiotics, with no residual effects.

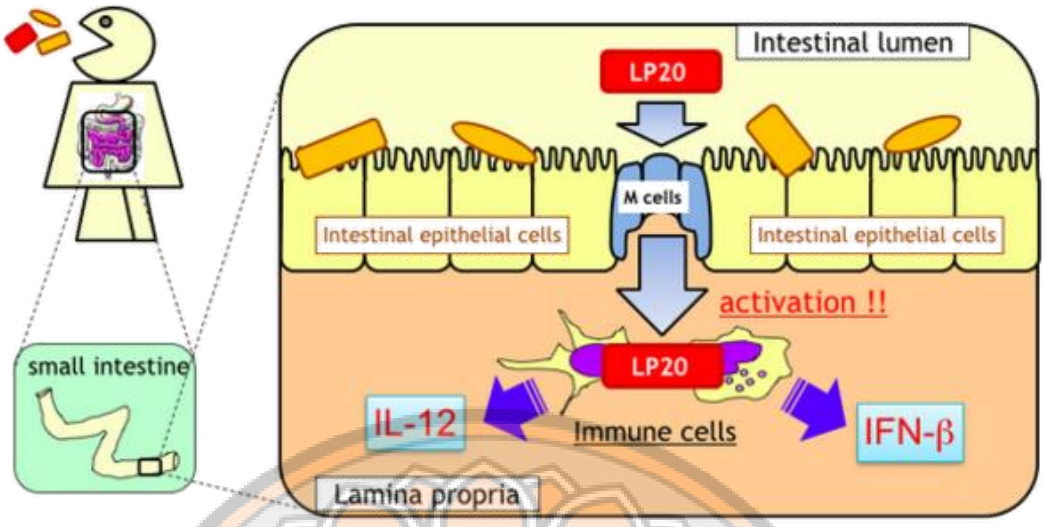

Figure 1 illustrates the mechanism of action of Heat-Killed Bacteria strain L-137 (HK L-137) in stimulating the gastrointestinal immune response.

When animals ingest *Lactobacillus plantarum* strain L-137 (HK L-137) or "immunobiotics," the bacteria reach the small intestine and interact with the villi, stimulating the M cells located within the villi. These M cells, responsible for capturing antigens or foreign substances entering the body, bind with *Lactobacillus plantarum* strain L-137 (HK L-137). This bacterium acts as a trigger, inducing the production of interleukin (IL-12), increasing interferon-beta (IFN-β) and T cells, and stimulating macrophages (Murosaki et al., 1998) to produce stronger immune cells to fend off future pathogens. In aquaculture reports, *Lactobacillus plantarum* has been shown to modulate immunity, improve growth performance, and enhance disease resistance and resilience in fish. Tartrakoon, et al. (2023) suggested that incorporating HKL137 in pig diets can enhance pro-duction performance and boost the immune system without the inconsistencies associated with traditional in-feed probiotics.

Microencapsulation Technique

Microencapsulation technology was developed in the 1950s through research in the printing industry (Green, & Scheicher, 1995). Today, this technology is continuously evolving and gaining wider acceptance in pharmaceuticals, chemicals, cosmetics, food, and other industries (Augustin et al., 2001; Heinzen, 2002).

Microencapsulation involves enclosing materials within a sealed capsule, allowing the controlled release of the core material. Microcapsules consist of a core material, which is often reactive or sensitive, such as flavors, vitamins, minerals, fats, and oils, that is coated with a suitable wall material. The wall material protects the core from degradation, facilitates transport, and allows the controlled release of the core material under specified conditions. The coating material used for encapsulation should not react with the core material and must be manageable in form, such as having low viscosity at high concentrations. It should also allow for complete solvent removal during the process, protect the core material from environmental factors, and provide a stable emulsion (Trubiao and Lacourse, 1988; Shahidi and Han, 1993).

1. Encapsulation Materials

1.1 Starch-Based Materials

Maltodextrin ($C_6H_{10}O_5$) is derived from the partial hydrolysis of starch using acid or enzymes, with the degree of hydrolysis indicated by the dextrose equivalent (DE) value. Maltodextrins with different DE levels provide varying stability as coating agents. Anandaraman and Reineccius (1986) found that maltodextrin at an appropriate DE level can help prevent the oxidation of oils, but the DE should not exceed 20.

Gum is highly soluble in water with low viscosity and acts as an effective emulsifier, efficiently encapsulating oil-based core materials. It reduces surface tension and protects the core material from environmental degradation. Beristain et al. (2001) studied the ability of gum to encapsulate cardamom oil into microcapsules by varying the core-to-coating ratio from 1:3 to 1:5 and using spray drying at an inlet temperature of 200°C and an outlet temperature of 110°C. They found that as the core-to-coating ratio decreased, the oil droplet size became smaller, surface oil content was reduced, and the encapsulation efficiency increased.

1.2 Protein-Based Materials

Proteins are widely used in microencapsulation due to their diverse chemical groups, amphiphilic nature (having both polar and non-polar properties), ability to interact with both water and oil, and their large molecular weight and flexibility. The advantages of proteins as coating materials include water solubility, emulsification properties, increased viscosity, and film formation. In the

microencapsulation process, proteins are rapidly absorbed and positioned at the oil-water interface, forming a stable emulsion that prevents the coalescence of water during production and storage (Dalgleish, 1997; Dickinson, 2001). Examples of proteins used as coating agents in microencapsulation include soy protein isolates, whey protein, and sodium caseinate. Hogan et al. (2001) studied the potential of sodium caseinate as a coating agent for the microencapsulation of soybean oil. They found that as the ratio of soybean oil to sodium caseinate increased, the viscosity of the emulsion increased, the particle size of the microcapsules grew larger, and the efficiency of microencapsulation decreased. The resulting microcapsules were spherical, had smooth surfaces, and exhibited particle agglomeration with large internal cavities.

2. Microencapsulation Techniques

Various techniques are used for microencapsulation, including chemical methods such as coacervation, co-crystallization, molecular inclusion, and interfacial polymerization, as well as mechanical methods like spray drying, spray chilling/cooling, extrusion, and fluidized bed coating. The resulting microcapsules vary in shape depending on the materials and processes used. These include simple spherical microcapsules where the core material is surrounded by a uniformly thick coating, irregularly shaped microcapsules, microcapsules consisting of numerous small droplets embedded in a coating matrix, multi-core microcapsules containing more than one core material, and multi-wall microcapsules with multiple layers of coating material. Among these techniques, spray drying is the most commonly used for microencapsulation (Deis, 1997) due to its availability, low production cost, versatility with different core materials, and high efficiency in encapsulating volatile substances.

Experimental Design Using Response Surface Methodology

Response Surface Methodology (RSM) is used to reduce the number of experimental runs needed to evaluate multiple variables. It is a mathematical method used to create models and visualize responses to different variables, with the goal of finding optimal conditions. RSM is considered an effective technique for complex processes, making them easier to manage and interpret compared to other methods (Box & Behnken, 1960; An et al., 2013). Additionally, a key advantage of RSM is its ability to reduce the number of experimental runs required to evaluate multiple variables,

saving labor and time compared to other methods for determining optimal conditions. This experiment was designed using the Box-Behnken Design (BBD), which offers fewer experimental runs compared to Fractional Factorial Design (FFD) or Central Composite Design (CCD) when the number of factors is the same.

The relationship between the factors under study and the response values can be calculated using the following equation:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{23}X_2X_3+\beta_{13}X_1X_3+\beta_{11}X_{12}+\beta_{22}X_{22}+\beta_{33}X_{32} \quad (1)$$

Where:

Y is the predicted response value,

$\beta_0$is the constant term,

X_1, X_2, X_3 are the independent variables,

β_1, β_2, β_3 are the linear coefficients,

β_{12}, β_{13}, β_{23} are the interaction coefficients,

β_{12}, β_{22}, β_{32} are the quadratic coefficients.

Omics Sciences and Technologies

Omics sciences and technologies are a part of systems biology. The term "omics" is derived from the Latin word “~omne,” meaning the whole or all components. It has been used as a suffix in English to describe technologies related to the study of the complete set of biomolecules, ranging from genetic information (genes) to gene expression at the RNA level (transcripts), protein synthesis (proteins), and the analysis of all metabolites in an organism or biological system (San and Mayuree, 2017; Mozzi et al., 2013). The results of these studies are presented in the form of biomolecular profiles, such as the complete genetic information (genome), focusing on identifying gene groups or the genetics of organisms to determine gene arrangements and functions. Other profiles include the complete set of gene expression or mRNA (transcriptome), protein synthesis (proteome), which involves the study of proteins in terms of their characteristics, functions, and locations, and the complete set of metabolites (metabolome) of a particular organism or biological system under specific conditions. The objective is to understand the functional mechanisms and interactions between these

biomolecules and to find correlations between these biomolecular profiles and the phenotype or specific characteristics of the organism or biological system under study.

Metabolomics Technology

Metabolomics, or metabolite analysis, is a branch of omics sciences and technologies that studies the chemical components of a system by analyzing metabolites or small biomolecules (typically less than 1.5 kDa) such as nucleic acids, nucleotides, amino acids, short-chain peptides, fatty acids, sugars, oligosaccharides, vitamins, alcohol compounds, carbonyl compounds, organic acids, sulfur compounds, phenolic compounds, aromatic hydrocarbons, etc., synthesized by organisms or present in a biological system. The aim is to collect data on the types and quantities of all metabolites, or the metabolome, including those synthesized within cells (intracellular) and those secreted outside cells (extracellular), which result from metabolic pathways. This holistic approach is intended to understand the biological system, as influenced by genetic expression and environmental adaptation. Common analytical techniques used in metabolomics include mass spectrometry (MS), often combined with separation techniques such as gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS) and nuclear magnetic resonance (NMR) spectroscopy.

1. **Gas Chromatography/Mass Spectrometry: GC/MS**

GC/MS combines gas chromatography and mass spectrometry, making it one of the most used separation techniques in food research (Karoui and De Baerdemaeker, 2007). It is particularly useful for analyzing volatile compounds, such as pesticides and other volatile substances. The advantages of GC/MS include high sensitivity and accuracy, along with extensive libraries for identifying and characterizing substances without needing standard reference materials (Putri et al., 2013).

2. **Liquid Chromatography/Mass Spectrometry: LC/MS**

LC/MS combines liquid chromatography with mass spectrometry (Putri et al., 2013). It is widely used in metabolomics studies due to its sensitivity and the ability to analyze a broader range of molecular masses compared to GC/MS. The most commonly used technique is high-performance liquid chromatography (HPLC), which

is employed to separate, identify, and quantify components in a sample, depending on the type of stationary phase. Different components are separated based on their chemical characteristics, such as molecular mass and charge (Karoui and De Baerdemaeker, 2007).

3. Nuclear Magnetic Resonance: NMR

NMR is a commonly used technique in metabolomics studies. Its advantages include rapid analysis, no need for sample preparation, and the fact that the sample is not destroyed during analysis, allowing for further studies afterward. NMR can analyze both solid and liquid samples. NMR is widely applied in organic chemistry and biochemistry to identify organic compounds and analyze the structure of biopolymers (Hu et al., 2004). It is highly specific and non-destructive, making it valuable despite lower sensitivity compared to some spectroscopic methods (Lindon, 2017). The most studied aspect in biochemical research is hydrogen or proton (1H) (Lindon, 2017).

Data Processing Using Bioinformatics

Chemometrics is a branch of chemistry that applies mathematics, statistics, and logical principles to design or select appropriate experimental processes and to prepare the most correlated chemical knowledge for data analysis. It is used to analyze patterns and compare differences in metabolomic data between samples using multivariate statistical analysis such as principal component analysis (PCA) or cluster analysis (Ebbels, & De, 2011).

1. Principal Component Analysis (PCA)

PCA is used to reduce the dimensionality of variables by combining related variables into new ones while preserving the total variance of the original variables, with the new variables being called principal components. However, if the factors are difficult to interpret, factor rotation can be used to make the factors more meaningful. Orthogonal rotation is a common method for reducing data dimensionality by considering factor loadings, which indicate the level or extent of the relationship between each variable.

2. Cluster Analysis

Cluster analysis is a technique that divides data units into at least two subgroups based on the criteria that units within the same group have similar or identical characteristics, while units in different groups have distinct characteristics. The type of cluster analysis is determined by the grouping process, methods, or criteria used for grouping. Two commonly used types are hierarchical cluster analysis and non-hierarchical cluster analysis. The grouping process is often illustrated by a dendrogram, which is convenient for visualizing groupings.

Application of Metabolomics Technology in Studying the Systemic Biochemical Composition of Milk

Metabolomics technology involves the study of metabolite composition in living organisms using advanced chemical analysis tools such as spectrophotometry, chromatography, and mass spectrometry. This technology can simultaneously analyze thousands of metabolites, enabling comprehensive studies of an organism's systemic biochemical composition. The application of metabolomics technology to study the systemic biochemical composition of milk aims to explore the relationship between milk composition and various factors such as animal breed, diet, environment, and health. This research can enhance our understanding of the mechanisms behind milk production in mammals and can be used to develop high-quality, nutritionally rich dairy products. An interesting example of such research is the study of the relationship between milk composition and animal health. It has been found that milk from healthy animals contains higher levels of metabolites associated with growth and immunity, whereas milk from unhealthy animals contains higher levels of metabolites related to inflammation and infection.

Moreover, this research can be applied to the development of nutritionally enhanced dairy products. For instance, studies have shown that supplementing dairy cows with omega-3 fatty acids increases the omega-3 content in their milk, which is essential for the brain and nervous system development in infants. Studying the systemic biochemical composition of milk using metabolomics is an effective approach to understanding the mechanisms of milk production in mammals and can be used to develop high-quality, nutritionally enriched dairy products. Since raw milk has a

complex chemical composition, metabolomics technology can analyze both volatile and non-volatile metabolites in milk. Volatile compounds include alcohols, carbonyl compounds, organic acids, sulfur compounds, and heterocyclic compounds, while non-volatile compounds include amino acids, carbohydrates, lipid derivatives, carbonyl compounds, sulfur compounds, and nucleosides, covering approximately 200 types.

CHAPTER III

EMULSION PROPERTIES AND OPTIMIZATION OF MICROENCAPSULATION OF MEDIUM CHAIN FATTY ACIDS BY SPRAY DRYER RESPONSE SURFACE METHODOLOGY

Abstract

The research was aimed at optimizing the microencapsulation condition of medium chain fatty acids (MCFAs) by using a spray drying technique and response surface methodology with Box- Behnken Design (BBD) experiment to study the optimum conditions. There were 3 factors studied including coating ratio between sodium caseinate (NaCas) and Maltodextrin DE18 (X1), ratio of wall coating material to core material (X2) and homogenizing level (X3). The experiment was repeated 3 times with a total of 17 Run. Each factor was defined as 3 levels, the ratio of coatings NaCas:Maltodextrin at 1:3, 1:4 and 1:5, ratio of wall material:Core material at 50:50, 60:40 and 30:70, homogenizing level at 12,000, 15,000 and 18,000 rpm. Total soluble solid, viscosity and emulsion stability of 3 replications were analyzed by response surface methodology (RSM). After that, the optimal condition for drying by spray-dryer were selected and various properties of microencapsulated powder were analyzed. The optimal condition of the ratio of NaCas:Maltodextrin coating materials, the ratio of the wall material:core material and homogenizing speed was 1:4.98, 30:70 and 16,367.75 rpm. The viscosity of the emulsion was 70.87 mPa.s, total soluble solids was 27.67% and the emulsion stability at 1, 4 and 8 h were 97.31%, 73.57% and 38.20%, respectively. Spray-drying at 200°C with a feed rate of 1.94 L/h resulted in microencapsulation efficiency of 83.13% and a yield of 98.85%. The microcapsules exhibited spherical shapes and multi-core structures, with the preservation of essential fatty acids such as caprylic and lauric acids. The optimized microencapsulation and spray-drying processes successfully enhanced the stability and retention of MCFAs, making them suitable for food, pharmaceutical, and cosmetic applications.

Introduction

The group MCFA comprises monocarboxylic fatty acids containing from 6 to 12 carbon atoms, including caproic (C6), caprylic (C8), capric (C10), and lauric (C12) acids (Marten et al., 2006; Petrović & Arsic, 2016). Their medium chain triacylglycerols (MCT) are usually used in animal feeds. In the animal digestive tract, MCT are preferentially hydrolyzed by preduodenal lipases (Dierick & Decuypere, 2004); thus, the resulting MCFA can be partly absorbed already through the stomach mucosa (Pfeuffer & Schrezenmeir, 2002). Moreover, MCT can be absorbed into intestinal epithelial enterocytes and then hydrolyzed into cells by microsomal lipases (Czernichow et al., 1996; Tashiro et al., 1998; Slight et al., 2004). Portion of resulting MCFA entered into mitochondria is probably independent of the carnitine acyltransferase system (Lamers, 1995). Also, their oxidation should be carnitine independent because they are activated within the mitochondrial matrix (Noland et al., 2009).

The efficacy of MCFAs in animal nutrition extends beyond mere energy provision and their unique metabolic pathways also suggest potential health benefits. Recent studies indicate that MCFAs, particularly when microencapsulated for stability and bioavailability, can enhance gut health by promoting beneficial microbial populations while suppressing pathogens (Yang et al., 2024). This is crucial not only for the immediate well-being of livestock but also for improving overall feed efficiency and growth rates. Additionally, the encapsulation process itself, such as using various wall materials like whey protein isolate or octenyl succinic anhydride starch, has been shown to significantly affect the release profile and effectiveness of these fatty acids during digestion (Korma et al., 2019; San et al., 2022). As such, optimizing microencapsulation techniques may lead to more effective formulations that harness the full spectrum of MCFAs' advantages, potentially paving the way for innovative dietary strategies in animal husbandry. Microencapsulation, which provides a physical barrier for bioactive compounds and separates the core material from the environment until release, is thought to improve the stability of bioactive compounds and enable the slow release of oil in animals (Champagne & Fustier, 2007; Kuang et al., 2010; Dias et al., 2015). Lipid matrix microencapsulation has been popularly used to deliver bioactive

compounds, for example essential oil, organic acids, and vitamins to the animal gut (Shen et al., 2011; de la Torre & de Pinho, 2015; Madureira et al., 2016).

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. It also has important applications in the design, development, and formulation of new products. In addition to the advantages of microencapsulation (Vining, 2008), the choice of wall materials plays a crucial role in determining the efficiency and stability of the encapsulated MCFAs. Recent studies have shown that using various combinations of wall materials, such as whey protein isolate (WPI) and prebiotic carbohydrates like maltodextrin or inulin, can significantly enhance the physicochemical properties of microcapsules (Korma et al., 2019; Teixeira et al., 2004). For instance, a specific formulation with WPI and inulin not only improved encapsulation efficiency but also provided better oxidative stability, which is vital for maintaining the bioactivity of MCFAs during storage and digestion (Korma et al., 2019). This optimization through RSM could lead to more effective delivery systems in animal nutrition, ensuring that these beneficial compounds remain intact until they reach their target site within the gut (San et al., 2022). Moreover, understanding how different emulsification techniques affect the final product's characteristics may open new pathways for improving feed formulations tailored to the nutritional needs of livestock (Yang et al., 2024).

This study focused on improving the microencapsulation of MCFAs using RSM for use in animal feed. The results from this research have the potential to greatly boost nutrient absorption efficiency, resulting in better growth rates and health in livestock.

Materials and Methods

1. Materials

Medium Chain Triglycerides (MCT) derived from palm kernel oil, intended for use with breeding sows. Since MCT, the primary ingredient, is obtained from a standardized manufacturing process, the quality is relatively consistent. However, the raw materials used in this project were procured from a certified vegetable oil factory in quantities sufficient for the one-year duration of the project. These materials were

stored in a cold room to maintain quality. For each batch of MCT prepared, random samples were collected for the analysis of other chemical components, particularly fatty acids. Palm kernel oil from Suksomboon Palm Oil Co., Ltd., Chonburi; soybean oil (Kook brand) from Thanakorn Vegetable Oil Products Co., Ltd., Samut Prakan; sodium caseinate (NaCas) from S.A. Chemical Co., Ltd., Bangkok; maltodextrin (DE18) from S.A. Chemical Co., Ltd., Bangkok; and lecithin (Food grade) from Vejakit Co., Ltd., Bangkok.

Each type of oil was subjected to a thermal treatment at a temperature of 50 °C for 1 h. Subsequently, the oils were amalgamated, ensuring that the resultant mixture possesses the requisite concentration of MCFAs suitable for the gestational and lactational stages of swine. The resultant composite is preserved in a refrigeration unit maintained at a temperature of 4 °C. Prior to application, it is extracted from the refrigeration unit and permitted to equilibrate to ambient temperature.

2. Experimental design and analysis

Preparation of oil–in–water (O/W) emulsions

The emulsion samples are prepared by mixing coating agents, namely NaCas and maltodextrin (DE18), with a total soluble solid content of 30% for the combined coating and core materials. The emulsion is then homogenized using a homogenizer (APV Gaulin, Inc., Model 15MR-8TA, USA) for 15 min. The variables studied include ratio of NaCas to maltodextrin (1:3, 1:4, 1:5); ratio of coating material to core material (50:50, 60:40, 70:30) and homogenization speed (12,000, 15,000, 18,000 rpm). This study utilized a Box-Behnken Design (BBD) to optimize the preparation of an O/W emulsion. A total of 17 experimental runs were conducted to investigate the relationships between the independent variables (oil droplet size, total soluble solids, viscosity, and emulsion stability) and the dependent responses. The details of the methods used for each parameter are as follows:

Total Soluble Solids in the Emulsion

The total soluble solids content in the emulsion samples was measured using a refractometer (Atago, Japan) following standard protocols for soluble solid determination. Samples were homogenized at15,000 rpm for 10 min, and the total soluble solids were expressed as a percentage. This measurement indicates the concentration of dissolved solids in the emulsion, which is crucial for understanding

the emulsifying ability of the formulation. Soluble solids content is a key factor in determining the final texture and consistency of the emulsion. Each sample was tested in triplicate to ensure the accuracy of the results.

Emulsion Viscosity

The viscosity of the emulsion was measured using a viscometer (Rheology International Shannon Ltd., Model RL:2, Ireland) at room temperature. Each sample was subjected to shear rates, and viscosity was recorded in milliPascal-seconds (mPa.s). Measurement of viscosity is essential for evaluating the flow properties and stability of the emulsion, as emulsions with higher viscosities tend to be more stable due to their resistance to phase separation. Three replicates were conducted for each sample to ensure the accuracy of the results.

Emulsion Stability

Emulsion stability was assessed by measuring the extent of phase separation over a 24-h period. Samples were stored at room temperature, and stability was monitored hourly. The percentage of the remaining stable emulsion phase was recorded at each time point. Emulsions with minimal phase separation are considered more stable, reflecting the ability of the emulsion to resist gravitational separation of oil and water phases. Stability assessments were repeated three times for each sample to ensure reproducibility.

Statistical Analysis

The experimental design followed a Box-Behnken Design (BBD) with 17 runs, including three replicates at the center points. This design allowed for the evaluation of the effects of independent variables on the response variables (total soluble solids, viscosity, and stability) with minimal experimental runs. Coded levels of the variables were used to analyze the statistical variance of the data using Analysis of Variance (ANOVA) at a 95% confidence level. The relationships between total soluble solids, viscosity, and emulsion stability were analyzed using Response Surface Methodology (RSM) via Design-Expert software (version 11.1). Optimal emulsion preparation conditions were determined based on the desirability function, prioritizing total soluble solids, emulsion viscosity, and stability in that order. Differences in mean values were evaluated using Duncan's New Multiple Range Test through SPSS software (version 24). This method allows for the comparison of means across different

groups and helps identify significant differences between treatment conditions. A significance level of $P < 0.05$ was used to determine statistical significance.

3. Spray drying of emulsions

The emulsion samples prepared under the conditions selected in the previous study were used for this section. The emulsion samples were randomly selected, and the oil was simply extracted to analyze the peroxide value. The emulsion was then spray-dried using a spray dryer (GEA Niro A/S, Model Mobile Minor 2000, Denmark).

The variables studied include feed rate at 1, 1.5, and 2 kg/h and inlet temperature at 160, 180, and 200°C. The experiment was designed using Central Composite Design (CCD). The following analyses were conducted on the microencapsulated samples:

Yield

The yield of the microencapsulation process was calculated as the percentage ratio of the weight of the microcapsules obtained after spray drying to the initial weight of the feed solids (core and wall materials) used in the emulsion. This method is crucial for evaluating the efficiency of the spray drying process.

Moisture Content

The moisture content of the microcapsules was determined using the AOCS Official Method Cd 8-53 (1998). Approximately 2 g of microcapsule powder were weighed and dried in a moisture analyzer at 105°C until a constant weight was achieved. This method provides a reliable measurement of the residual moisture present in microcapsules (Herrmann & Schlemm, 2007).

Water Activity

Water activity (aw) was measured using a water activity meter (Aqualab, Series 3, Decagon Devices, USA) at room temperature. The microcapsule sample was placed in a sealed chamber, and the water activity was recorded once the system reached equilibrium. Water activity is a key parameter for assessing the shelf stability of microencapsulated products, as lower water activity typically correlates with better stability.

Bulk and Tapped Density

Bulk and tapped densities of the microcapsules were determined using a graduated cylinder. Approximately 10 g of microcapsules were gently poured into a graduated cylinder, and the initial volume was recorded as bulk density. The cylinder was then tapped until no further volume reduction occurred, and the final volume was recorded as tapped density. This method provides insights into the flowability and packing properties of the microcapsules.

Solubility

The solubility of the microcapsules was determined following the method of Approximately 1 g of microcapsules was dispersed in 100 mL of distilled water at room temperature (25°C) and stirred for 5 min. The solution was filtered using Whatman No. 1 filter paper, and the undissolved residue was dried at 105°C for 24 h to determine the insoluble portion. This test helps to determine the ability of microcapsules to dissolve in water, which is essential for their application in food and beverage formulations.

Morphology of Microcapsules

The surface morphology of the microcapsules was analyzed using a Scanning Electron Microscope (SEM) (JEOL, Model JSM-S410LV, Japan). Microcapsules were mounted on aluminum stubs, coated with a thin layer of gold, and observed under high vacuum at different magnifications. The SEM images provided detailed information on the shape, surface structure, and any surface irregularities such as cracks or pores. Morphological characteristics like surface smoothness and particle size were noted, which are critical for evaluating the stability and release properties of microcapsules.

Extraction of Total Oil

Total oil content was determined by solvent extraction using Soxhlet extraction approximately 5 grams of microcapsules were placed in a Soxhlet apparatus, and petroleum ether was used as the solvent. The extraction was carried out for 8 h, after which the solvent was evaporated, and the extracted oil was weighed. This method measures the total amount of oil (both surface and encapsulated) within the microcapsules.

Extraction of Surface Oil

Approximately 5 g of microcapsules were washed with 50 mL of petroleum ether in a flask, stirred for 5 min, and filtered. The solvent evaporated, and the weight of the extracted oil was recorded. This method determines the amount of free oil present on the surface of the microcapsules, which can affect the product's stability and shelf life.

Extraction of Encapsulated Oil

Encapsulated oil was calculated by subtracting the surface oil from the total oil content. The encapsulated oil represents the amount of oil effectively retained within the microcapsule matrix. This parameter is essential for evaluating the efficiency of the encapsulation process.

Microencapsulation Efficiency (ME%)

Microencapsulation efficiency (ME%) represents the proportion of total oil encapsulated within the wall material, excluding surface oil. A high ME% indicates that a greater proportion of oil has been successfully encapsulated within the wall material, which is critical for the stability and controlled release of the oil.

Determination of Fatty Acid Composition by Gas Chromatography (GC)

The fatty acid composition of the extracted oil was determined using Gas Chromatography (GC) following the method of Park & Goins (1994). The oil samples were first converted to fatty acid methyl esters (FAMEs) by methylation with methanolic sodium hydroxide. The FAMEs were then injected into a GC (Agilent 6890, USA) equipped with a flame ionization detector (FID) and a capillary column. The fatty acids were identified by comparing their retention times to known FAME standards, and the relative concentrations were expressed as a percentage of the total fatty acids. This method provides detailed information on the fatty acid profile, which is essential for determining the nutritional and functional properties of the encapsulated oil.

Surface Characteristics and Internal Structure of the Microcapsules (SEM)

The surface and internal morphology of the microcapsules were analyzed using a Scanning Electron Microscope (SEM) (JEOL, Model JSM-S410LV, Japan). Samples were mounted on aluminum stubs with double-sided adhesive carbon tape, sputter-coated with a thin layer of gold, and observed under high vacuum. Images were captured at varying magnifications to assess surface texture, shape, and any potential

cracks or voids in the microcapsules. This method allows for detailed characterization of surface morphology and structural integrity, providing insights into the effects of encapsulation parameters.

3. Statistical and data analysis

Each sample was analyzed in triplicate. Coded levels for each experimental condition were established to analyze statistical variance (ANOVA) at a 95% confidence level. The optimal conditions for producing microencapsulated MCFAs-enriched oil by spray drying were determined using RSM, based on the relationship between physical properties and microencapsulation efficiency. Differences in mean values were analyzed using Duncan's New Multiple Range Test with SPSS software. The differences in various properties between the microcapsules produced under optimal spray drying conditions and those obtained by substituting coded levels into the statistical model equation from the RSM analysis, using Design-Expert software (version 11.1), were evaluated. The predicted values were validated through three repeated experiments to test the accuracy of the predictions using Regression Analysis with SPSS software (version 17). The optimal conditions were selected based on the priority order of yield, microencapsulation efficiency, moisture content, water activity, microcapsule size, surface characteristics, and internal structure of the microcapsules.

Results

1. Optimal Conditions for Emulsion Preparation Using Response Surface Methodology (RSM)

The optimal parameters for the production of a MCFAs emulsion were ascertained through the utilization of statistical software, specifically applying a Box-Behnken Design (BBD) within the framework of RSM. A total of three variables were examined: the ratio of sodium caseinate to maltodextrin (X1), the proportion of wall material to core material (X2), and the rate of homogenization (X3). The experimental design encompassed 17 trials, inclusive of three replicates at the central point, with each variable evaluated at three distinct levels: sodium caseinate to maltodextrin ratios of 1:3, 1:4, and 1:5; wall material to core material proportions of 50:50, 60:40, and 30:70; as well as homogenization speeds of 12,000, 15,000, and 18,000 rpm, as delineated in Table 1. The emulsion preparation revealed viscosities ranging from 73.33 to 386.4

mPa.s, water-soluble solids between 20.33% and 27.67%, and emulsion stability percentages at 1 h ranging from 61.06% to 100%, and at 4 h from 33.41% to 100%. The interactions among NaCas to maltodextrin ratio, wall material to core material ratio, and homogenization speed significantly influenced the emulsion's viscosity, water-soluble solids, and stability. Using Design Expert software (version 11.0.0), the optimal conditions predicted for emulsion preparation were a NaCas to maltodextrin ratio of 1:4.98, a wall material to core material ratio of 70:30, and a homogenization speed of 16,367.37 rpm. These conditions are ideal for subsequent microencapsulation using the spray drying method, as shown in Table 1. When the predicted optimal levels of the three factors were applied, the resulting responses were a viscosity of 70.87 mPa.s, water-soluble solids of 27.67%, and emulsion stability of 97.31%, 73.57%, and 38.20% at 1, 4, and 8 h, respectively, as shown in Table 2. It was observed that increasing the NaCas level in the NaCas to maltodextrin ratio resulted in higher viscosity, which in turn led to an increase in water-soluble solids due to the higher molecular weight of NaCas compared to maltodextrin. Conversely, reducing the NaCas ratio decreased both viscosity and water-soluble solids, as illustrated in Figure 2. This trend highlights the critical role of NaCas in influencing the textural properties of the emulsion, suggesting that careful optimization of its concentration is essential for achieving desired product characteristics. Decreasing the NaCas ratio also lowered the emulsion stability at 1, 4, and 8 h, while reducing the wall material to core material ratio increased stability over the same time periods, as shown in Figure 3. This indicates that both the composition and the ratio of ingredients play a significant role in determining the overall performance and quality of the emulsion, necessitating further investigation into their interactions. The average viscosity and water-soluble solids had standard errors of 21.17 and 0.37, respectively, while the standard errors for emulsion stability at 1, 4, and 8 h were 6.09, 7.89, and 6.43, respectively. The R-squared values ranged from 0.9994 to 0.9999, indicating that the predicted data closely matched the experimental results, as shown in Table 1. Moreover, the findings suggest that optimizing these parameters could lead to enhanced sensory attributes and shelf life, making it imperative to explore various combinations in future studies.

2. Optimal Conditions for Microencapsulation of Medium-Chain Fatty Acids Using Spray Drying

The optimal conditions for microencapsulating MCFAs via spray drying were determined using statistical software. A Completely Randomized Design (CRD) was used to study the effects of two factors: feed rate (X1) and inlet air temperature (X2). The experiment included 13 trials, with three replicates at the central point. Each factor was tested at three levels: feed rates of 1, 1.5, and 2 kg/h, and inlet air temperatures of 160, 180, and 200°C, as shown in Table 3. The optimal conditions predicted using Design Expert software (version 11.0.0) were an inlet temperature of 200°C and a feed rate of 1.94 kg/h. These conditions were deemed most suitable for microencapsulation using spray drying, as shown in Table 3. Applying the predicted optimal conditions resulted in a moisture content of 1.16%, water activity of 0.204, bulk density of 0.44 g/cm², microencapsulation efficiency of 83.13%, and a yield of 98.85%, as shown in Table 3. It was found that increasing the feed rate and inlet air temperature reduced moisture content, water activity, and bulk density, while improving microencapsulation efficiency and yield, as shown in Table 3. The fatty acid profile of the encapsulated powder revealed three essential fatty acids: caprylic acid, undecylic acid, and lauric acid. Lauric acid, a saturated MCFA, exhibits antimicrobial properties without harming beneficial gut microorganisms or contributing to antibiotic resistance, as shown in Table 5. Increasing the inlet air temperature while maintaining a constant feed rate resulted in smaller microcapsules with slightly reduced bulk density. This also led to lower moisture content and water activity. increasing the inlet air temperature from 160 to 210°C reduced particle density, as illustrated in Figure 4. At a constant inlet air temperature, increasing the feed rate reduced microencapsulation efficiency but increased the bulk density of the powder. Conversely, raising the inlet air temperature reduced bulk density while increasing microencapsulation efficiency, as shown in Figure 4. The average moisture content, water activity, and bulk density had standard errors of 0.19, 0.01, and 0.01, respectively, while microencapsulation efficiency and yield had standard errors of 0.50 and 0.28, respectively. The R-squared values ranged from 0.9774 to 0.9998, indicating that the predicted data closely matched the experimental results, as shown in Table 3.

3. Surface and Internal Structure of Medium-Chain Fatty Acid Microcapsules

The surface and internal structure of MCFA microcapsules, observed under different conditions, were generally spherical with uneven particle sizes and surfaces marked by air bubbles. The increased inlet air temperature caused rapid water evaporation, leading to surface indentations on the microcapsules. The internal structure of the microcapsules was consistent across all drying conditions, with small oil droplets embedded within, forming both multi-core and simple core structures. These structural differences did not significantly impact microencapsulation properties. The variation in surface structure may be attributed to the film-forming properties of the coating materials, the viscosity of the emulsion, and the drying conditions Figures 6-7.

Discussion

This study explored the optimization of functional nutrient products, specifically microencapsulated MCFAs, using both RSM and spray drying techniques to improve the stability and quality of the product. The findings provide significant insights into the impact of various processing parameters on emulsion preparation, microencapsulation efficiency, and the structural integrity of MCFA microcapsules. Furthermore, the results indicated that optimizing these parameters can lead to enhanced release profiles and bioavailability of the encapsulated nutrients, which is crucial for their application in functional foods and dietary supplements.

1. Optimal Conditions for Emulsion Preparation

The study highlights the importance of selecting appropriate wall materials to further enhance the protective properties of the microcapsules, ensuring that the MCFAs remain stable during storage and processing. The optimization of emulsion preparation using RSM, and a Box-Behnken Design revealed that the ratio of sodium caseinate (NaCas) to maltodextrin, the wall material to core material ratio, and homogenization speed were critical factors influencing the properties of the emulsion, including viscosity, water-soluble solids, and stability (O'Regan & Mulvihill, 2009; O'Regan & Mulvihill, 2010; Udomrati et al., 2013; Liang et al., 2014).

The optimal conditions predicted NaCas to maltodextrin ratio of 1:4.98, a wall material to core material ratio of 70:30, and a homogenization speed of 16,367.37

rpm led to favorable outcomes in terms of viscosity (70.87 mPa.s), water soluble solids (27.67%), and emulsion stability. The relationship between NaCas concentration and viscosity aligns with existing literature, which indicates that NaCas, as a protein, contributes significantly to the thickening and stabilizing properties of emulsions. The higher molecular weight of NaCas compared to maltodextrin increases viscosity (Dokić et al., 2004). As confirmed by previous research on protein-stabilized emulsions this observation underscores the importance of optimizing NaCas concentration to balance the emulsion's textural and stability properties. The results also demonstrated that reducing the wall material to core material ratio enhanced emulsion stability, particularly at longer time intervals (1, 4, and 8 h). This could be attributed to the protective nature of the wall material, which reduces the likelihood of phase separation or destabilization of the emulsion, as shown in similar studies on microencapsulation. Furthermore, the interaction between the wall material and the core components plays a crucial role in determining the overall performance of the emulsion, highlighting the need for further investigation into the specific mechanisms at play.

2. Optimal Conditions for Microencapsulation Using Spray Drying

The optimization of microencapsulation conditions through spray drying also demonstrated the importance of processing parameters. The use of a Completely Randomized Design allowed for the assessment of feed rate and inlet air temperature, with the optimal conditions being an inlet temperature of 200°C and a feed rate of 1.94 kg/h. These conditions led to a moisture content of 1.16%, water activity of 0.204, bulk density of 0.44 g/cm², microencapsulation efficiency of 83.13%, and a yield of 98.85%. The inverse relationship between feed rate and microencapsulation efficiency is consistent with previous research, which suggests that higher feed rates reduce the time available for solvent evaporation, leading to less efficient encapsulation. Additionally, the increased inlet air temperature reduced the moisture content and water activity, creating a drier product with higher stability. These findings emphasize the importance of fine-tuning spray drying conditions to maximize product quality and shelf life, particularly for thermally sensitive compounds like MCFAs. The analysis of the fatty acid profile in the encapsulated powder revealed the presence of essential fatty acids, including caprylic acid, undecylic acid, and lauric acid. The preservation of these fatty acids highlights the effectiveness of the encapsulation process in maintaining the

integrity of bioactive compounds. Lauric acid, in particular, is known for its antimicrobial properties, making it a valuable component in functional food products. The successful encapsulation of these fatty acids aligns with existing studies that emphasize the importance of preserving functional properties in microencapsulated products.

3. Characteristics, surface area, and internal picture structure of medium-chain fatty acid microcapsules

The surface and internal structure of the microcapsules were observed to vary depending on the spray drying conditions. Higher inlet air temperatures led to smaller particle sizes and reduced bulk density, as water evaporated more quickly during the drying process. These findings are consistent with other studies that have reported similar structural changes in spray-dried microcapsules at higher temperatures. The formation of multi-core and simple core structures within the microcapsules provides insight into the encapsulation process. The multi-core structure may enhance the retention of core material, potentially leading to a more controlled release of the encapsulated MCFAs, which could be beneficial for food, pharmaceutical, and cosmetic applications. The presence of air bubbles and surface indentations, particularly at higher temperatures, suggests that rapid moisture evaporation can cause surface roughness, which may influence the release kinetics of the microcapsules.

4. Implications for Functional Food Development

The successful optimization of emulsion preparation and microencapsulation processes has important implications for the development of functional nutrient products. The enhanced stability, encapsulation efficiency, and retention of bioactive compounds achieved in this study suggest that these microencapsulated MCFAs could be effectively applied in functional food products aimed at improving animal health (Champagne & Fustier, 2007a).

The antimicrobial properties of lauric acid, combined with the controlled release potential of the microcapsules, make them particularly suitable for use in nutraceuticals or fortified foods designed to promote gut health and immunity. Furthermore, the ability to fine-tune the processing parameters to achieve desired product characteristics opens new avenues for the customization of functional foods based on specific health benefits or consumer preferences. The findings also suggest

potential applications beyond the food industry, including pharmaceuticals and cosmetics, where controlled release and stability are critical. While this study has demonstrated significant advancements in the optimization of MCFAs microencapsulation, further research is needed to explore the long-term stability and sensory attributes of the encapsulated products. Additionally, studies on the bioavailability and release kinetics of the encapsulated fatty acids in vivo would provide valuable insights into their efficacy as functional ingredients.

Future research could also investigate the use of alternative wall materials or biopolymers to further enhance the stability and functionality of the microcapsules. The integration of these findings with other functional nutrients, such as prebiotics or antioxidants, could lead to the development of more comprehensive functional food products.

Conclusions

This study successfully optimized the production of microencapsulated MCFAs using RSM and spray drying techniques. The research identified key parameters, including the ratio of sodium caseinate to maltodextrin, wall material to core material ratio, and homogenization speed, which significantly influenced emulsion viscosity, water-soluble solids, and stability. The optimal conditions for microencapsulation were established, resulting in high encapsulation efficiency (83.13%) and product yield (98.85%), with favorable moisture content and bulk density for long-term stability. The surface and internal structure of the microcapsules, influenced by spray drying conditions, were generally spherical with multi-core and simple core structures, which are important for controlling the release of bioactive compounds such as caprylic and lauric acids. These optimized microencapsulation techniques can be applied to develop functional food products aimed at improving gut health and boosting immunity.

Table 1 Coded levels for independent variables used in the emulsion experiment design for microencapsulation of medium chain fatty acid

Run	Extraction condition										
	Code variables			Independent parameters			Dependent parameters				
	X1	X2	X3	NaCas:MD ratio	Wall:Core ratio	Homogenize (rpm)	Viscosity (cP)	Total soluble solid (°Brix)	Stability at 1 h (%)	Stability at 4 h (%)	Stability at 8 h (%)
1.00	-1.00	0.00	1.00	1:3	60:40	18000.00	322.80	21.00	65.98	38.77	36.54
2.00	0.00	0.00	0.00	1:4	60:40	15000.00	145.13	23.33	100.00	77.18	52.56
3.00	1.00	1.00	0.00	1:5	70:30	15000.00	89.80	27.67	100.00	100.00	34.20
4.00	0.00	0.00	0.00	1:4	60:40	15000.00	145.13	23.33	100.00	77.18	52.56
5.00	0.00	1.00	1.00	1:4	70:30	18000.00	73.33	23.33	71.85	36.63	33.41
6.00	0.00	-1.00	-1.00	1:4	50:50	12000.00	246.93	25.00	100.00	100.00	66.20
7.00	0.00	-1.00	1.00	1:4	50:50	18000.00	175.87	25.00	100.00	100.00	73.23
8.00	1.00	-1.00	0.00	1:5	50:50	15000.00	104.60	26.00	100.00	100.00	40.63
9.00	-1.00	1.00	0.00	1:3	70:30	15000.00	290.67	20.33	85.12	49.01	42.94
10.00	1.00	0.00	-1.00	1:5	60:40	12000.00	107.33	26.00	100.00	100.00	34.47
11.00	0.00	0.00	0.00	1:4	60:40	15000.00	145.13	23.33	100.00	77.18	52.56
12.00	0.00	1.00	1.00	1:4	70:30	18000.00	73.33	23.33	71.85	36.63	33.41
13.00	-1.00	-1.00	0.00	1:3	50:50	15000.00	386.40	24.00	100.00	100.00	100.00
14.00	0.00	1.00	-1.00	1:4	70:30	12000.00	107.07	23.67	76.88	41.62	39.24
15.00	0.00	0.00	0.00	1:4	60:40	15000.00	86.63	23.33	100.00	41.41	41.83

Run	Extraction condition						Dependent parameters				
	Code variables			Independent parameters							
	X1	X2	X3	NaCas:MD ratio	Wall:Core ratio	Homogenize (rpm)	Viscosity (cP)	Total soluble solid (°Brix)	Stability at 1 h (%)	Stability at 4 h (%)	Stability at 8 h (%)
16.00	1.00	0.00	1.00	1:5	60:40	18000.00	85.33	27.00	100.00	100.00	34.61
17.00	-1.00	0.00	-1.00	1:3	60:40	12000.00	310.67	21.00	88.30	53.27	49.16

Table 2 Analysis results between prediction values and actual experiments of response surfaces in emulsion preparation

Response	Prediction	SE mean	Actually	SE mean	R Square	Adjusted R square
Viscosity	70.87	21.17	70.81	0.56	0.9999	-0.0001
Total soluble solid	27.67	0.37	27.52	0.30	0.9998	-0.0002
Stability at 1 h	97.31	6.09	99.19	0.67	0.9999	-0.0001
Stability at 4 h	73.57	7.89	74.73	1.10	0.9998	-0.0002
Stability at 8 h	38.20	6.43	39.62	0.91	0.9994	-0.0006

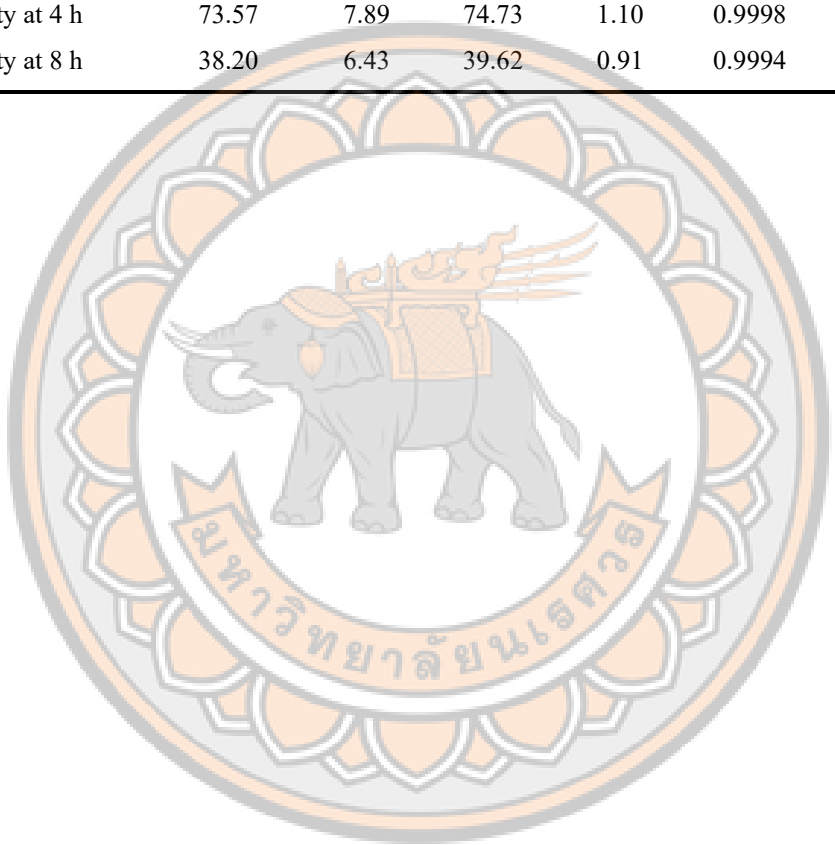

Table 3 Coded levels for independent variables used in spray dry experiment design for microencapsulation of medium chain fatty acid

Run	Code variables		Extraction condition Independent parameters		Dependent parameters				
	X1	X2	Temperature (ºC)	Flow rate (kg/h)	Microencapsulation efficiency (%)	Moisture (%)	Water activity	Density (g/cm^3)	Solubility (%)
1.00	1.00	-1.00	200.00	1.00	85.50	1.32	0.24	0.46	60.05
2.00	1.41	0.00	208.00	1.50	81.73	1.22	0.21	0.45	40.32
3.00	-1.00	-1.00	160.00	1.00	73.32	1.35	0.20	0.43	60.56
4.00	0.00	1.00	180.00	2.00	76.40	0.85	0.22	0.35	60.04
5.00	0.00	0.00	180.00	1.50	82.24	1.19	0.19	0.43	60.03
6.00	0.00	0.00	180.00	1.50	82.24	1.19	0.19	0.43	60.03
7.00	0.00	0.00	180.00	1.50	82.24	1.19	0.19	0.43	60.03
8.00	0.00	-1.41	180.00	0.79	86.88	1.33	0.27	0.46	60.07
9.00	0.00	0.00	180.00	1.50	82.24	1.19	0.19	0.43	60.03
10.00	0.00	0.00	180.00	1.50	82.24	1.19	0.19	0.43	60.03
11.00	0.00	1.41	180.00	2.21	82.71	0.92	0.16	0.34	36.28
12.00	1.00	1.00	200.00	2.00	96.01	1.02	0.20	0.44	40.35
13.00	-1.41	0.00	152.00	1.50	82.51	1.11	0.15	0.37	60.20

Table 4 Analysis results between prediction values and actual experiments of response surface in microencapsulation from medium fatty acid by the spray-drying method

Response	Prediction	SE mean	Actually	SE mean	R Square	Adjusted R square
Moisture	1.16	0.19	1.68	0.41	0.9774	-0.0226
a_w	0.20	0.01	0.26	0.04	0.9904	-0.0096
Bulk density	0.44	0.01	0.45	0.04	0.9978	-0.0022
Microencapsulation efficiency	83.13	0.50	80.55	1.41	0.9998	-0.0002
Yield	98.84	0.28	94.61	3.26	0.9992	-0.0008

a_w = water activity and ME = microencapsulation efficiency

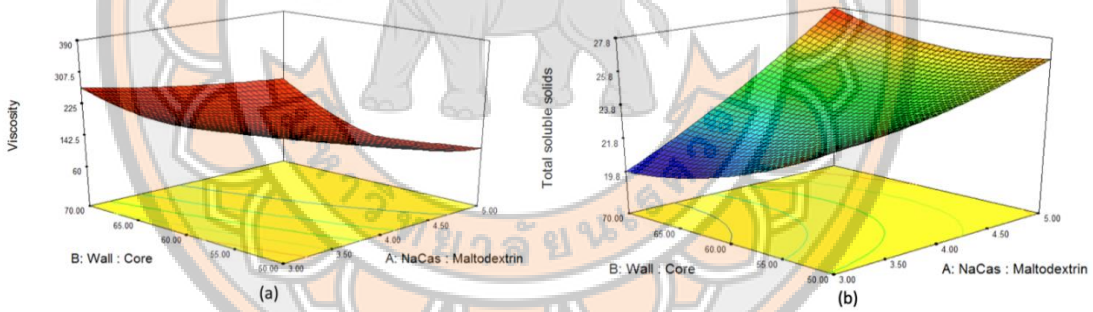

Figure 2 The 3D graph shows the relationship between coating ratio response values (NaCas:Maltodextrin), ratio of coating material (Wall material: Core material) effect on viscosity (a) and dissolved solids (b) by response surface methodology

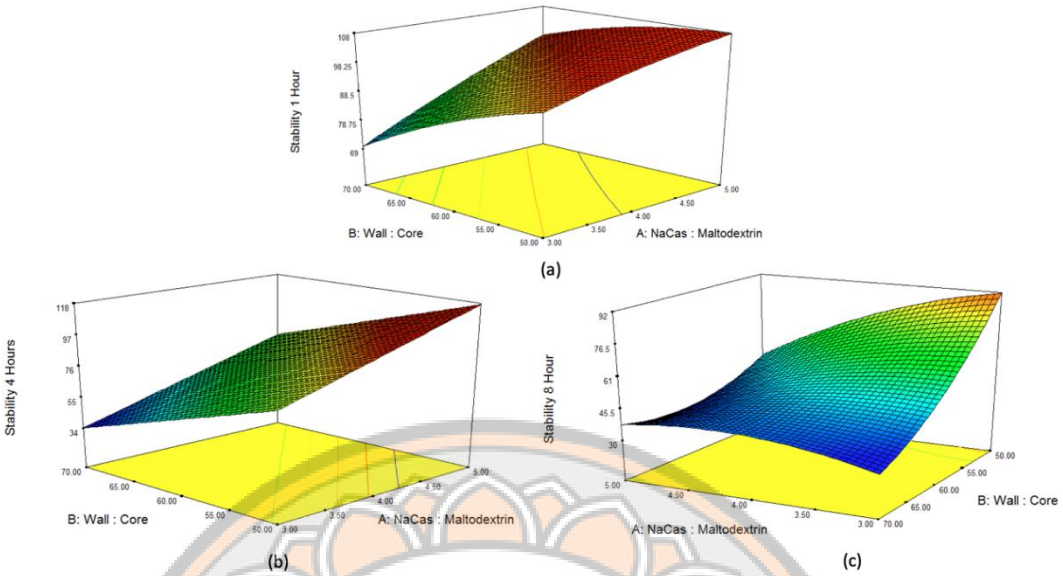

Figure 3 The 3D graph shows the relationship between coating ratio response values (NaCas: Maltodextrin), ratio of coating material (Wall material:Core material) to *1* h stability (a) *4* h stability (b) and *8* h stability (c) by response surface methodology

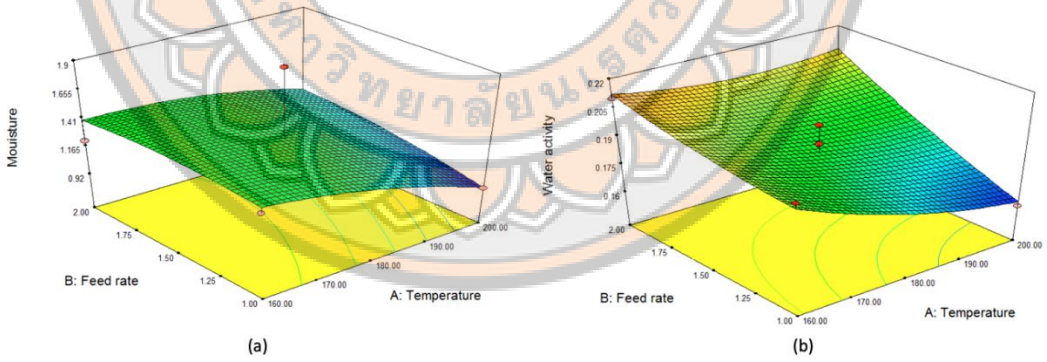

Figure 4 The 3D graph shows the relationship between coating ratio response values (NasCas: Maltodextrin), ratio of coating material (Wall material:Core material) effect on moisture (a) and water activity values (b) by response surface methodology

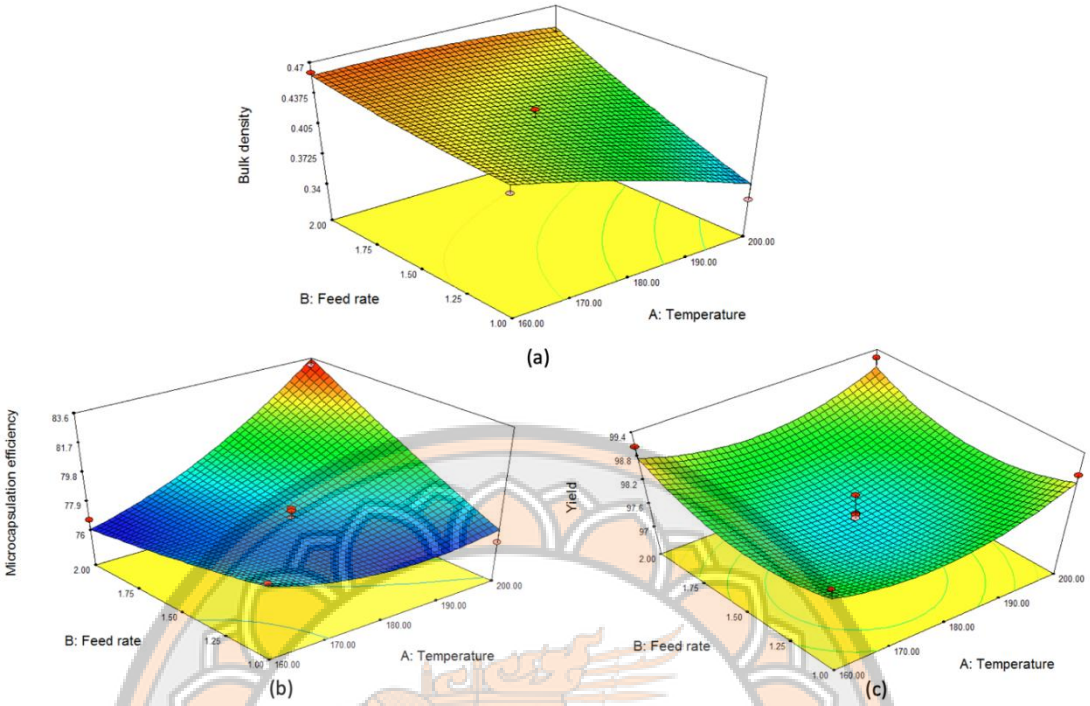

Figure 5 The 3D graph shows the relationship between coating ratio response values (NasCas: Maltodextrin), ratio of coating material (Wall material:Core material) to density (a), micro-capsule production efficiency (b) and yield (c) by response surface methodology

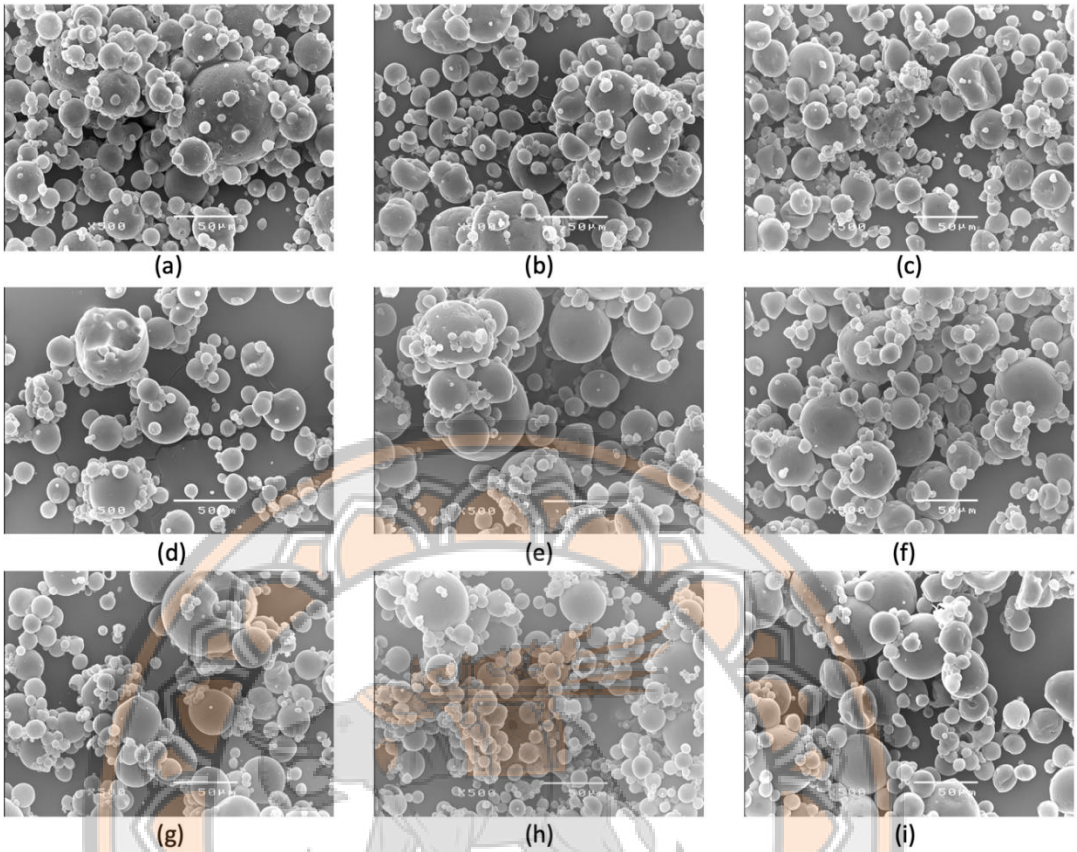

Figure 6 Surface area of microcapsulated medium chain fatty acid at various drying conditions (a), (b), (c) feed rate = 1 kg/h, inlet temperature 160°C, 180 C, 200°C; (d), (e), (f) feed rate = 1.5 kg/h, inlet temperature 160°C, 180°C, 200°C; (g), (h), (i) feed rate = 2 kg/h, inlet temperature 160°C, 180°C, 200°C (X500)

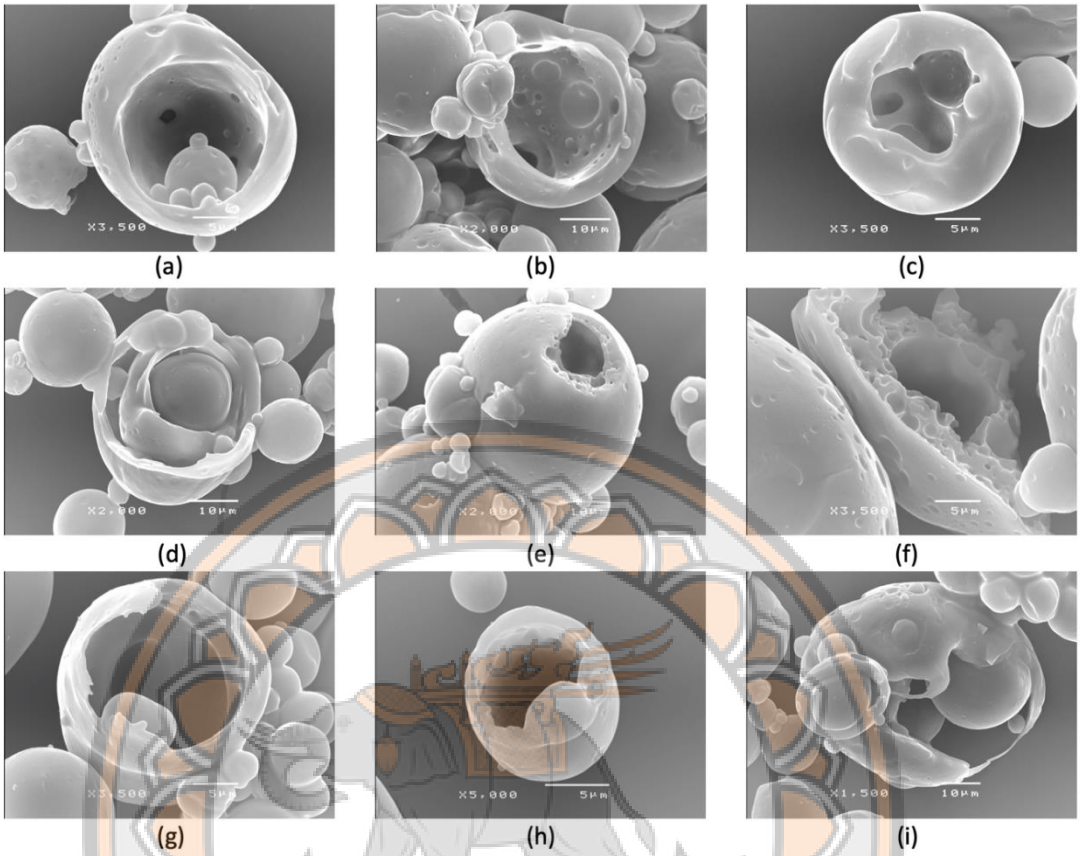

Figure 7 Structural characteristics of microencapsulate of medium-chain fatty acids at various drying conditions (a), (b), (c) feed rate = 1 kg/h, inlet temperature 160°C, 180°C, 200°C; (d), (e), (f) feed rate = 1.5 kg/h, inlet temperature 160°C, 180°C, 200°C; (g), (h), (i) feed rate = *2* kg/h, inlet temperature 160°C, 180°C, 200°C, respectively (*X*1500 - 5000)

Table 5 Fatty acid composition and nutritional factors

Fatty acid (g/100g)	Olils-mixed	Emulsion	miMCFA
Butyric acid (C4:0)	6.43	nd	nd
Caprylic acid (C8:0)	1.91	2.87	1.76
Capric acid (C10:0)	2.22	2.68	1.70
Undecylic acid (C11:0)	1.80	0.03	nd
Lauric acid (C12:0)	38.12	37.58	23.24
Tridecylic acid (C13:0)	0.03	0.05	nd
Myristic acid (C14:0)	13.74	13.74	8.59

Fatty acid (g/100g)	Olils-mixed	Emulsion	miMCFA
Palmitic acid (C16:0)	8.87	9.68	7.15
Stearic acid (C18:0)	2.14	0.03	2.02
Arachidic acid (C20:0)	0.12	nd	nd
Gondoic acid (C21:0)	0.05	nd	nd
Total SFAs	75.44	66.66	44.46
Palmitoleic acid (C16:1)	0.01	nd	nd
Oleic acid (C18:1 n9t)	15.62	15.15	12.31
Behenic acid (C22:1 n6)	0.04	nd	nd
Total MUFAs	15.66	15.15	12.31
Linoleic acid (C18:2 n6c)	9.34	2.98	9.94
Y-Linolenic acid (C18:3 n3)	0.04	0.28	0.03
a-linolenic acid (C20:2)	0.66	nd	nd
Total PUFAs	10.04	3.26	9.97
Tran fat	0.02	0.02	0.02
Omega 3	1.24	0.93	1.07
Omega 6	11.62	10.64	9.98
Omega 9	19.26	18.28	12.30
AI	3.57	3.82	2.77
TI	2.96	3.34	2.73
HH ratio	1.14	0.79	1.42
PUFA: SFA	0.13	0.05	0.22
Omega 6:3	9.35	11.41	9.32

nd = non-detection, AI = Atherogenic index, TI = Thrombogenic index,
HH = Hypocholesterolemic: Hypercholesterolemic

CHAPTER IV

EFFECT OF MICROENCAPSULATED MEDIUM-CHAIN FATTY ACIDS RICH-OILS POWDER SUPPLEMENTATION ON LACTATING SOW PERFORMANCE AND COLOSTRUM COMPOSITION

Abstract

This study evaluated the effects of supplementing lactating sow diets with microencapsulated medium-chain fatty acid (miMCFA) rich-oils on sow performance, colostrum composition, and piglet growth. Thirty sows (Large White × Landrace, parity 4.2 ± 0.31) were allocated to four groups: a control group and three treatment groups receiving 25 g/day, 50 g/day, and 75 g/day of miMCFA from day 100 of gestation to day 7 of lactation. Colostrum yield, sow body condition, and piglet growth were recorded, and colostrum samples were analyzed for fat, protein, lactose, and IgG content. The results showed that miMCFA supplementation significantly increased ($P < 0.05$) colostrum production in the 50 g/day and 75 g/day groups compared to the control group. The percentage of live-born piglets was also significantly higher ($P < 0.01$) in all miMCFA groups, with the 50 g/day and 75 g/day groups showing the greatest improvements. Piglet birth weight and litter weight at day 7 were significantly higher ($P < 0.05$) in the miMCFA-supplemented groups, particularly at 50 g/day and 75 g/day. Additionally, sows supplemented with 50 g/day and 75 g/day of miMCFA had significantly less back-fat loss during the lactation period ($P < 0.05$), indicating improved body condition maintenance. Colostrum composition analysis revealed that miMCFA supplementation increased fat content ($P < 0.05$), while protein, lactose, and IgG levels remained unaffected. These findings suggest that miMCFA supplementation supports improved colostrum quality and piglet growth, while helping to preserve sow body condition during lactation. The results indicate that supplementing lactating sows with 50 g/day to 75 g/day of miMCFA can enhance sow productivity and piglet survival, making it a promising nutritional strategy in modern swine production systems.

Keywords: medium-chain fatty acids; microencapsulation; fat powder; sows; sucking pig

Introduction

The modern swine industry is under increasing pressure to meet global demands for pork while maintaining sustainable and efficient production systems. In recent decades, genetic selection for improved productivity has led to the development of highly prolific sows that produce larger litters, often exceeding 14 piglets per farrowing. While this genetic improvement has contributed to increased pork production, it has also presented new challenges, particularly regarding sow nutrition during late gestation and lactation. The energy and nutrient demands during these critical periods have escalated, and improper management of these demands can negatively impact both sow and litter performance. Nutritional strategies that support sows during late gestation and lactation are essential for optimizing reproductive success and ensuring the well-being of both the sow and her offspring. During lactation, sows must consume adequate nutrients to support milk production and maintain their body condition, as insufficient nutrient intake can lead to a negative energy balance. This energy deficit may cause sows to mobilize their body reserves, particularly fat and muscle, to meet the high energy requirements for milk synthesis. Prolonged negative energy balance can result in excessive loss of body condition, which is associated with decreased reproductive performance in subsequent breeding cycles. To address these challenges, researchers have investigated various nutritional strategies, including the supplementation of fatty acids, which play a vital role in providing energy and supporting metabolic functions. Fatty acids are a major source of energy for sows, particularly during lactation when energy demands are at their highest. In addition to their role in energy metabolism, fatty acids possess a range of biological activities, including anti-inflammatory, antibacterial, and immune-modulating effects, making them essential components of the diet for lactating sows.

Medium-chain fatty acids (MCFAs), which are saturated fatty acids with chain lengths of 6 to 12 carbon atoms, have garnered increasing attention in recent years due to their unique properties and potential benefits in animal nutrition. Unlike long-chain fatty acids, which require complex digestion and transport mechanisms, MCFAs are rapidly absorbed and metabolized by the liver, providing a quick source of energy. This rapid metabolism makes MCFAs particularly suitable for supporting lactating sows, as they can help to reduce the severity of negative energy balance and support body

condition maintenance during lactation. Studies have demonstrated the positive effects of MCFAs on the performance of lactating sows and their piglets. Jean and Chiang (1999) investigated the impact of dietary fat supplementation on piglet survival rates and found that sows supplemented with medium-chain fatty acids had higher piglet survival rates compared to those supplemented with soybean oil. In their study, piglets weighing less than 1,100 g at birth had a survival rate of only 48% until day 3 post-birth when their dams were supplemented with 10% soybean oil. However, this survival rate increased to 80% and 98% when sows were supplemented with 10% coconut oil or MCFAs, respectively. Additionally, the hepatic glycogen content of piglets four hours after birth was higher in those born to sows fed medium-chain fatty acids or coconut oil from day 84 of gestation until farrowing. This finding suggests that MCFAs may provide piglets with a readily available energy source, thereby improving their early survival and growth. In the context of lactating sows, MCFAs not only provide an efficient energy source but may also have other physiological benefits. Research has shown that MCFAs exhibit antimicrobial properties, which can help reduce the bacterial load in the gastrointestinal tract and prevent the proliferation of pathogenic bacteria. Furthermore, MCFAs have been shown to modulate inflammatory responses, potentially reducing inflammation in the mammary glands and supporting overall under health. These properties make MCFAs an attractive supplement for improving sow performance and ensuring the health and growth of their litter. Despite the promising effects of MCFAs, one of the challenges in incorporating these fatty acids into animal diets is their instability. MCFAs are prone to oxidation and degradation when exposed to environmental factors such as heat, light, and air. To overcome this challenge, microencapsulation technology has been developed as a means of protecting bioactive compounds, including MCFAs, from degradation. Microencapsulation involves encapsulating the fatty acids within a protective coating, which serves as a physical barrie r that separates the core material from the surrounding environment. This coating not only enhances the stability of the fatty acids but also allows for controlled release in the animal's digestive system, ensuring that the bioactive compounds are delivered effectively to the target tissues.

Microencapsulation has been widely used to deliver bioactive compounds, such as essential oils, organic acids, and vitamins, to the gastrointestinal tract of animals. This technology is particularly beneficial for delivering MCFAs, as it ensures that the fatty acids remain intact until they reach the gut, where they can be absorbed and metabolized. In addition to enhancing the stability and bioavailability of fatty acids, microencapsulation also allows for the slow release of the fatty acids, providing a sustained energy source for the animal. Given the unique properties of MCFAs and the benefits of microencapsulation, there is growing interest in evaluating the potential of microencapsulated medium-chain fatty acid-rich oils as a dietary supplement for lactating sows. The combination of rapid energy provision, antimicrobial and anti-inflammatory properties, and the protective effect of microencapsulation makes this a promising nutritional strategy for improving sow and litter performance. Previous studies have shown that supplementing sow diets with microencapsulated fatty acids can improve various aspects of reproductive and lactational performance, including increased milk production, enhanced litter growth, and improved sow body condition. However, there is still limited information on the effects of microencapsulated MCFAs on colostrum composition, which plays a critical role in providing newborn piglets with essential nutrients and immune factors during the first hours of life (Devillers , 2004). Colostrum not only supplies piglets with energy but also provides immunoglobulins, proteins, and other components that are vital for immune protection and growth. Therefore, any dietary strategy that can enhance colostrum quality and yield may have a profound impact on piglet survival and development.

The aim of the present study was to investigate the effects of supplementing lactating sow diets with different levels of microencapsulated medium-chain fatty acid-rich oils on sow performance, colostrum composition, and litter growth. Specifically, the study aimed to determine whether the inclusion of microencapsulated MCFAs in sow diets would improve colostrum yield and composition, support piglet growth and survival, and help maintain sow body condition during the lactation period. The findings from this study will contribute to the growing body of knowledge on the use of MCFAs in swine nutrition and may provide new insights into effective nutritional strategies for improving the productivity and welfare of lactating sows.

Materials and Methods

1. Animal Use and Care

The animal use and care protocols were approved by Naresuan University Animal Care and Use Committee (NUACUC) (NU-AG630102).

2. Microencapsulated Medium-Chain Fatty Acids Powder

The microencapsulated medium-chain fatty acids (miMCFA) powder was produced using a spray-drying process as described by Lozinska et al. (2020). The nutrient composition of the miMCFA product is shown in Table 7. The product contained 0.78 g/100g ash, 3.78 g/100g moisture, 54.60 g/100g carbohydrates, 9.69 g/100g protein, 31.20 g/100g fat, and provided 537.96 kcal/100g. The fatty acid composition included caprylic acid (C8:0) at 1.76 g/100g, capric acid (C10:0) at 1.70 g/100g, lauric acid (C12:0) at 23.24 g/100g, and oleic acid (C18:1) at 12.31 g/100g, among others.

3. Experimental Design and Animals

40 sows (Large White × Landrace; 4.2 ± 0.31 parity) were assigned to one of four experimental treatments using a randomized complete block design (RCBD). The sows were initially grouped by body condition score (BCS) and back-fat thickness (BF) on day 100 of gestation and were balanced for parity among treatments. The four experimental groups included:

1. Control group (n=10) without miMCFA supplementation
2. Test group supplemented with 25 g/day miMCFA (n=10)
3. Test group supplemented with 50 g/day miMCFA (n=10)
4. Test group supplemented with 75 g/day miMCFA (n=10)

The miMCFA was top-dressed onto the sows' morning meal during the experimental period, which lasted for 21 days, from day 100 of gestation until 7 days after farrowing. Nutrients in the diet met the recommended requirements (NRC, 2012), and the nutrient content of the basal diets used during gestation and lactation periods is detailed in Table 6. The sows were housed in conventional farrowing crates (2.0 × 2.5 m^2) in an environmentally regulated facility, where the temperature was maintained at 25.6 (± 1.5°C). Heat lamps were used to provide supplemental warmth for the piglets. Sows were fed 3.5 kg/day of the gestating diets from day 100 of gestation to farrowing,

after which they were allowed ad libitum access to feed and water throughout the lactation period. Fresh feed was provided at 07:00, 13:00, and 17:00 each day.

4. Records and Sampling

Feed intake was recorded daily to calculate the average daily feed intake (ADFI) for each sow. Back-fat thickness was measured using an ultrasonic detection machine (Preg-Alert Pro, Renco Corp., Minneapolis, USA) at three time points: day 100 of gestation, farrowing, and day 7 of lactation. Back-fat loss during the study period was calculated based on these measurements.

Within 24 hours after farrowing, litter size was standardized to approximately 11 piglets per sow. Piglet body weight was recorded at birth and weaning to determine average daily weight gain (ADG). Colostrum yield (CY) was calculated as the sum of individual piglets' colostrum intake (CI) within a litter, following the method described by Devillers et al. (2004).

Colostrum samples were collected from sows within 24 hours of the first piglet's parturition. Approximately 15 mL of colostrum was collected from each gland and immediately frozen at −20°C for later analysis. Colostrum composition, including total solids (TS), fat, protein, lactose, salt content, and IgG levels (Brix%).

Statistical Analyses

Data were analyzed using the GLM procedure in SPSS (SPSS Software Release 23, IBM Inc., USA). Differences between treatment means were determined using Duncan's multiple range tests. Results are reported as means ± standard error of the mean (SEM), and statistical significance was considered at $P < 0.05$ or $P < 0.01$.

Results

1. Effects of miMCFA on sow performance

The total feed intake during gestation and lactation as well as the overall 21-day period did not differ significantly between the control group and the groups supplemented with 25 g/day, 50 g/day, or 75 g/day of microencapsulated medium-chain fatty acids (miMCFA) ($P > 0.05$) (Table 8). Sow in all groups consumed similar amounts of feed during the experiment. Specifically, the total feed intake at gestation day 100 was 46.00 kg in the control group, compared to 45.75 kg, 45.81 kg, and 45.91 kg in the 25 g/day, 50 g/day, and 75 g/day miMCFA groups, respectively ($P = 0.148$). Lactation

feed intake at day 7 was also similar across treatments, ranging from 32.79 kg in the control group to 34.03 kg in the 75 g/day group (P = 0.630). Over the entire 21-day lactation period, feed intake ranged from 78.79 kg to 80.71 kg, with no significant differences observed among the treatment groups (P = 0.659). Similarly, average daily feed intake (ADFI) during gestation and lactation periods did not differ significantly between the control and miMCFA-supplemented groups (P>0.05). ADFI at gestation day 100 was approximately 3.29–3.28 kg across all groups, and during lactation day 7, it ranged from 4.68 to 4.77 kg. The back-fat thickness (P2) at late gestation (day 100), farrowing (Lac1), and lactation day 7 (Lac7) was unaffected by the miMCFA supplementation (P > 0.05). Back-fat thickness at gestation day 100 ranged from 20.45 mm to 20.75 mm, and at lactation day 7, it was 19.50 mm in the control group and between 20.00 mm and 20.27 mm in the miMCFA groups. However, there was a significant difference in back-fat loss between gestation day 100 and lactation day 7. Sows supplemented with 50 g/day (P = 0.012) and 75 g/day (P = 0.012) of miMCFA showed significantly less back-fat loss (1.38 mm and 1.27 mm, respectively) compared to the control group (2.25 mm), indicating that miMCFA supplementation maintained back-fat during lactation. Sow body condition scores (BCS) at gestation day 100, farrowing, and lactation day 7 did not show significant differences between the control and miMCFA groups (P > 0.05). The BCS ranged from 2.94 to 2.97 at gestation day 100, 2.81 to 2.90 at farrowing, and 2.71 to 2.81 at lactation day 7. Notably, colostrum production per sow was significantly increased (P < 0.05) in the 50 g/day and 75 g/day miMCFA groups (5.66 kg and 4.73 kg, respectively) compared to the control group (3.53 kg). This suggests that miMCFA supplementation may enhance colostrum production in lactating sows. (Table 8)

2. Piglet Growth Performance

Piglet Growth Performance showed in Table 9 The total number of piglets born, live-born piglets, and birth weights were analyzed across the control and miMCFA-supplemented groups. The total number of piglets born was not significantly different between groups, with the control group producing 11.63 piglets compared to 13.63, 11.65, and 11.75 piglets in the 25 g/day, 50 g/day, and 75 g/day miMCFA groups, respectively (P > 0.05). Similarly, the number of live-born piglets was not significantly affected by miMCFA supplementation (P > 0.05). However, the percentage of live-born

piglets was significantly higher ($P < 0.01$) in the 25 g/day, 50 g/day, and 75 g/day miMCFA groups (85.33%, 87.83%, and 87.92%, respectively) compared to the control group (67.32%).

The stillborn and mummified piglet percentages were not significantly affected by miMCFA supplementation, although the mummified piglet percentage tended to reduce in the miMCFA-supplemented groups ($P = 0.052$). Piglet birth weight tended to increase in the miMCFA-supplemented groups compared to the control group, though this increase was not statistically significant ($P = 0.081$). At birth, piglets in the 50 g/day and 75 g/day groups weighed 1.64 kg and 1.74 kg, respectively, compared to 1.41 kg in the control group. By 7 days of age, the differences became more pronounced, with the 50 g/day and 75 g/day groups having significantly higher weights (3.22 kg and 3.32 kg, respectively) compared to the control group (2.67 kg, $P<0.006$). Litter weight followed a similar trend, with significantly higher weights in the miMCFA-supplemented groups. At birth, litter weights ranged from 11.59 kg in the control group to 16.53 kg in the 75 g/day group ($P = 0.046$). By 7 days of age, litter weight increased significantly in the 50 g/day and 75 g/day groups (32.38 kg and 32.48 kg, respectively), compared to the control group (21.79 kg, $P = 0.042$). Piglet weight gain between days 0 and 7 was not significantly different among groups ($P > 0.05$), although piglets in the miMCFA-supplemented groups tended to show higher weight gains. Average daily gain (ADG) was also not significantly different among groups; however, piglets in the miMCFA groups had slightly higher ADG compared to the control group at 7 days ($P > 0.05$). Fecal scores of piglets remained consistent across all groups, with no significant differences observed ($P = 0.634$).

The mortality rate of piglets across the different treatment groups is presented in Table 10. There were no significant differences in the total number of piglets born and the number of live-born piglets between the control and miMCFA-supplemented groups ($P > 0.05$). However, the live-born piglet percentage was significantly higher ($P < 0.01$) in the miMCFA-supplemented groups, with the 25 g/day, 50 g/day, and 75 g/day groups showing live-born rates of 85.83%, 87.83%, and 87.92%, respectively, compared to 67.32% in the control group. Although no statistically significant differences were observed in the causes of piglet mortality, there were noticeable trends. The percentage of stillborn piglets was lower in the miMCFA groups,

ranging from 5.52% to 5.92%, compared to 9.45% in the control group (P = 0.736). Similarly, the percentage of mummified piglets showed a decreasing trend in the miMCFA groups, particularly in the 50 g/day and 75 g/day groups, with mummified piglet percentages of 1.29% and 1.39%, respectively, compared to 8.47% in the control group (P = 0.052). The number of piglets that were crushed by the sow, disabled piglets, and piglets that died due to weakness were not significantly different among the groups (P > 0.05). Piglets crushed by the sow occurred less frequently in the miMCFA-supplemented groups (0.00% in the 50 g/day and 75 g/day groups) compared to the control group (2.08%, P = 0.283). Additionally, the percentage of weak piglets was reduced in the miMCFA groups, with the lowest percentage observed in the 50 g/day group (1.49%) compared to the control (6.63%, P = 0.175), although the difference was not statistically significant.

3. Effects of miMCFA on colostrum composition in sow

The effect of microencapsulated medium-chain fatty acids (miMCFA) supplementation on colostrum composition is shown in Table 11. The fat content in colostrum was significantly increased (P < 0.05) in the 50 g/day and 75 g/day miMCFA groups (5.72% and 5.78%, respectively) compared to the control group (3.47%). The fat content in the 25 g/day group (4.58%) was also higher than the control, although not significantly different from the other miMCFA groups. Lactose content showed no significant difference between the groups, with values ranging from 9.97% to 11.27% across all treatments (P = 0.583). Similarly, protein content was unaffected by miMCFA supplementation, with the control group at 7.47% and miMCFA groups ranging from 7.06% to 7.99% (P = 0.588). The density of colostrum and solid non-fat content also showed no significant differences among the groups (P > 0.05). The density ranged from 66.40% to 75.37%, and the solid non-fat content ranged from 18.85% to 21.22%. Salt content and IgG (Brix%) levels were not significantly influenced by miMCFA supplementation. IgG levels in the control group were 23.14%, while the miMCFA groups ranged from 24.63% to 25.80% (P = 0.646).

Discussion

The present study explored the effects of microencapsulated medium-chain fatty acids (miMCFA) supplementation on sow and litter performance, including feed intake, back-fat thickness, colostrum production, and piglet growth, as well as colostrum composition. The results, detailed in Tables 8 – 10 provide important insights into the potential benefits of miMCFA supplementation during late gestation and lactation.

lactating sows typically experience a negative energy balance, as they are unable to consume sufficient feed to meet their energy requirements for maintenance and milk production. This energy deficit can lead to the mobilization of body protein and fat stores, which over time may compromise reproductive performance and increase the risk of excessive body condition loss. Specifically, excessive loss of back-fat during gestation and lactation has been linked to poor reproductive outcomes and an increased percentage of stillborn piglets.

In this study, back-fat thickness was significantly better maintained in sows supplemented with 50 g/day and 75 g/day of miMCFA compared to the control group ($P < 0.05$). The reduction in back-fat loss suggests that miMCFA supplementation may help sows sustain their body condition during the lactation period, thereby minimizing the detrimental effects associated with excessive mobilization of energy reserves. This finding aligns with the understanding that maintaining an optimal back-fat thickness is crucial for ensuring the reproductive health of sows and improving the overall survival rate of piglets. Moreover, miMCFA supplementation significantly increased colostrum production, with the 50 g/day and 75 g/day groups showing the highest levels of production ($P < 0.05$). Colostrum is essential for the early growth and survival of piglets, as it provides not only nutrients but also immunological protection. Higher colostrum production in miMCFA-supplemented sows may contribute to improved early piglet growth and survival, as observed in this study.

The percentage of live-born piglets was significantly increased ($P < 0.01$) in all miMCFA groups compared to the control group, with the highest percentages observed in the 50 g/day and 75 g/day groups. This improvement in live-born piglet percentage is likely a reflection of the enhanced maternal condition and energy balance in miMCFA-supplemented sows, which may result in more robust pregnancies and higher piglet viability. Similar findings have been reported in previous studies, where fatty acid

supplementation in sow diets improved piglet survival rates and overall litter performance.

Litter weight at birth and at 7 days of age was also significantly increased in miMCFA-supplemented sows ($P < 0.05$), with the most notable improvements seen in the 50 g/day and 75 g/day groups. Piglet birth weight is a critical factor influencing piglet survival, growth, and weaning weight. The increased birth weights observed in this study are consistent with previous research, which has demonstrated the positive effects of fatty acid supplementation on the growth performance of suckling piglets . supplementing sow diets with 10% medium-chain triglycerides (MCTs) significantly increased the average daily body weight gain of piglets during the suckling period. The increased piglet weight gain from birth to 7 days of age in the miMCFA-supplemented groups further supports the notion that miMCFA supplementation enhances piglet growth performance. Although feed intake was not significantly affected by miMCFA supplementation in this study, it is possible that the improved colostrum and milk composition in miMCFA-supplemented sows contributed to the increased growth performance of their piglets. Colostrum and milk are the primary sources of nutrition for piglets during the first weeks of life, and their composition can have a significant impact on piglet development.

The effects of miMCFA supplementation on colostrum composition are detailed in Table 11. Notably, the fat content in colostrum was significantly increased ($P < 0.05$) in the 50 g/day and 75 g/day miMCFA groups compared to the control group. This increase in fat content is particularly important, as colostrum fat is a critical energy source for newborn piglets, supporting both thermoregulation and growth in the early stages of life. These findings are consistent with previous studies that have demonstrated the influence of dietary fatty acid supplementation on colostrum and milk composition. For example, Azain (1993) found that supplementing sow diets with 10% MCTs significantly increased the fat content in sow milk, similar to the results of this study. In addition to increased fat content, miMCFA supplementation appeared to have no significant effect on other components of colostrum, such as lactose, protein, density, solid nonfat, salts, or IgG (Brix%) levels. This suggests that the primary benefit of miMCFA supplementation in terms of colostrum composition lies in the increased energy density provided by the higher fat content. The lack of significant changes in

other colostrum components is consistent with findings from previous studies, where the primary impact of fatty acid supplementation was observed in the fat content of colostrum and milk, rather than in other nutrients.

The positive effects of miMCFA supplementation on colostrum fat content may also contribute to the increased piglet growth performance observed in this study. Fatty acids, especially medium-chain fatty acids (MCFAs), are rapidly metabolized and provide a readily available source of energy for both sows and piglets. By increasing the fat content in colostrum, miMCFA supplementation may improve the energy status of piglets, promoting more rapid growth and development during the critical early postnatal period (Azain, 1993). In addition to the benefits for piglets, the increased fat content in colostrum may also benefit sows by improving their overall lactational performance. The ability to produce high-fat colostrum and milk can help sows meet the energy demands of lactation more effectively, thereby reducing the risk of excessive body condition loss and supporting better long-term reproductive performance. The preservation of back-fat thickness in miMCFA-supplemented sows, as observed in this study, further underscores the potential benefits of miMCFA in supporting both maternal and offspring health.

Conclusion

This research demonstrated that miMCFA supplementation during late gestation and lactation has positive effects on both sow and piglet performance. Specifically, miMCFA supplementation improves colostrum production, increases colostrum fat content, enhances piglet growth performance, and helps maintain back-fat thickness in sows. These findings highlight the potential of miMCFA as a dietary supplement for improving reproductive and lactational performance in sows, ultimately leading to better piglet survival and growth. Future research should explore the long-term effects of miMCFA supplementation on sow reproductive performance across multiple parities, as well as its impact on the health and productivity of piglets through to weaning and beyond

Table 6 Nutritional content of the basal diets analyzed during the gestation and lactation period

Nutritional Content	Gestation Diet	Lactation Diet
[a]Metabolizable energy, kcal/kg	3050	3315
Crude protein, %	14.56	18.76
Crude fat, %	4.95	7.53
Crude fiber, %	6.62	4.72
Ash, %	6.46	6.55
Moisture, %	12.37	11.42
Lysine, %	1.61	2.74

[a]Metabolizable energy was provided higher nutriennt requirements than the NRC2012

Table 7 Nutrient composition and Fatty acid composition analysis of microencapsulated medium-chain fatty acids rich-oils powder supplementation

Item	miMCFA
Proximate composition analysis	
Ash (g/100g)	0.78
Moisture (g/100g)	3.78
Carbohydrates (g/100g)	54.60
Protein (g/100g)	9.69
Fat (g/100g)	31.20
Energy (kcal/100g)	537.96
Crude fiber (g/100g)	nd
Fatty acid (g/100g)	
Caprylic acid (C8:0)	1.76
Capric acid (C10:0)	1.70
Lauric acid (C12:0)	23.24
Myristic acid (C14:0)	8.59
Palmitic acid (C16:0)	7.15
Stearic acid (C18:0)	2.02
Total SFAs	44.46
Palmitoleic acid (C16:1)	nd
Oleic acid (C18:1 n9t)	12.31
Behenic acid (C22:1 n6)	nd
Total MUFAs	12.31

Item	miMCFA
Linoleic acid (C18:2 n6c)	9.94
Y-Linolenic acid (C18:3 n3)	0.03
a-linolenic acid (C20:2)	nd
Total PUFAs	9.97
Tran fat	0.02
Omega 3 (mg/100g)	1070.99
Omega 6 (mg/100g)	9983.07
Omega 9 (mg/100g)	12297.52

nd= non-detection

Table 8 Effect of microencapsulated medium-chain fatty acids rich-oils powder supplementation on feed intake and back-fat thickness

Item	Control	miMCFA			SEM	P-Value
		25 g/d	50 g/d	75 g/d		
Total feed intake (kg)						
gestating 100 d	46.00	45.75	45.81	45.91	0.041	0.148
lactating 7 d	32.79	33.40	34.80	34.90	0.660	0.614
overall 21d	78.79	79.28	80.71	80.81	0.657	0.630
Average daily feed intake (kg)						
gestating 100 d	3.29	3.27	3.28	3.28	0.008	0.541
lactating 7 d	4.68	4.77	4.88	4.99	0.094	0.703
overall 21d	3.75	3.78	3.75	3.85	0.031	0.659
Backfat thickness (P2) (mm)						
Late gestating 100 d (gas100)	20.75	17.63	19.40	19.50	0.762	0.561
Farrowing 1 d (Lac1)	17.75	19.75	19.40	19.50	0.570	0.606
Lactating 7 d (Lac7)	19.50	20.63	20.77	20.88	0.772	0.923
BF changes during ges100- Lac1	-3.00	2.13	-0.10	0.00	0.775	0.133
BF changes during Lac1- Lac7	1.75	0.88	1.27	1.38	0.736	0.983
BF changes during ges100 - Lac7	-1.25^{b}	3.00^{a}	1.27^{ab}	1.38^{ab}	0.554	<0.05
Sows body scoring						
Body scoring at gestating 100 d	2.94	3.19	2.90	3.00	0.046	0.114
Body scoring at farrowing	2.81	2.94	2.90	3.00	0.054	0.675
Body scoring at lactating 100 d	2.75	2.81	2.71	2.81	0.044	0.819
Colostrum production per sow (kg)	3.53^{b}	5.66^{a}	4.63^{ab}	4.73^{ab}	0.252	<0.05

P<0.05, <0.01 indicates a significant difference. [a]SEM means standard error of the mean. n = 10

Table 9 Effect of microencapsulated medium-chain fatty acids rich-oils powder supplementation on piglets growth performance

Item	Control	miMCFA			SEM[a]	P-Value
		25 g/d	50 g/d	75 g/d		
Total born piglets (n)	11.63	13.63	11.65	11.75	0.621	0.620
Live-born piglets (n)	8.25	11.63	10.27	10.38	0.620	0.291
Live-born piglet percentage (%)	67.32[b]	85.83[a]	87.83[a]	87.92[a]	2.511	<0.01
Still-born percentage (%)	9.45	5.92	5.52	5.63	1.416	0.736
Mummy piglet percentage (%)	8.47	2.27	1.29	1.39	1.093	0.052
Birth weight (kg)						
At birth	1.41	1.52	1.64	1.74	0.049	0.081
24 hr	1.49[b]	1.61[ab]	1.81[ab]	1.91[a]	0.051	0.010
3-d	1.80[b]	1.87[b]	2.16[ab]	2.26[a]	0.057	0.005
7-d	2.67[b]	2.81[ab]	3.22[ab]	3.32[a]	0.082	0.006
Litter weight (kg)						
At birth	11.59[b]	18.22[a]	16.24[ab]	16.35[ab]	0.883	0.046
24 hr	12.18[b]	20.10[a]	17.93[ab]	18.03a[b]	0.937	0.014
3-d	14.67[b]	22.54[a]	21.37[ab]	21.47[a]	1.118	0.044
7-d	21.79	32.51	32.38	32.48	1.803	0.082
Piglet weight gain (g)						
0-1-d	125.44	105.33	171.73	171.83	12.087	0.119
1-3-d	310.71	282.96	347.62	347.72	12.326	0.181
3-7-d	866.99	857.39	921.84	921.94	17.382	0.406
Average daily gain of piglet (g)						
3-d	155.36	141.48	173.76	173.86	6.162	0.181
7-d	216.75	214.35	223.95	224.05	4.810	0.707
Fecal scores of piglets	0.94	1.00	0.84	0.94	0.044	0.634

P<0.05, <0.01 indicates a significant difference. [a]SEM means standard error of the mean. n = 10.

Table 10 Effect of microencapsulated medium-chain fatty acids rich-oils powder supplementation on mortality rate

Item	Control	miMCFA			SEM[a]	P-Value
		25 g/d	50 g/d	75 g/d		
Total born piglets (n)	11.63	13.63	11.65	11.75	0.621	0.620
Live-born piglets (n)	8.25	11.63	10.27	10.38	0.620	0.291
Live-born piglet percentage (%)	67.32[b]	85.83[a]	87.83[a]	87.92[a]	2.511	<0.01
Cause of mortality						
Still-born percentage	9.45	5.92	5.52	5.63	1.416	0.736
Mummified piglet percentage	8.47	2.27	1.29	1.39	1.093	0.052
Crushing	2.08	0.78	0.00	0.00	0.444	0.283
Disable piglets	6.04	0.89	3.37	3.47	0.823	0.180
Weakness piglets	6.63	4.31	1.49	1.59	0.957	0.175

P<0.05, <0.01 indicates a significant difference. [a]SEM means standard error of the mean. n = 10.

Table 11 Effect of microencapsulated medium-chain fatty acids rich-oils powder supplementation on sow colostrum composition

Item	Control	miMCFA			SEM	P-Value
		25 g/d	50 g/d	75 g/d		
Fat (%)	3.47[b]	4.58[ab]	5.72[ab]	5.78[a]	0.334	<0.05
Lactose (%)	10.39	11.27	9.97	10.03	2.535	0.583
Protein (%)	7.47	7.99	7.06	7.12	0.362	0.588
Density (%)	70.11	75.37	66.40	66.46	0.689	0.616
Solid non fat (%)	19.67	21.22	18.85	18.91	0.266	0.613
Salts (%)	1.81	1.96	1.71	1.76	0.062	0.536
IgG (Brix%)	23.14	25.80	24.63	24.68	0.707	0.646

CHAPTER V

EFFECT OF MICROENCAPSULATED MEDIUM-CHAIN FATTY ACIDS, LIGNO-CELLULOSE, AND HEAT-KILLED *LACTOBACILLUS PLANTARUM* L-I37 SUPPLEMENTATION ON LACTATING SOW PERFORMANCE, AND NUTRITIONAL AND IMMUNOLOGICAL PARAMETERS IN COLOSTRUM

Abstract

This experiment aimed to evaluate the effects of supplementing miMCFA, lignocellulose, and HKL137 on lactating sow performance and nutritional composition and immunity in colostrum. Fifty 3–4 parity sows (Large White × Landrace) were randomly assigned to one of five treatments: 1) control (CON) without supplementation, 2) miMCFA (S1), 3) miMCFA + lignocellulose (S2), 4) miMCFA + HKL137 (S3), and 5) miMCFA + lignocellulose + HKL137 (S4). Supplements were daily added to the morning meal for 21 days (day 100 of gestation to day 7 post-farrowing). Compared with CON, the S1 and S4 groups had improvements ($P<0.01$) in live-born piglet numbers, and colostrum yield. Fat content in colostrum was significantly increased ($P<0.01$) in the S3 and S4 groups than those of CON group. Additionally, IgG levels were significantly greatest ($P < 0.05$) in the S3 and S4 groups. Likewise, IgM levels were notably highest ($P < 0.05$) in the S2 and S4 groups. These results indicated that the synergistic administration of miMCFA, lignocellulose, and HKL137 resulted in significant enhencements in both sow performance indices and colostrum metrics, en-compassing both quantitative and qualitative parameters.

Introduction

Feeding and nutrition of sows during late-gestation and lactation are crucial for maximizing piglet production and survival. During this stage, sows face increased nutritional demands as they support both their own needs and the development of their offspring. Adequate nutrition during this period has been associated with enhanced birth weights, higher survival rates, and improved overall health of piglets, which ultimately impacts the productivity of the swine industry (Liu et al., 2023). Among the various nutritional components, fatty acids play a pivotal role. They are not only a

primary energy source but also contribute to various physiological functions including metabolic regulation, antibacterial activity, and anti-inflammatory responses (Chen et al., 2022; Liu et al., 2021; Maybodi et al., 2022).

Previous studies have shown similar improvements in colostrum composition and piglet growth following supplementation with fatty acid supplementation including β-hydroxy β-methyl butyrate, polyunsaturated fatty acids, and medium-chain fatty acids (Craig et al., 2019; Lv et al., 2021; Zou et al., 2020). However, medium-chain fatty acids (MCFAs) are particularly important due to their unique chemical structure, characterized by fatty acids with 6 to 12 carbon atoms. These MCFAs occur naturally in milk fat and various feed materials in the form of medium-chain triglycerides (MCTs). Coconut oil and palm kernel oil are prominent sources of MCTs (Amer et al., 2021; Son et al., 2022). The hydrolysis of MCTs is faster than long-chain triglycerides partly due to their enhanced water solubility regardless of emulsifiers such as bile salts. MCT oils and fatty acids are efficiently absorbed and digested, making them viable energy sources for sow diets (Tian et al., 2017; Wang et al., 2022; Yuan et al., 2022). Recent studies indicated that supplementing sows with 10% coconut oil, a rich source of MCFAs, from day 84 of gestation until farrowing can significantly increase the survival rates of newborn piglets (Choi & Kim, 2020). However, the challenge of rancidity in high oil content feeds necessitates alternative delivery methods. The use of oil in powdered form, through microencapsulation, offers a promising solution. Microencapsulation provides a protective barrier around the core material, allowing for delayed release and better preservation of bioactive compounds until they reach the desired site of action in the animal's body (Choi et al., 2020). The encapsulation technology has directly delivered numerous bioactive substances (vitamins, organic acids, essential oils) to the intestines of animals, thus, improving their efficiency (Gardiner et al., 2020).

Recently, dietary fiber (DF) plays a crucial role in sow nutrition. It is a carbohydrate polymer that resists digestion in the small intestine and undergoes partial or complete fermentation in the large intestine. This fermentation process enhances gut microbiota profiles, improves barrier function, and boosts immunity, particularly in weaning and growing pigs (Shi et al., 2021). Some studies have focused on the supplementation of fiber sources such as lignocellulose into the diets of lactating sows.

These studies have demonstrated that the inclusion of fiber can affect positively performance and colostrum (Jin et al., 2018) by promoting digestion, weight management, and gut microbiota health (Loisel et al., 2013). Most of the research on DF has focused on its impact on sow welfare, colostrum production, physiology, and performance (Farmer & Quesnel, 2009; Ferreira Dos Santos et al., 2016). However, there is limited information on how DF influences the quantity and quality of colostrum and milk, as well as the immune status of the offspring. Because colostrum is the primary source of maternal antibodies for newborn piglets, understanding the impact of DF on colostrum composition is critical for enhancing piglet health and growth outcomes.

Another promising area of research involves the use of heat-killed *Lactobacillus plantarum* strain L137 (HKL137), a non-viable lactic acid bacterium isolated from fermented foods. HKL137 is known for its remarkable resistance to high temperature and pressure, which makes it a stable supplement in animal feed (Ferreira Dos Santos et al., 2016; Petrov et al., 2021). HKL137 has been suggested as a candidate for use as an immunobiotic. It also plays a significant part in innate immune responses, resistance to disease and stress, and stimulation of growth in a wide variety of mammalian and aquatic animals such as mice (Murosaki et al., 1998), pigs (Arimori et al., 2012), kuruma shrimp (Thanh Tung et al., 2010), and red sea bream (Dawood et al., 2015a, 2015b). In pigs, HKL137 has shown potential in enhancing immune responses and promoting growth, yet research on its effects in sows and their piglets remains scarce (Arimori et al., 2012). Tartrakoon et al. (2023) suggested that incorporating HKL137 in pig diets can enhance production performance and boost the immune system without the inconsistencies associated with traditional in-feed probiotics. Given its immunomodulatory properties, HKL137 supplementation during late-gestation and lactation could offer significant benefits, potentially enhancing sow health, improving colostrum quality, and boosting piglet immunity. On the other hand, research on sows and piglets remains exceptionally limited.

In the current research, we aimed to fill the existing knowledge gaps by providing insights into how these dietary components interact to influence sow and piglet health, potentially leading to improved outcomes in swine production. Thus, the objective of this study was to explore the effects of supplementing sow diets with

microencapsulated medium-chain fatty acids, lignocellulose, and HKL137 on lactation performance and nutritional composition and immunity in colostrum. We hypothesized that the combination of these dietary supplements would synergistically enhance sow lactation performance by improving colostrum yield and composition, resulting in improved piglet health status.

Materials and Methods

1. Ingredients

Microencapsulated medium-chain fatty acids (miMCFA) powder was produced from a mixture of crude palm kernel and soybean oils at optimized ratios. Maltodextrin and sodium caseinate served as encapsulating agents following a modified protocol based on (Ghosh et al., 2022). Finally, the microencapsulation of MCFA within the wall material was achieved through a continuous spray-drying process. The main nutrient composition per 100 g of miMCFA was: protein 9.69 g, fat 31.2 g, and energy 537.96 kcal. The fatty acids in miMCFA included caprylic acid (C8:0) 1.76 g, capric acid (C10:0) 1.70 g, lauric acid (C12:0) 23.24 g, myristic acid (C14:0) 8.59 g, palmitic acid (C16:0) 7.15 g, stearic acid (C18:0) 2.02 g, oleic acid (C18:1n9t) 12.31 g, linoleic acid (C18:2n6c) 9.94 g, and α-Linolenic acid (C18:3n3) 0.03 g.

Lignocellulose (Opticell, Agromed Austria GmbH, Kremsm¨unster, Austria) is a feed additive primarily composed of a natural fiber complex derived from plant cell walls. It contains 30-50% cellulose, 20-30% hemicellulose, and 15-25% lignin**.**

HKL137 (Feed LP20, House Wellness Foods, Itami, Japan) contains 20% HKL137, and the remaining 80% dextrin on a dry-weight basis with a concentration of approximately 1.0×10^{11} cells g^{-1}, were used. The product was stored at room temperature (25 ± 2 °C) until use.

2. Animal ethics

The current investigation was conducted at the swine research farm of the Faculty of Agriculture, Natural Re-sources, and Environment, Naresuan University (Phitsanulok province, Thailand). Animal procedures were approved by the Naresuan University Agricultural Animal Care and Use Committee (Approval ID 6301001), in

accordance with the Ethics of Animal Experimentation of the National Research Council of Thailand.

3. Experimental design

A total of 50 multiparous Large White × Landrace sows (average parity 3-4) were assigned to five experimental groups in a completely randomized block design based on parity, body condition score (BC), backfat thickness (BF), and body weight on day 100 of gestation. The control group (CON) was fed a basal diet without supplement, while other four treatments received the basal diet with diferent supplements as follows: 50 g of miMCFA (S1 group), 50 g of miMCFA + 30 g of lignocellulose (S2 group), 50 g of miMCFA + 0.10 g of HKL137 (S3 group), and 50 g of miMCFA + 30 g of lignocellulose + 0.10 g of HKL137 (S4 group). Each supplemental group was administered daily in the morning meal as a topping. The experimental period commenced on day 100 of gestation and continued until seven days post-farrowing, spanning a total of 21 days.

4. Animals, diets and management

The experiment was conducted at a commercial pig farm (Vilaipron Pig Farm, Nakhon Sawan Province, Thailand). The sows were maintained under a standard evaporative cooling system and housed in conventional farrowing crates measuring 2.0 × 2.5 m². The environmental temperature was controlled and maintained at 25.6 ± 1.5°C throughout the experimental period. From day 100 of gestation until farrowing, the sows in the experiment were daily fed with 3.5 kg of a standard diet. Following farrowing, the lactating sows were given *ad libitum* access to feed, offered three times daily at 07:00 A.M., 01:00 P.M., and 05:00 P.M. The feed consisted of a corn-soybean-based diet, formulated to meet or exceed the nutritional requirements for lactating sows according to the National Research Council (NRC) (Council, 2012). The analyzed chemical composition of the basal diet is presented in Table 12.

Sow reproductive and litter performance

During the experiment, several sow performance parameters were recorded, including the duration of parturition, total feed intake, average daily feed intake, body weight, total piglets born, live-born piglets, survival percentage, piglet birth weight, stillborn piglets, colostrum production, and colostrum intake per piglet. Backfat thickness was measured at farrowing and again on day 7 of lactation using an ultrasonic

detection device (Leanmeater®, Series 12, Renco Corp., Golden Valley, MN, USA) at the P2 point, which is located 60 mm to the left of the dorsal midline at the last rib. The average backfat thickness for each sow was calculated from three separate measurements, recorded in millimeters. The change in backfat thickness was determined by subtracting the measurement on day 7 of lactation from the value recorded at farrowing. Litter sizes were standardized to 11 piglets per sow 24 hours post-farrowing. Also, diarrhea scores were noted individually based on the appearance of piglet feces, categorized as normal (score = 0), soft (score = 1), or runny/watery (score = 2), and assessed throughout the lactation period (Yu et al., 2020).

Determination of Colostrum Consumption and Colostrum Yield

Colostrum intake (CI) for each piglet was estimated using a method that considers the piglet's birth weight and its weight gain during the first 24 hours postpartum. This approach has been widely used in research to approximate individual piglet colostrum consumption by factoring in early energy requirements and colostrum transfer efficiency. Colostrum yield (CY) for each sow was determined as the total amount of colostrum consumed by all piglets in the litter within the first 24 hours after birth. This formula, as outlined by Theil et al. (Theil et al., 2014) is expressed as: Colostrum consumption (g) = − 106 + 2.26WG + 200BWB + 0.111D – 1414WG/D + 0.0182WG/BWB. Here, WG represents the piglet's weight gain over 24 h (g), BWB stands for birth weight (kg), and D indicates the duration of colostrum suckling (min).

Collection of Colostrum and Milk Samples

Colostrum was manually collected from all functional teats within the first piglet parturition period, and mature milk was gathered from the same teats on day 7 of lactation (Nicolas et al., 2016). To aid in milk let-down for the mature milk collection, sows were given an intravenous injection of 0.2 mL oxytocin (10 IU/mL, VetOne®, Boise, ID, USA). Prior to both colostrum and milk collection, the udders were cleaned with sterile water and dried with a towel to minimize contamination. About 30 mL of colostrum and mature milk were collected from all functional mammary glands into centrifuge tubes. The samples were filtered through gauze, transferred into clean 30 mL bottles, and stored in a Styrofoam box at 4°C during collection. Upon arrival at the lab, the samples were centrifuged at 2700× g for 20 minutes at 4°C and stored at -20°C for later analysis (Paludetti et al., 2018).

Determination of Major Chemical Composition in Sow Colostrum and Milk

The colostrum and mature milk composition parameters included fat, solids-non-fat (SNF), density, protein, lactose, salt and pH using Master Eco, Milkotester LTD, Belovo, Bulgaria. Colostrum immunoglobulin G (IgG) was measured as Brix percent using 0.3 mL of colostrum sample and a digital Brix refractometer. This device is designed to measure the percentage of sucrose in liquids, approximates the total solids (TS%) content when used with non-sucrose-containing liquids. A commercial digital refractometer with a range of 0 to 55% Brix was used for measurements, specifically the Digital Brix Meter Sugar Refractometer from Deltatrak, CA, USA (Hasan et al., 2016).

Analysis of Sow Colostrum Immunoglobulin

The concentrations of immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) in sow colostrum was quantified using commercially available immunoglobulin-specific enzyme-linked immunosorbent assay (ELISA) kits (IgG, IgM, IgA quantitation kit; Sanwei Biological Engineering Co., Ltd., Shandong, China). The ELISA technique allows for the precise measurement of these immunoglobulins by utilizing specific antibodies that bind to the target proteins in the samples (Nuntapaitoon et al., 2019). An automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY, USA) was employed for the quantification process. This automated system enhances the reproducibility of the results by standardizing the reaction conditions and ensuring consistency across all sample analyses. This method was critical for assessing the immune properties of colostrum as immunoglobulins such as IgG, IgA, and IgM are key components in passive immunity transfer from the sow to her piglets. Their concentrations provide insight into both maternal immune function and the effectiveness of colostrum in conferring immunity to newborn piglets (Papatsiros et al., 2022).

Statistical analysis

Descriptive statistics (i.e., means and standard error of the means) were calculated using the statistical package SAS 9.4 (SAS Institute, Cary, NC, USA). Sow performance was analyzed using multiple analyses of variance via the general linear model (GLM) procedure in SAS. Diarrhea scores were analyzed using the general linear mixed model procedure in SAS. The effects of treatment groups were analyzed using

the general linear model procedure, and least-squares means were obtained for each parity class (3–4). Statistical significance was determined at $P < 0.05$. The statistical model formula for this study can be expressed as follows, tailored for the experimental design described:

$$Y_{ijk} = \mu + T_i + P_j + B_k + \epsilon_{ijk}$$

Where Y_{Ijk} is The response variable (e.g., sow performance metrics, colostrum yield, piglet birth weight), μ is The overall mean of the response variable, Ti is Fixed effect of the treatment (i = 1, 2, 3, 4, 5 for control and four supplement groups), P_j is Fixed effect of parity (j = 3, 4 for sow parity classes), B_k is Fixed block effect (e.g., initial body condition score or backfat thickness), ϵ_{ijk} is Random error associated with each observation, assumed to be independently and normally distributed ($\epsilon \sim N(0,\sigma^2)$).

Results

1. Sow Performance Parameters

The effects of miMCFA, lignocellulose, and HKL137 supplementation on sow reproductive performance are presented in Table 13. The live-born piglets and yield of colostrum production in the S1 and S4 group was significantly greater than those of the CON group. Conversely, no notable differences ($P > 0.05$) were observed in the duration of gestation, length of parturition, length of parturition per piglet, total feed intake, average daily feed intake, body weight, backfat thickness, total born piglets, alive percentage, piglet birth weight, stillborn piglets, mummified piglets or colostrum intake per piglet.

2. Major Chemical Composition of Sow Colostrum and Milk

Effect of miMCFA, lignocellulose, and HKL137 supplementation on the nutritional composition of sow colostrum and mature milk are displayed in Figure 8 and 9, respectively . Fat content in colostrum was significantly increased in the S3 and S4 group ($P<0.01$) than those of CON group. Additionally, IgG levels, measured using the Brix percentage, were significantly greater in the S3 and S4 groups compared to

CON groups ($P < 0.05$). However, there were no differences in terms of protein, lactose, density, and solids-non-fat content in colostrum. By day 7 of lactation, the mature milk from all groups exhibited no significant variations in fat, protein, lactose, density, IgG levels, or solids-non-fat content.

3. Changes in Immunoglobulins of Sow Colostrum

Effect of miMCFA, lignocellulose, and HKL137 supplementation on the immunoglobulins alteration of sow colostrum showed in Figure 10. The S3 and S4 groups demonstrated markedly elevated IgG levels compared to the CON group and other supplementation groups ($P < 0.05$). Likewise, IgM levels were notably higher in the S2 and S4 groups compared to the CON group and other treatments ($P < 0.05$). In contrast, IgA levels in sow colostrum showed no significant differences across the groups ($P > 0.05$).

Discussion

The results of the present study suggest that supplementing lactating sows with a combination of miMCFA, lignocellulose, and HKL137 (S4 group) can positively impact sow performance and colostrum composition. Specifically, the supplementation of miMCFA+Lig+HKL137 enhanced the amount of colostrum yield. Furthermore, the colostrum from supplemented sows had greater levels of beneficial fatty acids and immune-modulating compounds, potentially contributing to improved piglet growth and health. Previous studies suggest that dietary supplementation with medium-chain fatty acids (MCFA) positively affects sow performance and colostrum composition. According to Chen et al. (2019), MCFA supplementation shortened the weaning-to-estrus interval of sows and increased the fat and protein content in colostrum. Lan and Kim (2018) also observed improved performance in sows and their piglets with MCFA supplementation, including increased Lactobacillus counts and decreased Escherichia coli counts in faecal samples. Additionally, Jin et al. (2017) reported that supplementation with various fat sources, including MCFA, improved growth performance of nursing piglets and increased the concentration of specific fatty acids in colostrum and milk.

Previous studies indicated that dietary supplementation with MCFA positively affects sow performance and colostrum composition. The benefits of fatty acids on the

overall growth performance of piglets nursing from the sow are well documented . Supplementing sow's diet with fatty acids during the latter stages of pregnancy and lactation increased piglet's daily average body weight gain after weaning (Goh et al., 2022; Peltoniemi et al., 2019; Roszkos et al., 2020). Previous research indicated that supplementing sows with 10% MCTs resulted in increased average daily body weight gain of in nursing piglets. Feed intake and milk composition were identified as the primary parameters affecting growth performance (Chen et al., 2021; Lv et al., 2021; Szyndler-Nędza et al., 2022). Thus, the greater proportion of live-born piglets could be attributable to changes in fatty acid or calorie intake in addition to changes in sow milk composition due to dietary supplementation with the miMCFA+Lig+HKL137. The elevated levels of miMCFA in this study did not significantly impact the average daily feed consumption of sows. This study had a positive impact on sow backfat from late gestation through 7 days into of lactation.

Supplementation of miMCFA+Lig+HKL137 improved the number of live piglets. These findings suggest that incorporating miMCFA, lignocellulose, and HKL137 into sow diets positively influences piglet survival. These results are consistent with previous research highlighting the potential of dietary supplements and additives to enhance piglet growth and health (Qi et al., 2020; Wang et al., 2017). Previous studies suggested that dietary fiber supplementation in sow diets can have various effects on sow performance and colostrum composition. Yang et al. (2021) reported that high-fiber diets increased the number of piglets born alive and improved litter weight. observed that high-fiber diets improved weaning weight and colostrum immunoglobulin levels. Gao et al. (2023) found that different fiber sources had varying effects on farrowing performance and colostrum dry matter concentration. Feyera et al. (2021) reported that high-fiber diets increased colostrum lipid content and colostrum intake in low-birth-weight piglets.

Fiber can play a role volatile fatty acid (VFAs) production in the digestive system of sows. Fibers are complex carbohydrates that are not fully digestible in the small intestine of monogastric animals like pigs. Instead, they undergo fermentation in the hindgut (cecum and colon) by the microbial population present in the gastrointestinal tract (Niu et al., 2022; Varel et al., 1987). During fermentation, microbes break down complex carbohydrates such as fiber into simpler compounds

including VFAs. The primary VFAs produced in this process are acetic acid, propionic acid, and butyric acid. These VFAs serve as an energy source for the pig and can be absorbed and utilized (Lowell et al., 2015). The relationship between VFAs and milk fat production is not straightforward. While VFAs contribute to the overall energy supply for sows, the actual synthesis of milk fat occurs in the mammary glands. Triglycerides, the main components of milk fat, are produced within mammary cells from fatty acids obtained from dietary fats and mobilized body fat reserves. Despite fiber potentially contributing to VFA production and energy for sows, their direct influence on milk fat synthesis remains unclear (Lv et al., 2018; Yan et al., 2017). However, supplementing of sow diets with lignocellulose has been shown to improve gut health and an increase in the number of goblet cells in piglets, offering several benefits including improved gut health.

Supplementation of with miMCFA+Lig+HKL137 also demonstrated potential for enhancing piglet immune response. Additionally, HKL137 has shown promise in boosting the immune components of sow colostrum and milk. These findings suggest that supplementation with HKL137 in sow diets can potentially enhance the immune factors in both colostrum and milk. A previous study indicated that the effects of heat-killed *Lactobacillus plantarum* on sow performance and colostrum composition were influenced by various factors. Nardone et al. (1997) reported that heat stress in cows during late pregnancy and the early postpartum period resulted in colostrum with lower concentrations of immunoglobulins and other components. Betancur et al. (2021) highlighted factors such as parity, genotype, endocrine status, and nutrition can affect colostrum yield and composition in sows and demonstrated that oral administration of *Lactobacillus plantarum* CAM6 to breeding sows improved the content of lactose, nonfat solids, mineral salts, and density of sow's milk, while reducing milk fat. Additionally, Shang et al. (2019) reported that fermented liquid feeding in sows resulted in lower coliform populations in feces and higher levels of lactic acid bacteria, which may contribute to improved colostrum quality. These findings suggest that heat-killed *Lactobacillus plantarum* and dietary factors can influence sow performance and colostrum composition.

Overall, the observed results suggest that the interactive mechanisms among miMCFA, lignocellulose, and HKL137 supplementation likely contribute to the

enhanced performance of sows and improved colostrum composition. The miMCFA may play a role in providing a readily available energy source and modulating lipid metabolism, while lignocellulose could influence gut health through enhanced fiber fermentation and VFAs production. In addition, HKL137 may further enhance immune function as a heat-killed probiotic by modulating the immune components of colostrum and milk. These interactions collectively likely account for the improved sow productivity and piglet health. However, further investigations into these synergistic effects and underlying mechanisms are warranted to fully elucidate the observed outcomes.

Conclusions

The administration of miMCFA demonstrated significant enhancement of live-born piglet numbers, and elevated colostrum yield. Furthermore, the synergistic application of miMCFA in conjunction with lignocellu-lose and HKL137 exhibited additional neonatal piglet benefits and sow performance, specifically in terms of fat content and immunoglobulin G and M (IgG and IgM) concentrations in colostrum. These findings suggest potential mechanisms for enhanced maternal-offspring immune transfer, which may consequently lead to improved maternal health outcomes, superior colostrum quality, and enhanced immunological competence and growth performance in piglets. Finally, these results suggest that supplementing sow diets with miMCFA, lignocellulose, and HKL137 can significantly improve piglet survival and growth, offering a practical strategy for swine producers.

Table 12 Nutritional content of the basal diets during the gestation and lactation period

Nutritional Content	Gestation Diet	Lactation Diet
[a]Metabolizable energy, kcal/kg	3050	3315
Crude protein, %	14.56	18.76
Crude fat, %	4.95	7.53
Crude fiber, %	6.62	4.72
Ash, %	6.46	6.55
Moisture, %	12.37	11.42
Lysine, %	1.61	2.74

[a]Metabolizable energy was provided higher nutriennt requirements than the NRC2012

Table 13 Effect of microencapsulated medium-chain fatty acids, lignocellulose, and HK L-137 supplementation on sow reproductive performance

Item	CON	Dietary supplement treatment				SEM	P-value
		S1	S2	S3	S4		
	n=10	n=10	n=10	n=10	n=10		
Duration of gestation, day	112.6	112.4	112.5	113	112.8	0.12	0.536
Length of parturition, min	465.7	357.6	381.0	364.8	447.7	27.75	0.647
Length of parturition per piglet, min	42.8	23.7	27.9	31.9	32.0	2.86	0.294
Total feed intake							
Gestation day 100-114, kg	49.8	48.9	50.1	52.1	51.6	0.40	0.051
Lactation day 1-7, kg	23.1	23.7	24.8	23.5	26.4	0.89	0.792
Average daily feed intake							
Gestation day 100-114, kg	3.7	3.7	3.8	3.7	3.7	0.03	0.842
Lactation day 1-7, kg	2.9	3.0	3.1	2.9	3.3	0.11	0.792
Body weight							
Late-gestation day 100, kg	226.0	233.2	230.5	220.7	228.7	4.44	0.938
Lactation day 7, kg	208.4	216.4	216.7	197.8	209.3	3.61	0.542
BW changes, kg	-17.6	-16.8	-13.8	-22.9	-19.4	3.44	0.963
BW changes, %	-7.2	-6.8	-4.1	-10.2	-8.4	1.47	0.829
Backfat thickness (P2)							
Late-gestation day 100, mm	21.0	21.8	19.7	18.8	18.4	0.55	0.202
Lactation day 7, mm	18.6	21.2	19.3	18.8	18.8	0.54	0.460
BF changes, mm	-2.4	-0.6	-0.4	0.00	0.4	0.46	0.395
BF changes, %	-10.2	-2.3	-1.9	1.9	2.5	2.11	0.349
Total born piglets, n	14.7	17.6	15.7	14.6	16.1	0.51	0.335
Live-born piglets, n	11.5^{b}	15.3^{a}	13.9^{ab}	12.8^{ab}	14.4^{a}	0.41	0.026
Alive percentage, %	79.7	87.2	88.6	80.7	90.0	1.62	0.147
Piglet birth weight, kg	1.4	1.4	1.5	1.5	1.4	0.03	0.679
Stillborn piglets, %	2.6	2.2	1.3	2.2	1.40	0.34	0.727
Mummified piglet, %	1.4	0.9	1.0	1.0	0.70	0.11	0.358
Colostrum production, kg/sow	4.3^{c}	6.5^{ab}	5.4^{bc}	4.5^{c}	6.9^{a}	0.26	0.001
Colostrum intake per piglet, g/d/piglet	389.2	435.4	390.8	411.1	480.5	13.66	0.185

Data are expressed as means with the standard errors of the means **(**n=10**).** CON, animals did not receive the supplement; S1, animals daily received 50 g microencapsulated medium-chain fatty acid in the morning meal as a topping; S2, animals daily received 50 g microencapsulated medium-chain fatty acid + 30 g lignocellulose in the morning meal as a topping; S3, animals daily received 50 g microencapsulated medium-chain fatty acid + 0**.**10 g heat-killed *Lactobacillus plantarum* strain L137 in the morning meal as a topping; S4, animals daily received 50 g microencapsulated medium-chain fatty acid + 30 g lignocellulose + 0**.**10 g heat-killed *Lactobacillus plantarum* strain L137 in the morning meal as a topping**.** Means with different superscripts within each row are significantly different (P < 0.05). SEM = standard errors of means**.**

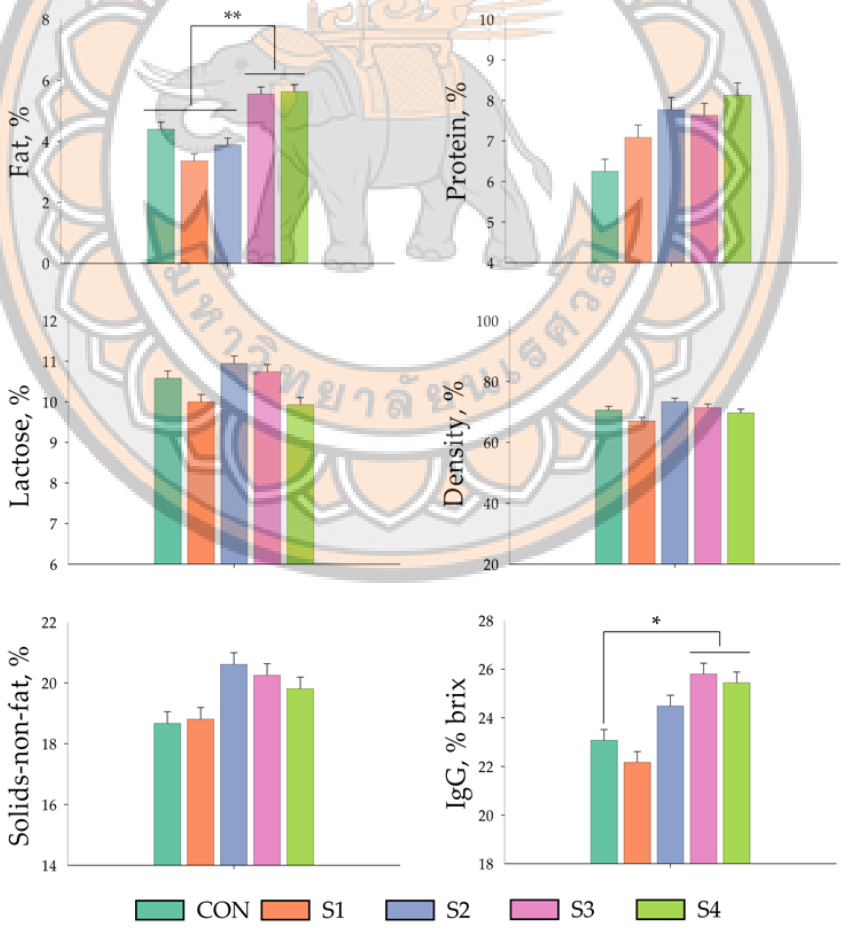

Figure 8 Effect of miMCFA, lignocellulose, and HKL137 supplementation on the nutritional composition of sow colostrum. ** = **P<0.01,** * = **P<0.05**

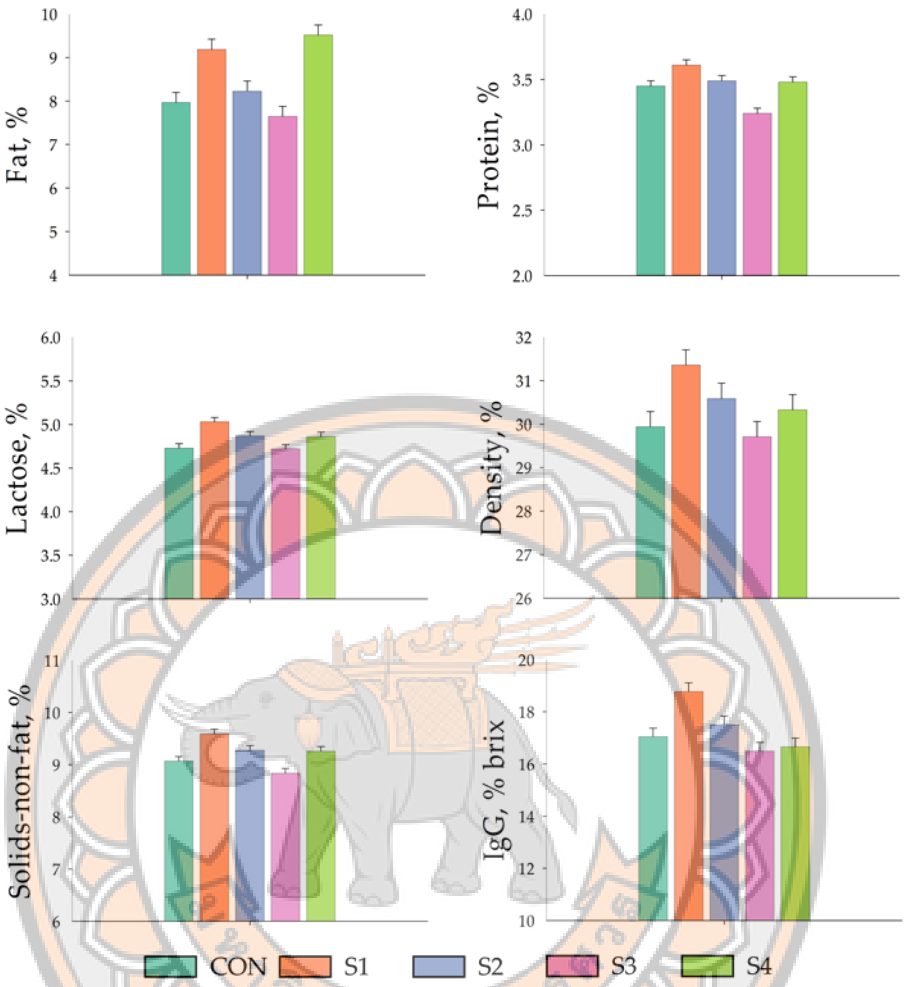

Figure 9 Effect of miMCFA, lignocellulose, and HKL137 supplementation on the nutritional composition of mature milk.

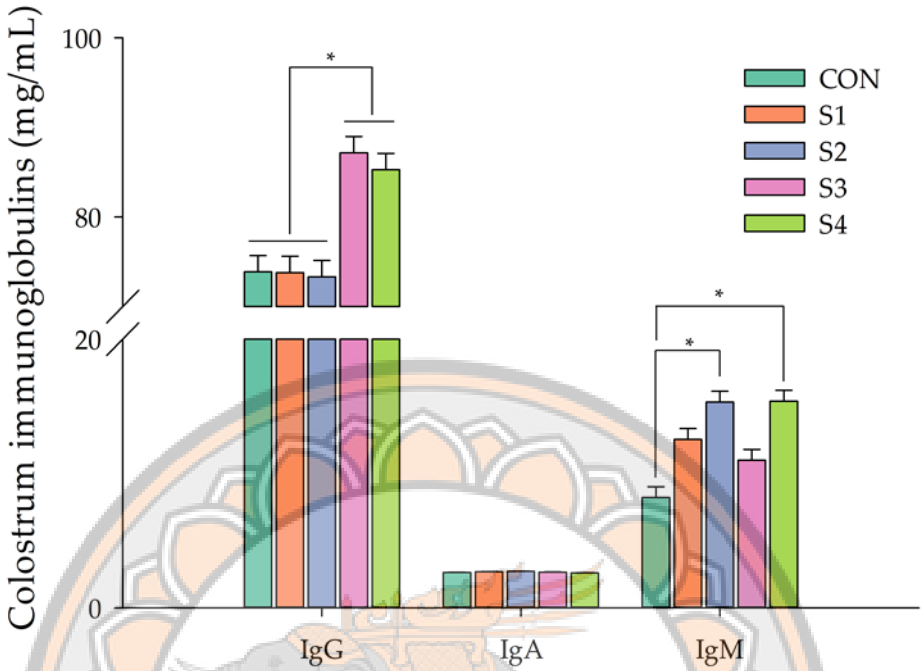

Figure 10 Effect of miMCFA, lignocellulose, and HKL137 supplementation on the immunoglobulin alteration of sow colostrum. * = P<0.05

CHAPTER VI

OPTIMIZING LEVELS AND TIMING OF FUNCTIONAL NUTRIENT SUPPLEMENTATION

Abstract

This study investigated how different levels of functional nutrient supplementation (FNS) during gestation and lactation influence sow and piglet performance, as well as colostrum and milk quality, immunoglobulin levels, fatty acid profiles, and IGF-1 gene expression. Sixty sows were assigned to four groups: a Control group (no FNS) and three supplementation levels—Low (40 g/day), Mid (80 g/day), and High (120 g/day). Overall, FNS had minimal effects on sow performance and piglet growth, as litter size, birth weight, and weaning weight were not significantly different among groups. However, FNS notably influenced colostrum quality. Sows in the Low and Mid groups produced colostrum with significantly higher fat content and IgG concentrations compared to the Control and High groups, indicating improved energy supply and passive immunity transfer to piglets. IgA and IgM levels were not significantly affected by supplementation. Fatty acid profiles also showed no major changes, although a slight increase in omega-3 fatty acids was observed in the Low Lactation group. Functional nutrients affected IGF-1 gene expression, particularly during gestation. The Mid and High groups showed significantly higher IGF-1 expression, suggesting enhanced fetal growth potential. In lactating sows, IGF-1 levels remained highest in the Mid group, though the differences were smaller than those observed during gestation. In summary, FNS supplementation had modest effects on reproductive and growth outcomes but improved specific colostrum components and stimulated IGF-1 expression. These results indicate that functional nutrients may strengthen passive immunity and support fetal development. Further research is needed to refine supplementation levels and timing to maximize benefits in commercial swine production.

Introduction

The pig farming industry, particularly in Thailand, plays a crucial role in the economy, contributing significantly through the production of millions of live pigs annually. As industry evolves, there is an increasing focus on improving the productivity and welfare of sows to meet the growing demands for high-quality pork. One of the major challenges faced by pig farmers today is ensuring that sows produce adequate quantities of high-quality milk to support the healthy growth and development of piglets. This challenge is particularly pronounced in prolific sows, which have been genetically selected for their ability to produce large litters, often exceeding 20 piglets per litter. While these genetic advancements have been successful in increasing litter size, they have also introduced new challenges, particularly in the areas of neonatal mortality and piglet growth. The neonatal period is a critical phase for piglets, with high mortality rates often attributed to factors such as inadequate nutrition, poor colostrum intake, and insufficient immune protection. To address these issues, there has been a growing interest in the use of functional nutrient products that go beyond basic nutrition to enhance the health and productivity of sows and their litter. These functional nutrients, including medium-chain fatty acids (MCFAs), lignocellulose, and heat-killed *Lactobacillus plantarum* strain L-137 (HK L-137), have shown promise in improving milk quality, boosting the immune system, and supporting overall sow health during gestation and lactation.

In particular, the timing and level of supplementation with these functional nutrients are critical factors that can influence their effectiveness. Previous studies have indicated that the appropriate timing of nutrient supplementation can significantly impact the physiological responses of sows, including milk production, colostrum quality, and the transfer of immunity to piglets. Similarly, the levels at which these nutrients are provided can determine the extent of their benefits, with both under-supplementation and over-supplementation potentially leading to suboptimal outcomes. Despite the recognized importance of functional nutrient supplementation, there remains a gap in the literature regarding the optimal levels and timing of these supplements. This study seeks to address this gap by systematically evaluating the effects of varying levels and timing of functional nutrient supplementation on sow productivity, milk production, and piglet growth and immunity. By doing so, the study

aims to identify the most effective strategies for maximizing the benefits of functional nutrients in sow diets, ultimately contributing to improved sow and piglet health and enhanced productivity in the pig farming industry.

This chapter will provide an in-depth analysis of the influence of different levels and timing of functional nutrient supplementation on sow performance. It will explore how these variables affect key indicators such as milk yield, milk composition, piglet growth rates, and immune status. Additionally, the chapter will discuss the potential mechanisms through which these nutrients exert their effects, drawing on the latest research in animal nutrition and physiology. The findings from this study are expected to provide valuable insights into the optimal use of functional nutrients in sow diets, offering practical recommendations for pig farmers and feed manufacturers. These recommendations will be particularly relevant in the context of reducing the reliance on antibiotics and other synthetic additives, in line with the global trend towards more sustainable and health-conscious animal production practices.

The study presented in this chapter aims to advance our understanding of the role of functional nutrients in enhancing sow productivity and piglet survival. By investigating the interplay between nutrient levels, timing of supplementation, and their physiological impacts, this research will contribute to the development of more effective feeding strategies that support the health and well-being of sows and their offspring.

Materials and Methods

Experimental Design

The study was structured to evaluate the optimal level and duration of functional nutrition supplementation (FNS) for pregnant and lactating sows. The experimental design followed a 3x2 factorial arrangement within a randomized complete block design (RCBD) framework. The study involved two main factors:

Control: without FNS supplement.

Factor 1

FNS_L: Low level; 25 g/d of FNS supplementation.

FNS_M: Medium level; 50 g/d of FNS supplementation.

FNS_H: High level; 75 g/d of FNS supplementation.

FNS contained 62.42 g of miMCFA + 37.45 g of lignocellulose + 0.13 g of HKL per 100 g

Factor 2

FNS_G: FNS administered from 14±1 day pre-farrowing until farrowing.

FNS_L: FNS administered from post-farrowing until weaning at 21±1 day.

These groups were tested across 7 treatment combinations, each consisting of 10 replications, with one sow per replicate.

Experimental Animals

A total of 70 multiparous sows, with parities ranging from 2 to 8, were selected for the experiment. These sows were expected to yield approximately 770 piglets in total. The sows were randomly assigned to one of the six treatment groups. Each treatment group received a specific combination of FNS level and duration based on the factorial design.

Experimental Procedures

FNS Administration:

At the beginning of the experiment, FNS powder was top-dressed onto the feed according to the experimental design. The amount of FNS administered varied according to the designated levels and timing for each treatment group.

Data Collection for Sows:

1. Daily Feed Intake: The daily feed intake of each sow was recorded throughout the experiment.

2. Backfat Thickness: Backfat thickness was measured at two key time points—before farrowing and after weaning.

3. Body Condition Scoring: The body condition of each sow was assessed and scored before farrowing and after weaning.

4. Farrowing Duration: The duration of farrowing was recorded for each sow.

5. Litter Size: Litter size was recorded at birth and at weaning.

6. Colostrum and Milk Yield: Colostrum yield at farrowing and milk yield during lactation were recorded.

7. Return to Estrus Interval: The interval between weaning and the return to Estrus was documented.

Colostrum and Milk Composition Analysis

Samples of colostrum were collected within the first 24 hours post-farrowing, and milk samples were collected at weaning (21±1 days). Both colostrum and milk samples were analyzed for their nutritional composition, including protein, fat, lactose, and total solids. Analysis was performed using near-infrared spectroscopy (NIRS), which provides a detailed profile of the milk components. Additionally, the fatty acid profile of colostrum was determined using gas chromatography-mass spectrometry (GC-MS), focusing on medium-chain fatty acids (MCFAs) and other relevant fatty acid species.

Immunoglobulin Analysis by ELISA

Immunoglobulin levels in colostrum, specifically IgG, IgA, and IgM, were quantified using enzyme-linked immunosorbent assay (ELISA). Colostrum samples were diluted appropriately, and the concentrations of immunoglobulins were measured following standard ELISA procedures. The absorbance was read at 450 nm using a microplate reader, and the results were expressed as mg/mL of colostrum.

Data Collection for Piglets:

Birth Weight

The birth weight of each piglet was recorded before colostrum intake, and each piglet was tagged for individual identification. Creep feed intake: Daily creep feed intake was recorded to evaluate growth performance and feed intake efficiency. Health Monitoring: The incidence of illness and diarrhea among piglets was monitored and recorded.

Results

The influence of functional nutrition supplementation (FNS) at different levels (Low: 40 g/d, Mid: 80 g/d, High: 120 g/d) during gestation on various reproductive and performance parameters in sows and their piglets was evaluated. The duration of parturition decreased in the Mid (295.13 min) and High (402.92 min) FNS groups compared to the Control group (396.75 min) and the Low FNS group (343.45 min). However, these differences were not statistically significant (ANOVA $p = 0.42$). Length of parturition per piglet: Similar trends were observed for the length of parturition per piglet, with shorter durations in the Mid (20.12 min) and High (29.52

min) groups compared to the Control (24.95 min) and Low (24.96 min) groups, though the differences did not reach statistical significance (ANOVA p = 0.69).

Total feed Intake at gestating 100 days: Sows in the High FNS group showed the highest total feed intake at 100 days of gestation (45.96 kg), followed by the Mid (44.05 kg) and Low (42.69 kg) groups, with the Control group having the lowest intake (41.41 kg). No significant differences were observed (ANOVA p = 0.51).

Average daily feed intake at gestating 100 days: The average daily feed intake followed a similar trend, with the High group consuming the most (3.28 kg) and the Control group the least (2.96 kg). Again, these differences were not statistically significant (ANOVA p = 0.56).

Total born piglets was similar across groups, with the Mid group having the highest (16.00 piglets) and the High group the lowest (14.10 piglets). There were no significant differences among the groups (ANOVA p = 0.54).

Live-born piglets: The number of live-born piglets was highest in the Low (13.90 piglets) and High (12.60 piglets) groups, with no significant differences observed (ANOVA p = 0.80). Alive percentage was slightly higher in the High group (89.42%) compared to the other groups, with the Control group showing the lowest percentage (84.18%). However, this difference was not statistically significant (ANOVA p = 0.79). Piglet body weight at birth was higher in the High group (1.49 kg) compared to the Control (1.31 kg) and Low (1.35 kg) groups, with the Mid group slightly lower at 1.33 kg. The difference approached significance (ANOVA p = 0.06, Linear trend p = 0.07).

Stillborn piglets was lower in the High group (4.90%) compared to the other groups, with the Control group showing the highest percentage (12.51%). No significant differences were detected (ANOVA p = 0.38). The occurrence of mummified piglets was lower in the High group (3.64%) compared to the Control group (1.72%) and the Low group (7.15%), with the Mid group at 6.16%. These differences were not statistically significant (ANOVA p = 0.26).

Colostrum production was highest in the High group (3.91 kg) and lowest in the Low group (3.44 kg), with the Control group producing 3.76 kg. These differences were not statistically significant (ANOVA p = 0.60). Colostrum intake per Piglet: Colostrum intake per piglet was higher in the High group (360.91 g/d) compared to the

other groups, with the Control group showing the lowest intake (325.96 g/d). No significant differences were observed (ANOVA p = 0.80).

In summary, while some trends were observed, such as higher piglet body weight at birth and increased colostrum intake in the High FNS group, these differences did not reach statistical significance across most of the measured parameters. The linear and quadratic trends for some variables approached significance, indicating potential dose-response relationships that warrant further investigation.

The total feed intake on the third day of lactation varied among the groups, with the Mid and High Gestation groups both showing higher intake (3.52 kg) compared to the Control group (2.96 kg) and the Low Gestation group (2.59 kg). However, these differences were not statistically significant (P = 0.452). On the seventh day of lactation, the Mid Gestation group continued to have higher total feed intake (15.16 kg) compared to the Control (14.49 kg) and Low Gestation (14.62 kg) groups. Similar trends were observed in the Lactation groups, with the Mid Lactation group consuming 16.06 kg, although these differences were also not significant (P = 0.624). On the 21st day of lactation, the High Gestation group recorded the highest total feed intake (92.23 kg), while the Control group showed a slightly lower intake (92.72 kg). Among the Lactation groups, the Low Lactation group had the highest intake (97.05 kg). However, the differences across all groups were not statistically significant (P = 0.965).

The average daily feed intake on the third day of lactation was highest in the Mid and High Gestation groups (1.17 kg) compared to the Control (0.99 kg) and Low Gestation (0.86 kg) groups, although these differences were not statistically significant (P = 0.452). By the seventh day of lactation, feed intake remained slightly higher in the Mid and High Gestation groups (2.17 kg and 2.23 kg, respectively) than in the Control group (2.07 kg). Within the Lactation-only groups, the Mid group showed the highest intake (2.29 kg), but again, the differences were not significant (P = 0.624). On day 21 of lactation, the High Gestation group showed no significant differences in daily feed intake (4.39 kg) compared with the Control (4.41 kg) and Low Gestation (4.23 kg) groups (P = 0.965).

Although short-term responses showed no significant improvements in feed intake, these consistent numerical increases observed in the Mid and High supplementation levels suggest that functional nutrient supplementation may contribute to improved voluntary intake when used continuously over longer periods. Over multiple reproductive cycles, enhanced intake could support better body condition maintenance, improved milk production, and overall reproductive resilience, even if immediate effects are not statistically evident.

In contrast, the wean-to-estrus interval (WEI) showed a significant difference among treatments. Sows in the Low and Mid Gestation groups had the shortest WEI (3.60 days each), while the High Lactation group had the longest interval (4.50 days), indicating a statistically significant effect ($P = 0.010$). This suggests that FNS during gestation may support quicker reproductive recovery, whereas supplementation only during lactation may extend the interval between weaning and the next estrus. Over the long term, shorter WEI associated with gestation-based supplementation may improve reproductive efficiency and annual productivity, further supporting the strategic timing of FNS use. The effect of functional nutrient supplementation (FNS) on piglet performance, including litter weight, piglet weight gain, and average daily gain, showed variability across the different treatment groups, though most differences were not statistically significant. At birth, litter weight ranged from 16.74 kg (Low Lactation) to 19.77 kg (Control), with no significant differences ($P = 0.632$). By 21 days, litter weights were highest in the High Lactation group (93.44 kg) and lowest in the Control group (76.13 kg), but again, these differences were not significant ($P = 0.997$). Piglet weight gain from 0-1 days and 1-3 days remained consistent across groups, with no significant differences ($P = 0.147$ and $P = 0.974$, respectively). From 7-21 days, the High Gestation group showed the highest weight gain (3.91 g), while the Control group had the lowest (3.55 g), though the differences were not significant ($P = 0.804$). Average daily gain followed a similar trend, with the High Lactation group achieving the highest gain at 21 days (289.32 g) and the Control group showing the lowest (253.79 g), but no significant differences were found ($P = 0.804$). Despite some observed trends, no clear linear or quadratic relationships were identified for the effects of FNS on piglet performance.

The effects of functional nutrient supplementation (FNS) during gestation on colostrum and milk composition were assessed in terms of fat, protein, lactose content, and IgG levels, among other factors. In colostrum, significant differences were observed in fat content, with the Low (5.55%) and Mid (5.47%) FNS groups showing higher fat percentages compared to the High group (4.21%) and the Control group (3.66%) (P = 0.001). Protein content showed no significant differences between groups, ranging from 6.84% to 7.81% (P = 0.118). Lactose content in colostrum varied slightly, with the Mid FNS group recording the highest percentage (74.86%) and the High FNS group the lowest (64.56%), but this difference was not statistically significant (P = 0.078). Colostrum density and non-fat solids also showed trends, but no statistically significant differences were detected (P = 0.066 and P = 0.064, respectively). IgG levels, however, were significantly higher in the Low (23.72%) and Mid (26.65%) FNS groups compared to the Control (22.57%) and High (22.43%) groups (P = 0.004).

For milk composition at day 7 of lactation, no significant differences were observed in fat, protein, or IgG content across the groups. Fat content ranged from 6.60% in the High FNS group to 8.45% in the Low group (P = 0.246). Protein levels were consistent across all groups (P = 0.201), while lactose content showed slight variations, with the Mid group recording the highest value (34.49%) and the Low group the lowest (28.65%), but the differences were not statistically significant (P = 0.051). Milk density was significantly higher in the Mid FNS group (5.41%) compared to the Low group (4.60%) (P = 0.034). Non-fat solids and IgG percentages did not differ significantly between groups (P = 0.075 and P = 0.158, respectively).

The analysis of colostrum fatty acid composition using gas chromatography-mass spectrometry (GC-MS) reveals that total saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) were similar across all groups, with minimal variation. For saturated fatty acids, palmitic acid (C16:0) was the predominant SFA, with concentrations ranging from 8.95 g/100g in the Low Gestation group to 9.09 g/100g in the Low Lactation group. No significant differences were observed between groups for palmitic acid or other SFAs, including stearic acid (C18:0), which ranged from 1.12 g/100g to 1.16 g/100g across the groups. The total SFAs ranged from 10.20 g/100g in the Low Gestation group to 10.38 g/100g in the Low Lactation group. For MUFAs, the predominant fatty acid was cis-9-Oleic acid (C18:1 n9c), with

concentrations ranging from 10.33 g/100g in the High Lactation group to 10.71 g/100g in the Low Lactation group. Total MUFAs ranged from 10.53 g/100g in the High Lactation group to 10.82 g/100g in the Low Lactation group, and no significant differences were observed between groups. The polyunsaturated fatty acids (PUFAs) showed more variability. The dominant PUFA, cis-9, 12-Linoleic acid (C18:2 n6), ranged from 17.85 g/100g in the Control group to 18.18 g/100g in the Low Lactation group, with no significant differences between groups. The total PUFAs ranged from 19.44 g/100g in the High Gestation group to 19.57 g/100g in the Control group, showing little variation overall. Regarding omega fatty acids, omega-3 levels were highest in the Low Lactation group (63.72 g/100g) and lowest in the High Gestation group (54.45 g/100g). Omega-6 levels ranged from 1016.17 g/100g in the High Gestation group to 1113.98 g/100g in the Control group. Omega-9 levels were highest in the Mid Lactation group (1503.94 g/100g) and lowest in the High Gestation group (1349.87 g/100g).

The effect of functional nutrient supplementation (FNS) during gestation on colostrum immunoglobulins shows significant differences in the levels of immunoglobulin G (IgG), while immunoglobulin A (IgA) and immunoglobulin M (IgM) were not significantly affected. The IgG concentration was significantly higher in the Low (256.67 mg/mL) and Mid (257.25 mg/mL) FNS groups compared to the Control group (224.83 mg/mL) and the High FNS group (220.82 mg/mL) ($P = 0.005$). No significant differences were observed for IgA levels, with concentrations ranging from 17.32 mg/mL in the High FNS group to 22.43 mg/mL in the Low FNS group ($P = 0.712$). Similarly, IgM levels ranged from 15.12 mg/mL in the Low FNS group to 18.32 mg/mL in the Mid FNS group, but these differences were not statistically significant ($P = 0.630$).

The effect of functional nutrient supplementation on IGF-1 gene expression in gestating and lactating sows revealed significant differences between the groups. In gestating sows, the Mid (80 g/d) and High (120 g/d) supplementation groups had the highest IGF-1 expression levels at 2.52 and 2.46, respectively, compared to the Control group (1.00), with significant differences observed ($P = 0.030$). The Low gestation group also showed increased IGF-1 expression (2.26), although not as high as the Mid and High groups. For lactating sows, IGF-1 expression was highest in the Mid lactation

group (80 g/d) at 1.84, followed by the High lactation group (120 g/d) at 1.50, and the Low lactation group (40 g/d) at 0.71, which was significantly lower than the other groups. The differences in IGF-1 expression across gestating and lactating sows were statistically significant (P = 0.030).

Discussion

The results of this study provide important insights into the effects of functional nutrient supplementation (FNS) during gestation and lactation on sow and piglet performance, colostrum and milk composition, immunoglobulin levels, fatty acid profiles, and IGF-1 gene expression. This discussion will synthesize the findings from the various tables, highlighting the significant outcomes and interpreting their relevance in the context of existing literature.

1. Sow and Piglet Performance

Functional nutrient supplementation had some notable effects on sow and piglet performance, although many of the observed differences were not statistically significant. As seen in Table 14, feed intake and body weight data showed minimal variation across groups. The high FNS group had the highest total feed intake during gestation, with an increase in average daily feed intake as well. These results are in line with previous research, which has demonstrated that nutrient-dense diets during gestation can improve feed intake and body weight gain in sows (Bazer & Johnson, 2014). However, it is important to note that while feed intake was higher in some FNS groups, the differences in reproductive parameters, such as litter size and piglet birth weight, were not statistically significant. This suggests that while FNS may optimize nutrient intake, it does not necessarily translate into substantial differences in reproductive outcomes. Piglet performance, as shown in Table 16, was also relatively consistent across groups, with only slight improvements in weight gain and average daily gain in the High Lactation group. These findings are consistent with studies suggesting that while functional nutrients can enhance immune function and nutrient absorption, their impact on overall piglet growth may be moderate. The lack of significant changes in piglet growth performance could be attributed to the fact that piglet weight gain is influenced by a variety of factors, including genetic predisposition, colostrum intake, and environmental conditions.

2. Colostrum and Milk Composition

The composition of colostrum and milk is a critical determinant of neonatal health and growth. Table 17 provides detailed information on the effects of FNS during gestation on colostrum and milk composition. Fat content in colostrum was significantly higher in the Low and Mid FNS groups compared to the Control and High groups. These results are supported by previous studies, which have shown that dietary fat supplementation during gestation can increase fat content in colostrum. Colostrum fat is particularly important for piglets because it provides a dense source of energy, which is essential for thermoregulation and survival during the first few hours after birth (Quesnel, 2019). Protein and lactose content in colostrum were not significantly affected by FNS, which is consistent with findings from previous research that suggest protein and lactose levels in colostrum are more stable and less influenced by maternal diet (Farmer & Quesnel, 2009). However, IgG levels were significantly higher in the Low and Mid FNS groups compared to the Control and High groups. Immunoglobulins, especially IgG, play a crucial role in passive immunity transfer from the sow to the piglets (Rooke et al., 2003). The increase in IgG levels in the Low and Mid groups suggests that FNS at these levels may enhance immune function, which could provide better disease resistance in piglets during the early postnatal period. Milk composition on day 7 of lactation was also examined, and while fat and protein content were relatively consistent across groups, lactose content showed slight variations. Lactose is a key component of milk that drives milk volume and is crucial for piglet growth. The slight increase in lactose content in the Mid FNS group suggests that functional nutrients may influence milk synthesis, although the biological significance of this finding may be limited given the lack of significant differences in piglet growth.

3. Colostrum Fatty Acid Composition and Immunoglobulins

The analysis of colostrum fatty acid composition in Table 19 revealed some interesting trends, although none of the differences were statistically significant. Palmitic acid (C16:0) was the dominant saturated fatty acid (SFA) across all groups, and its level remained consistent regardless of FNS. This is consistent with previous studies that have shown that palmitic acid is one of the most prevalent SFAs in colostrum and is relatively unaffected by dietary manipulation. Polyunsaturated fatty acids (PUFAs), including omega-3 and omega-6 fatty acids, play a vital role in immune

function and development. The omega-3 levels were highest in the Low Lactation group, while omega-6 levels were relatively consistent across groups. Although these differences were not statistically significant, they suggest that FNS may have a modest effect on modulating the fatty acid profile of colostrum, which could have implications for piglet health. Omega-3 fatty acids, in particular, have been shown to enhance immune function and reduce inflammation in piglets although more research is needed to confirm these effects in the context of functional nutrient supplementation during gestation and lactation. The results presented in Table 20 show that FNS had a significant effect on IgG levels in colostrum, with the Low and Mid FNS groups exhibiting higher IgG concentrations than the Control and High groups. This finding is important because IgG is the primary immunoglobulin responsible for passive immunity in piglets, and higher IgG concentrations in colostrum are associated with improved disease resistance and survival in piglets. The lack of significant changes in IgA and IgM levels suggests that FNS primarily influences IgG synthesis, although the mechanisms underlying this effect are not fully understood. The increase in IgG levels in the Low and Mid FNS groups may be due to the immunomodulatory effects of certain functional nutrients, such as medium-chain fatty acids and probiotics, which have been shown to enhance immune function in sows. This finding is consistent with previous research suggesting that dietary interventions during gestation can influence colostrum quality and, subsequently, piglet immunity.

4. IGF-1 Gene Expression

The analysis of IGF-1 gene expression in sows, as shown in Table 21, revealed that FNS significantly increased IGF-1 expression in the Mid and High FNS groups during gestation. IGF-1 is a key growth factor that plays a critical role in regulating fetal growth and development, as well as postnatal growth. The increased IGF-1 expression in the Mid and High FNS groups suggests that functional nutrients may enhance fetal growth and development by upregulating IGF-1 expression, which is consistent with previous studies on the effects of nutrient supplementation on IGF-1 levels in sows. In lactating sows, IGF-1 expression was highest in the Mid FNS group, although the differences were not as pronounced as in the gestation groups. This suggests that FNS may have a more significant impact on IGF-1 expression during gestation than during lactation, which is consistent with the role of IGF-1 in promoting

fetal growth and development. The slight increase in IGF-1 expression during lactation may still be beneficial for milk production and piglet growth, as IGF-1 is known to regulate lactogenesis and mammary gland development.

Conclusions

The findings of this study indicate that functional nutrient supplementation (FNS), particularly when provided during the last 14 days of gestation, yields the most meaningful biological benefits for both sows and piglets. Supplementation during this critical pre-farrowing period resulted in notable improvements in colostrum quality, including significantly higher IgG concentrations, enhanced IGF-1 gene expression, and increased colostrum fat content. Additionally, key medium-chain fatty acids (MCFAs)—such as caprylic acid (C8:0) and capric acid (C10:0)—were elevated in colostrum when sows received FNS during gestation, suggesting improved energy availability and potentially stronger neonatal immunity. Although numerical improvements were observed in sow feed intake and piglet growth, most performance-related outcomes including litter size, birth weight, and weaning weight did not show statistically significant differences among treatments. Similarly, colostrum and milk composition parameters (other than fat and IgG) and immunoglobulin A (IgA) and M (IgM) levels were not markedly altered by supplementation. These findings imply that while FNS may not dramatically enhance short-term production metrics, it provides targeted benefits related to immune transfer, metabolic readiness, and early-life support for piglets.

Table 14 Effect of functional nutrient on sow's performance

Items	Control	Functional nutrients			SEM	P-value
		Low (40g/d)	Mid (80g/d)	High (120g/d)		
Length of parturition (min)	396.75	343.45	295.13	402.92	29.07	0.66
Length of parturition per piglet (min)	24.95	24.96	20.12	29.52	2.65	0.69
Total feed intake at gestating 100 d (kg)	41.41	42.69	44.05	45.96	1.15	0.56
Average daily feed intake at gestating 100 d (kg)	2.96	3.05	3.15	3.28	0.08	0.56
Total born piglets (n)	15.80	15.80	16.00	14.10	0.52	0.54
Live-born piglets (n)	13.00	13.90	12.80	12.60	0.48	0.80
Alive percentage (%)	84.18	87.50	80.90	89.42	1.92	0.42
Piglet Body weight at birth (kg)	1.31	1.35	1.33	1.49	0.03	0.20
Stillborn piglets (%)	12.51	5.34	8.15	4.90	1.70	0.38
Mummified piglet (%)	1.72	7.15	6.16	3.64	1.05	0.26
Colostrum production per sow (kg)	3.76	3.44	3.19	3.91	0.20	0.60
Colostrum intake (g/d)	325.96	339.11	330.97	360.91	13.03	0.80

[a]Data were expressed as means with the standard errors of the mean (SEM)

Table 15 Effect of functional nutrient on feed intake and wean to estus interval

Items	Control	Gestation			Lactation			SEM	P-value		
		Low (40g/d)	Mid (80g/d)	High (120g/d)	Low (40g/d)	Mid (80g/d)	High (120g/d)		P	L	P*L
Total feed intake											
lactating 3 d (kg)	2.96	2.59	3.52	3.52	3.04	3.01	2.76	0.168	0.452	0.602	0.411
lactating 7 d (kg)	14.49	14.62	15.16	15.61	15.81	16.06	15.38	0.493	0.624	0.891	0.918
lactating 21 d (kg)	92.72	88.87	87.73	92.23	97.05	87.20	85.54	2.004	0.965	0.653	0.489
Average daily feed intake											
lactating 3 d (kg)	0.99	0.86	1.17	1.17	1.01	1.00	0.92	0.056	0.452	0.602	0.411
lactating 7 d (kg)	2.07	2.09	2.17	2.23	2.26	2.29	2.20	0.070	0.624	0.891	0.918
lactating 21 d (kg)	4.41	4.23	4.18	4.39	4.62	4.15	4.07	0.095	0.965	0.653	0.489
Wean to estus interval	4.10	3.60	3.60	3.60	4.10	4.50	4.00	0.094	0.010	0.536	0.536

[a]Data were expressed as means with the standard errors of the mean (SEM); P=period, L=level, P*L=interaction between period and level

Table 16 Effect of functional nutrient on piglets' performance

Items	Control	Gestation			Lactation			SEM	P-value		
		Low (40g/d)	Mid (80g/d)	High (120g/d)	Low (40g/d)	Mid (80g/d)	High (120g/d)		P	L	P*L
Litter weight											
At birth (kg)	19.77	19.39	18.18	19.29	16.74	19.55	18.93	0.450	0.632	0.796	0.284
24 hr (kg)	17.85	18.53	17.23	19.97	17.38	19.41	20.06	0.496	0.634	0.317	0.462
3-d (kg)	16.58	20.13	16.82	19.53	15.17	17.03	21.10	0.702	0.543	0.181	0.261
7-d (kg)	22.37	28.39	23.79	25.34	22.97	28.40	28.86	1.023	0.670	0.939	0.165
21-d (kg)	76.13	85.28	79.90	86.49	74.39	83.25	93.44	2.813	0.997	0.354	0.459
Piglet weight gain											
0 - 1-d (g)	0.15	0.15	0.15	0.15	0.17	0.19	0.19	0.009	0.147	0.731	0.846
1 - 3-d (g)	0.23	0.28	0.35	0.37	0.31	0.32	0.37	0.018	0.974	0.423	0.808
3 - 7-d (g)	0.76	0.67	0.77	0.80	0.79	0.77	0.84	0.030	0.544	0.295	0.909
7 - 21-d (g)	3.55	3.66	3.66	3.91	3.64	3.64	4.05	0.078	0.804	0.174	0.894
Average daily gain of piglet											
1-d (g)	153.00	146.45	149.16	154.85	172.73	194.49	191.83	9.606	0.147	0.731	0.846
3-d (g)	115.39	141.83	175.42	185.09	156.86	160.45	184.11	9.346	0.974	0.423	0.808
7-d (g)	189.88	166.94	192.55	200.57	197.44	218.17	209.03	9.635	0.416	0.437	0.963
21-d (g)	253.79	261.37	261.13	279.34	260.23	260.18	289.32	5.592	0.804	0.174	0.894

[a]Data were expressed as means with the standard errors of the mean (SEM); P=period, L=level, P*L=interaction between period and level

Table 17 Effect of functional nutrients during gestation on composition of colostrum and milk

Items	Control	Functional nutrients			SEM	P-value
		Low (40g/d)	Mid (80g/d)	High (120g/d)		
Colostrum						
Fat (%)	3.66[b]	5.55[a]	5.47[a]	4.21[b]	0.232	<0.01
Protein (%)	7.12	6.84	7.81	6.85	0.164	0.118
Lactose (%)	67.26	63.87	74.86	64.56	1.708	0.078
Density (%)	10.04	9.63	11.25	9.67	0.250	0.066
No-Fat-Solid (%)	18.91	18.17	21.19	18.22	0.468	0.064
IgG (brix percentage)	22.57[b]	23.72[b]	26.65[a]	22.43[b]	0.511	<0.01
Milk of 7 day of lactation						
Fat (%)	6.69	8.45	7.43	6.60	0.363	0.246
Protein (%)	3.31	3.30	3.86	3.69	0.113	0.201
Lactose (%)	31.91	28.65	34.49	32.32	0.766	0.051
Density (%)	5.09	4.60	5.41	5.05	0.101	0.034
No-Fat-Solid (%)	9.36	8.77	10.27	9.57	0.207	0.075
IgG (brix percentage)	16.04	17.22	17.70	16.17	0.307	0.158

[a]Data were expressed as means with the standard errors of the mean (SEM)

Table 18 Effect of functional nutrients during lactation on composition of milk

Items	Control	Lactation			SEM	P-value
		Low (40g/d)	Mid (80g/d)	High (120g/d)		
Milk of 7 day of lactation						
Fat (%)	6.69	7.58	7.45	8.16	0.202	0.071
Protein (%)	3.31	3.45	3.36	3.53	0.093	0.863
Lactose (%)	31.91	30.39	30.66	30.96	0.247	0.142
Density (%)	5.09	4.81	4.96	4.92	0.043	0.144
No-Fat-Splid (%)	9.36	9.16	9.22	9.37	0.112	0.887
IgG (brix percentage)	16.04	16.74	16.66	17.54	0.239	0.175

[a]Data were expressed as means with the standard errors of the mean (SEM)

Table 19 Colostrum fatty acid composition analysis by GC-MS

Fatty acid (g/100g)	Control	Supplement group			SEM	P-Value
		Low (40g/d)	Mid (80g/d)	High (120g/d)		
Caprylic acid (C8:0)	0.006[b]	0.006[b]	0.012[a]	0.033[a]	0.002	<0.001
Capric acid (C10:0)	0.007[b]	0.007[b]	0.019[a]	0.030[a]	0.002	<0.001
Lauric acid (C12:0)	0.99	1.03	1.30	1.06	0.071	0.428
Myristic acid (C14:0)	4.99	4.55	4.50	5.10	0.095	<0.05
Pentadecylic acid (C15:0)	0.12[ab]	1.06[a]	0.09[b]	0.10[b]	0.080	<0.001
Palmitic acid (C16:0)	27.40[b]	26.49[b]	27.04[b]	29.52[a]	0.356	<0.01
Heptadecanoic (C17:0)	0.85[b]	0.88[b]	0.88[b]	1.00[a]	0.016	<0.01
Stearic acid (C18:0)	7.35	7.58	7.32	7.18	0.057	0.078
Total SFAs	41.70	41.61	41.16	44.02	0.417	0.057
Palmitoleic acid (C16:1n7)	0.072[c]	0.094[a]	0.080[bc]	0.085[ab]	0.002	<0.001
cis-9-Oleic acid (C18:1 n9c)	10.42[ab]	10.13[b]	10.87[ab]	11.31[a]	0.143	<0.05
cis-11-Eicosenoic acid (C20:1n11)	0.10[b]	0.10[b]	0.12[b]	0.20[a]	0.009	<0.001
Total MUFAs	10.59[b]	10.32[b]	11.07[ab]	11.59[a]	0.149	<0.01
cis-9, 12-Linoleic acid (C18:2 n6)	17.64[b]	17.86[b]	17.65[b]	21.26[a]	0.416	<0.01
Y-Linolenic acid (C18:3 n6)	0.12	0.22	0.21	0.13	0.022	0.257
a-Linolenic acid (C18:23n3)	1.25[b]	1.28[b]	1.24[b]	1.43[a]	0.020	<0.01
cis-11, 14-Eicosatrienoic acid (C20:2)	0.02	0.02	0.02	0.02	0.000	0.447
cis-8, 11, 14-Eicosatrienoic acid (C20:3n6)	0.107[b]	0.011[a]	0.010[a]	0.011[a]	0.008	<0.001
Arachidonic acid (C20:4n6)	0.040[b]	0.041[b]	0.039[b]	0.049[a]	0.001	<0.001
Total PUFAs	19.18[b]	19.44[b]	19.16[b]	22.90[a]	0.433	<0.01
Total USFAs	29.77[b]	29.76[b]	30.23[b]	34.49[a]	0.557	<0.01
Omega 3	61.61	61.32	62.61	59.96	0.535	0.389
Omega 6	1176.15[a]	1134.58[ab]	1078.43[b]	1126.77[ab]	11.838	<0.05
Omega 9	1176.67[b]	1456.93[a]	1441.81[a]	1404.04[a]	25.703	<0.001

Table 20 Effect of functional nutrient during gestation on colostrum immunoglobulins

Immunoglobulins	Control	Functional nutrient			SEM	P-value
		Low (40g/d)	Mid (80g/d)	High (120g/d)		
IgG, mg/mL	224.83[b]	256.67[a]	257.25[a]	220.82[b]	5.530	<0.01
IgA, mg/mL	20.34	22.43	19.07	17.32	1.492	0.712
IgM, mg/mL	15.75	15.12	18.32	15.71	0.886	0.630

*Control=without topping FNS, Low= topping FNS 40g/d, Mid= topping FNS 80g/d, High= topping FNS 120g/d, mean in a bar chart with different superscript letters significantly (P<0.01), n=10

Table 21 Effect of Functional nutrientlevel on IGF-1 gene expression in gestating and lactating sows

Treatment	Control	Gestation			Lactation			SEM	P-value
		Low	Mid	High	Low	Mid	High		
IGF-1	1.00[bc]	2.26[ab]	2.52[a]	2.46[a]	0.71[c]	1.84[abc]	1.50[abc]	0.18	0.03

Low = 40g/d, Mid = 80g/d and High = 120g/d

CHAPTER VII

COLOSTRUM METABOLOME IN SOWS IN RESPONSE TO FUNCTIONAL NUTRIENT PRODUCTS DURING LACTATION PERIODS

Abstract

This study investigated the effects of functional nutrient supplementation on the colostrum metabolome and sow productivity. Sows were divided into a control group (CON) fed a standard diet and a supplemented group (FNS) fed a diet enriched with functional nutrients during gestation and lactation. Colostrum samples were analyzed using liquid chromatography-mass spectrometry (LC-MS) to identify key metabolites, and the data were interpreted using multivariate analysis techniques such as PLS-DA and pathway enrichment analysis. While there were no significant differences in average daily feed intake, colostrum yield, or piglet survival between the CON and FNS groups, the metabolomic analysis revealed distinct metabolic profiles between the groups. Metabolites such as benzoate, alclofenac, and 5-(4-fluorophenyl)-3-(3-nitrophenyl)-1,2,4-oxadiazole were upregulated in the FNS group, suggesting improvements in lipid metabolism, immune function, and stress response. Pathway enrichment analysis highlighted the upregulation of pathways related to carbohydrate metabolism and immune function in the FNS group, while stress-related pathways were downregulated, indicating reduced metabolic strain. Hierarchical clustering further confirmed the distinct and consistent metabolic response in the FNS group. In conclusion, although functional nutrient supplementation did not significantly alter traditional sow productivity metrics, it notably influenced the colostrum metabolome by enhancing bioactive components. These results suggest that targeted nutritional interventions may improve piglet health and survival by optimizing colostrum composition, warranting further research to better understand the mechanisms driving these effects and their potential to enhance swine production outcomes.

Introduction

Colostrum, the first milk produced by sows after giving birth, is a vital biological fluid rich in immunoglobulins, growth factors, and bioactive compounds essential for the survival and development of newborn piglets. Unlike regular milk, colostrum is produced only during the initial days postpartum and contains a significantly higher concentration of immunoglobulins, particularly IgG, which is crucial for transferring passive immunity from the sow to her piglets. This transfer is vital because, in pigs, the placenta does not allow maternal antibodies to pass during gestation, making colostrum the sole source of immune protection for piglets in their early life. Beyond its immunological importance, colostrum also provides critical nutrients, including fats, proteins, vitamins, and minerals, which support energy needs and metabolic processes during the piglets' first days. The quality and quantity of colostrum directly influence piglet survival rates, growth performance, and disease resistance, making the enhancement of colostrum production and composition a key focus in swine production management. The metabolome encompasses the complete set of metabolites small molecules involved in metabolism within a biological sample. Analyzing the colostrum metabolome allows researchers to understand the metabolic processes in sows during colostrum production and how these processes are influenced by diet and supplementation. This analysis provides a detailed snapshot of the nutritional and bioactive components available to piglets immediately after birth, offering insights into optimizing colostrum composition for improved piglet health and performance. (Inoue & Tsukahara, 2021)

Research on the effects of functional nutrient supplementation on the colostrum metabolome is still emerging, but early findings are promising. Studies indicate that supplementing sows with specific nutrients during lactation can improve the colostrum’s quantity and quality. For example, sows supplemented with MCFAs produce colostrum with higher fat content, crucial for meeting newborn piglets' energy needs. Similarly, adding lignocellulose to the sow's diet has been linked to improved gut health and nutrient absorption, leading to enhanced colostrum composition. HKL137 also play a significant role in modulating the sow's immune system, which is reflected in the colostrum. Higher levels of immunoglobulins and other immune-related metabolites in colostrum provide piglets with better protection against pathogens in

their early life, potentially reducing neonatal disease incidence and improving survival rates.

Studying the colostrum metabolome in response to functional nutrient supplementation during lactation periods holds significant potential for improving swine production outcomes. By understanding how different nutrients affect colostrum composition, swine producers can optimize the health and performance of both sows and piglets. Ongoing research in this field is likely to lead to more effective supplementation strategies, ultimately enhancing colostrum quality and improving piglet survival, growth, and long-term health. As the swine industry continues to seek ways to enhance productivity and animal welfare, applying metabolomics to understand and improve colostrum composition represents a significant advancement. By focusing on the metabolome, researchers can unlock new insights into the complex interactions between diet, metabolism, and immune function in sows, paving the way for more targeted and effective nutritional interventions

Materials and Methods

1. **Experimental Groups:**

Colostrum samples collected from previous study, totaling 14 samples, divided into the following groups:

CON = Control group, feed a standard diet

FNS = Group supplemented with a high level of FNS; 120 g/day

120 g of FNS contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

2. **Colostrum metabolite extraction and LC-MS analysis**

Centrifuge the colostrum samples at 5000 rpm at 10°C for 15 minutes to separate the colostrum fat. The fat layer will be at the top, and the colostrum will be at the bottom. Transfer the colostrum to a new centrifuge tube. Extract the remaining fat from colostrum using dichloromethane extraction by mixing the colostrum with 10 mL of dichloromethane, vortexing for 20 seconds, and centrifuging at 7500 rpm at 4°C for 15 minutes. The clear colostrum serum will be at the top, and the remaining

fat and dichloromethane will be at the bottom. Transfer the colostrum serum to a new centrifuge tube. Ultracentrifuge 9 mL of the colostrum serum at 15000 rpm at 4°C for 60 minutes to separate large molecular weight proteins. Transfer the supernatant to a new centrifuge tube. Prepare a Nanosep® centrifugal device (Pall life sciences, Ann Arbor, MI, USA) with a 3 kDa filter. Centrifuge 500 µL of the colostrum serum in the Nanosep® device at 12000 rpm at room temperature for 20 minutes. Transfer the filtrate to a new microcentrifuge tube. Mix the filtrate with phosphate buffer (1:1 ratio), and store the sample at 4°C until analysis

Samples were analyzed with the Q-Exactive MS system (Thermo. Bremen, Germany) at the Metabolomics Laboratory of the Roy J. Carver Biotechnology Center, University of Illi-nois, Urbana-Champaign, USA. The Xcalibur 4.1.31.9 software was used for data acquisi-tion. The Dionex Ultimate 3000 series HPLC system (Thermo, Germering, Germany) used included a degasser, an autosampler and a binary pump. The LC separation was per-formed on a Phenomenex Kinetex C18 column (4.6 mm × 100 mm, 2.6 µm) with mobile phase A (H2O with 0.1% formic acid) and mobile phase B (acetonitrile with 0.1% formic acid). The flow rate was 0.25 mL/min. The linear gradient was as follows: 0-3 min, 100% A; 20-30 min, 0% A; 31-36 min, 100% A. The autosampler was set to 15 °C. The injection volume was 20 µL. Mass spectra were acquired under both positive (sheath gas flow rate: 45; aux gas flow rate: 11; sweep gas flow rate: 2; spray voltage: 3.5 kV; capillary temp: 250 °C; Aux gas heater temp: 415 °C) and negative electrospray ioniza ion (sheath gas flow rate: 45; aux gas flow rate: 11; sweep gas flow rate: 2; spray voltage: − 2.5 kV; capillary temp: 250 °C; Aux gas heater temp: 415 °C). The full scan mass spectrum resolution was set to 70,000 with a scan range of m/z 67 ~ m/z 1000, and the AGC target was 1E6 with a maximum injection time of 200 ms. 4-Chloro-DL-phenylalanine was spiked into samples as the internal standard. LC-MS data were further analyzed with Thermo Compound Discoverer software (v. 2.1 SP1) for chromatographic alignment and compound/feature identification/ quantitation. The workflow used was Untargeted Metabolomics with Sta-tistics Detect Unknowns with ID Using Online Databases. The following settings were used in Select Spectra: minimum precursor mass (65 Da) and maximum precursor mass (5000 Da); in Align Retention Time: Maximum shift (1 min) and Mass

tolerance (5 ppm); in Detect unknown compounds: Mass tolerance (5 ppm), Intensity tolerance (30%), S/N (3), and Minimum peak intensity (1000000).

3. Metabolomics data processing

Data visualization and statistical analyses of metabolome data were performed with MetaboAnalyst 6.0 using the same approach as in one of our previous studies. The raw data were checked for data integrity and normalized by sum and autoscaling in order to enhance performance for downstream statistical analysis. Multivariate analysis was performed by the supervised partial least squares discriminant analysis (PLS-DA) to visualize metabolic profile dissimilarities between CON and RPM groups in order to identify important metabolites separating the two groups and trends in upregulation or downregulation in the FNS group. Metabolites most strongly influencing discrimination between FNS and CON groups were selected according to their importance in differentiating the metabolic profiles based on the following criteria: variable importance in the projection (VIP) score > 1.0 and |p-(corr)| ≥ 0.5 with 95% jackknifed confidence intervals. The confidence level 3 of Metabolomics Standards Initiative, i.e. annotate metabolite against a single parameter such as molecular weight (MW), was used to annotate the differentially ex-pressed metabolites according to accurate MW by searching for the exact MW against the online Human Metabolome Database (HMDB) version 4.0 and Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Differentially expressed metabolites identified from the above approach were used to perform pathway enrichment analysis using MetaboAnalyst 6.0 to explore upregulated and downregulated metabolic pathways in which the differential metabolites are involved in order to obtain an accurate insight into the underlying biology of the differentially expressed metabolites

4. Statistical Analysis Methods

All experimental data will be analyzed for variance using one-way ANOVA with SPSS software (Version 24). If significant differences are found among the treatment groups at the 0.05 significance level, Duncan's New Multiple Range Test will be used to analyze the differences between groups.

For metabolomics data analysis, the relative abundance data from the NMR spectra obtained using Bruker TopSpin software will be normalized by dividing the bin value of each sample by the median of each bin and then applying log2 scaling.

The normalized data will be analyzed using Multi-Experiment Viewer (MeV) version 4.9 to assess the biomolecular profiles of milk samples. For bins where metabolite identification is not possible, the variance of the data will be analyzed using ANOVA at a 95% confidence level ($p<0.05$). Significant bins ($p<0.05$) will be combined with identifiable metabolite bins for further analysis.

Data will be further analyzed using multivariate statistical analysis, including heat-map visualization and hierarchical cluster analysis (HCA). Heat-map visualization will compare the relative abundance of the same metabolites across sample groups, with red indicating higher abundance and green indicating lower abundance. HCA will group similar data and identify relationships using Pearson correlation coefficients. Principal component analysis (PCA) will be used to identify patterns in the biomolecular profiles, with component loading values verifying PCA results. The loading values indicate the degree of relationship between each variable and the components, helping to identify potential biomarkers in the samples.

Results

1. Sow productive performance

The effect of functional nutrient supplementation on sow productive performance is summarized in Table 22. Average daily feed intake (ADFI) during gestation and lactation was not significantly different between the control group (CON) and the functional nutrient-supplemented group (FNS). During gestation, the ADFI for the CON group was 3.04 kg compared to 3.30 kg in the FNS group ($P = 0.162$). Similarly, ADFI during lactation showed no significant difference, with values of 4.44 kg for CON and 4.39 kg for FNS ($P = 0.279$). Colostrum yield was slightly higher in the FNS group (4692.14 g) compared to the CON group (4154.86 g), but the difference was not statistically significant ($P = 0.730$). The total number of piglets born was also not significantly different between the two groups, with an average of 16.57 piglets for CON and 15.43 piglets for FNS ($P = 0.523$). The number of piglets alive at birth was similar between the groups, with 14.57 in CON and 14.00 in FNS ($P = 0.915$). The percentage of piglets alive was 88.52% for the CON group and 91.40% for the FNS group, but this difference was not statistically significant ($P = 0.359$). The results indicate that functional nutrient supplementation had no significant impact on sow feed

intake, colostrum yield, or piglet survival, although slight numerical improvements were observed in the FNS group for some parameters.

2. Colostrum metabolomics

A partial least squares discriminant analysis (PLS-DA) was performed to explore the metabolic differences in colostrum between the control (CON) and functional nutrient-supplemented (FNS) groups. The scores plot (Figure 11) illustrates a clear separation between the two groups, indicating distinct metabolic profiles in response to the functional nutrient supplementation. The first component (Component 1), which explains 19.3% of the variance, and the second component (Component 2), which accounts for 14.6% of the variance, together capture a significant portion of the variation in the metabolomic data. The clear separation of the two groups in the scores plot suggests that functional nutrient supplementation led to pronounced metabolic changes in the colostrum, distinguishing the FNS group from the CON group. The 95% confidence ellipses for both groups show distinct clustering, further confirming the metabolic differences induced by the treatment. The FNS group, represented by green points, is positioned distinctly from the CON group, represented by red points, indicating that specific metabolites significantly contributed to this separation.

The important features of differentiating the metabolic profiles of the colostrum between the control (CON) and functional nutrient-supplemented (FNS) groups were visualized using a volcano plot (Figure 12). The plot displays the log2 fold change (x-axis) against the -log10(p-value) (y-axis), highlighting metabolites that were significantly upregulated or downregulated in response to nutrient supplementation. The vertical lines represent the fold change thresholds, and the horizontal line marks the t-test significance threshold. Several metabolites were found to be significantly upregulated in the FNS group, with notable features such as benzoxale, 5-(4-fluorophenyl)-3-(3-nitrophenyl)-1,2,4-oxadiazole, and alclofenac showing substantial fold changes and low p-values, indicating their importance in the metabolic response to supplementation. Conversely, metabolites such as zolmitriptan N-oxide were downregulated in the FNS group.

Further analysis of the PLS-DA results allowed for the identification of specific metabolites contributing to the observed differences between the CON and FNS groups. As shown in Table 23, metabolites such as thiocyanin, hydrogen

phosphate, and D-ribulose were significantly increased in the FNS group. These metabolites are involved in various metabolic pathways, including carbohydrate metabolism and lipid metabolism. Conversely, metabolites such as D-allothreonine, melatonin, and DG (18:1[9Z]) were significantly decreased in the FNS group, indicating changes in amino acid metabolism and lipid-related processes. The partial least squares discriminant analysis (PLS-DA) identified several key metabolites contributing to the discrimination between the control (CON) and functional nutrient-supplemented (FNS) groups, as shown in Figure 13. The Figure presents the variable importance in projection (VIP) scores for the top metabolites, indicating their relative importance in differentiating the two groups. The colored boxes on the right side of the plot show the relative concentrations of each metabolite in the CON and FNS groups. Metabolites such as benzoxale, 5-(4-fluorophenyl)-3-(3-nitrophenyl)-1,2,4-oxadiazole, and alclofenac were among the most important features, with the highest VIP scores, indicating that these compounds contributed significantly to the metabolic differentiation between the two groups. The corresponding colored boxes indicate that these metabolites were found at higher concentrations in the FNS group compared to the CON group, suggesting that functional nutrient supplementation induced notable changes in their levels.

Additionally, metabolites such as dimemorfan, libenzapril, and cyclobrassinol showed moderate VIP scores and displayed varying concentrations between the two groups. The heatmap illustrates that some metabolites, such as telatinib and lysoSM(d18:1), were elevated in the CON group but present at lower levels in the FNS group. The VIP scores and concentration patterns of these key metabolites provide insights into the underlying biochemical pathways affected by nutrient supplementation. The distinct metabolic profiles observed between the two groups suggest that the functional nutrient products influenced both lipid and carbohydrate metabolism, as well as the immune and stress-related metabolic pathways, contributing to the differentiation in colostrum composition. The hierarchical clustering analysis of metabolites in sow colostrum from the control (CON) and functional nutrient-supplemented (FNS) groups is illustrated in Figure 14. The heatmap displays the clustering of metabolites based on their relative abundance, with the color gradient representing the z-scores of metabolite concentrations across samples (blue indicates

lower concentrations, and red indicates higher concentrations). The clustering was performed using a distance measure based on correlation and a clustering algorithm to group similar metabolites.

The heatmap clearly shows two distinct clusters separating the CON and FNS groups, indicating substantial differences in the metabolic profiles of colostrum between the two treatments. Metabolites such as PG(20:5(6E,8Z,11Z)), benzoate, L-enantiomer, and anazolone were present at higher concentrations in the FNS group (depicted in blue for the CON group and red for the FNS group), suggesting that functional nutrient supplementation leads to the upregulation of these metabolites. In contrast, metabolites such as cyclobrassinol, dimemorfan, and alclofenac were more abundant in the CON group (depicted in red for the CON group and blue for the FNS group), indicating that these metabolites were downregulated in response to functional nutrient supplementation. The clustering dendrogram at the top of the heatmap further reinforces the grouping of the samples, with FNS-treated samples clustering separately from the control samples. This separation indicates that functional nutrient supplementation significantly alters the colostrum's metabolite composition, influencing several metabolic pathways.

3. Colostrum metabolomics enrichment analysis

To further explore the metabolic pathways affected by functional nutrient supplementation, pathway enrichment analyses were conducted on the metabolites in sow colostrum. The results of the enrichment analysis are presented in Figures 15, 16, and 17. Figure 15 highlights the top 25 enriched metabolite sets in colostrum from sows fed functional nutrient products. Metabolites involved in carbohydrate and carbohydrate conjugate metabolism, diphenylmethanes, and Strychnos alkaloids were among the most significantly enriched pathways, as indicated by their low p-values and high enrichment ratios. These results suggest that functional nutrient supplementation primarily impacts pathways related to carbohydrate metabolism and specific bioactive compounds. In Figure 16, the enrichment analysis of metabolites that were upregulated in the FNS group is presented. The most significantly enriched pathways for upregulated metabolites include indoles, morphinans, and estrane steroids. These findings suggest that nutrient supplementation leads to an upregulation of metabolites involved in immune modulation, stress response, and hormonal pathways, which may

contribute to improved colostrum quality and piglet development. Conversely, Figure 17 shows the enriched pathways for downregulated metabolites in the FNS group. Pathways such as indoles, benzenes, and quaternary ammonium salts were among the most significantly downregulated. The downregulation of these metabolites might indicate a reduction in metabolic pathways related to stress and oxidative processes, suggesting that the nutrient supplementation may help to reduce metabolic strain during lactation.

Discussion

The present study investigated the effects of functional nutrient supplementation on sow colostrum composition and piglet performance. Multiple analytical approaches, including PLS-DA, volcano plot analysis, pathway enrichment, and hierarchical clustering, were utilized to explore the underlying metabolic changes induced by nutrient supplementation. The results revealed that functional nutrient supplementation significantly modulated several metabolic pathways, with implications for both colostrum quality and sow productivity (Miguel et al., 2021)

1. Impact on Sow Performance and Colostrum Yield

The analysis of sow performance, as presented in Table 22, showed that functional nutrient supplementation did not result in statistically significant differences in average daily feed intake (ADFI) during both gestation and lactation. However, the slightly higher colostrum yield observed in the FNS group suggests that supplementation may enhance the production of colostrum, although this difference was not statistically significant (Declerck et al., 2015) While the total number of piglets born and the number of piglets alive at birth did not differ significantly between the CON and FNS groups, the increased percentage of piglets alive in the FNS group indicates a potential improvement in piglet survival, possibly related to enhanced colostrum quality (Quesnel et al., 2019)

2. Metabolomic Changes Induced by Functional Nutrient Supplementation

The PLS-DA analysis revealed clear separation between the CON and FNS groups, highlighting distinct metabolic profiles in colostrum. The clustering of samples within their respective treatment groups suggests that functional nutrient supplementation induced a consistent metabolic response (El-Loly, 2022; Kuzmin et

al., 2022) This was further supported by the volcano plot, which identified several metabolites that were significantly upregulated or downregulated in response to supplementation. Key metabolites, such as benzoate, 5-(4-fluorophenyl)-3-(3-nitrophenyl)-1,2,4-oxadiazole, and alclofenac, were found to be upregulated in the FNS group, indicating that these compounds may play important roles in the observed metabolic changes (Lemonakis et al., 2022) The VIP scores identified benzoxale, alclofenac, and cyclobrassinol as the most influential metabolites in distinguishing between the CON and FNS groups. The higher concentrations of these metabolites in the FNS group suggest that functional nutrient supplementation may have stimulated metabolic pathways associated with immune response, lipid metabolism, and oxidative stress management. These results align with previous research suggesting that nutrients such as medium-chain fatty acids (MCFAs) and lignocellulose can modulate colostrum composition and improve its bioactive properties (Opgenorth et al., 2020) The hierarchical clustering results demonstrated distinct grouping of metabolites between the CON and FNS groups, further emphasizing the metabolic changes induced by functional nutrient supplementation (Sampey et al., 2012) Metabolites such as PG(20:5(6E,8Z,11Z)), benzoate, and L-enantiomer were significantly upregulated in the FNS group, while cyclobrassinol and dimemorfan were more abundant in the control group. These findings suggest that functional nutrients can modulate the lipid and carbohydrate metabolism in sows, potentially improving colostrum's nutritional value (Eastwood et al., 2014) The clustering analysis also revealed that the FNS group exhibited more consistent metabolic responses compared to the control group, as evidenced by the tighter clustering samples. This consistency in metabolic response may reflect the positive effects of supplementation on the overall metabolic stability of the sow during lactation, leading to improved colostrum composition and enhanced piglet health outcomes.

3. Enrichment of Metabolic Pathways

The enrichment analysis provided further insights into the biochemical pathways affected by supplementation. The top 25 enriched metabolite sets highlighted those pathways involved in carbohydrate and carbohydrate conjugate metabolism, diphenylmethanes, and Strychnos alkaloids were significantly impacted by the functional nutrient supplementation (Hoegen et al., 2022) These pathways are crucial

for energy metabolism and the production of bioactive compounds that support immune function and growth. Additionally, the upregulated metabolites in the FNS group were enriched in pathways related to indoles, morphinans, and estrane steroids, suggesting a potential enhancement in immune modulation and stress resilience in sows (Liu et al., 2023; Suradhat, 2006) On the other hand, the downregulated metabolites (Figure 17) showed enrichment in pathways associated with benzenes, quaternary ammonium salts, and indoles, which may indicate a reduction in oxidative stress and metabolic load during lactation (Sun et al., 2017) The downregulation of these pathways could be beneficial in reducing metabolic strain, allowing for more efficient nutrient utilization during the lactation period (Fan et al., 2020; Pawlowski et al., 2019) Pathway enrichment analysis indicated that supplementation impacts a wide range of metabolic processes, including carbohydrate metabolism, lipid metabolism, and immune-related pathways. The upregulation of metabolites in pathways such as indoles and morphinans points to a potential improvement in the immunological properties of colostrum, while the downregulation of stress-related pathways suggests that functional nutrients may alleviate metabolic strain during lactation.

The results of this study provide compelling evidence that functional nutrient supplementation can modulate the colostrum metabolome, with potential benefits for both sow productivity and piglet health (Cheng et al., 2023) While the direct impact on productivity metrics such as feed intake and litter size was not significant, the observed changes in colostrum composition suggest that supplementation may improve piglet survival and performance by enhancing the immunological and nutritional quality of colostrum (Manzke et al., 2018) Furthermore, the implications of these findings extend beyond immediate sow productivity and piglet health, as they suggest a need for further exploration into breed-specific responses to functional nutrient supplementation. Different pig breeds may exhibit varying metabolic pathways that respond uniquely to nutritional interventions, potentially influencing colostrum quality and subsequent offspring development (King et al., 2006; Cheng et al., 2023) For instance, studies have shown that certain metabolites in colostrum, such as acetate and taurine, are positively correlated with litter weight gain and survival rates, highlighting the necessity for tailored dietary strategies based on genetic backgrounds (Picone et al., 2018). This approach could enhance not only the efficiency of pig

production systems but also improve animal welfare by ensuring optimal nutrition during critical early life stages, ultimately leading to healthier herds and more sustainable farming practices. The modulation of specific metabolic pathways, particularly those related to immune function and stress response, highlights the potential for targeted nutritional interventions to optimize colostrum composition. As the swine industry continues to focus on improving sow and piglet health, the application of metabolomic approaches to understand the effects of dietary supplementation offers new opportunities for advancing swine production efficiency and welfare (Cheng et al., 2023; Wu et al., 2021; Zhou et al., 2022)

Conclusions

The results of this study demonstrate that functional nutrient supplementation has a significant influence on the colostrum metabolome of sows, potentially improving the overall quality of colostrum and the health of piglets. Although no significant changes were observed in sow performance metrics such as feed intake, colostrum yield, or piglet survival rates, the metabolomic analyses revealed distinct differences between the control and supplemented groups. Key metabolites associated with immune function, lipid metabolism, and stress response were upregulated in the FNS group, suggesting that functional nutrient supplementation enhances the bioactive properties of colostrum.

Table 22 Effect of functional nutrient products on sow productive performance

Items	Treatment		SEM	P-value
	CON	FNS		
ADFI during gestation (kg)	3.04	3.30	0.125	0.162
ADFI during lactation (kg)	4.44	4.39	0.353	0.279
Colostrum yields (g)	4154.86	4692.14	401.758	0.730
Total born (n)	16.57	15.43	1.212	0.523
Piglets alive (n)	14.57	14.00	0.985	0.915
Alive (%)	88.52	91.40	3.175	0.359

FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

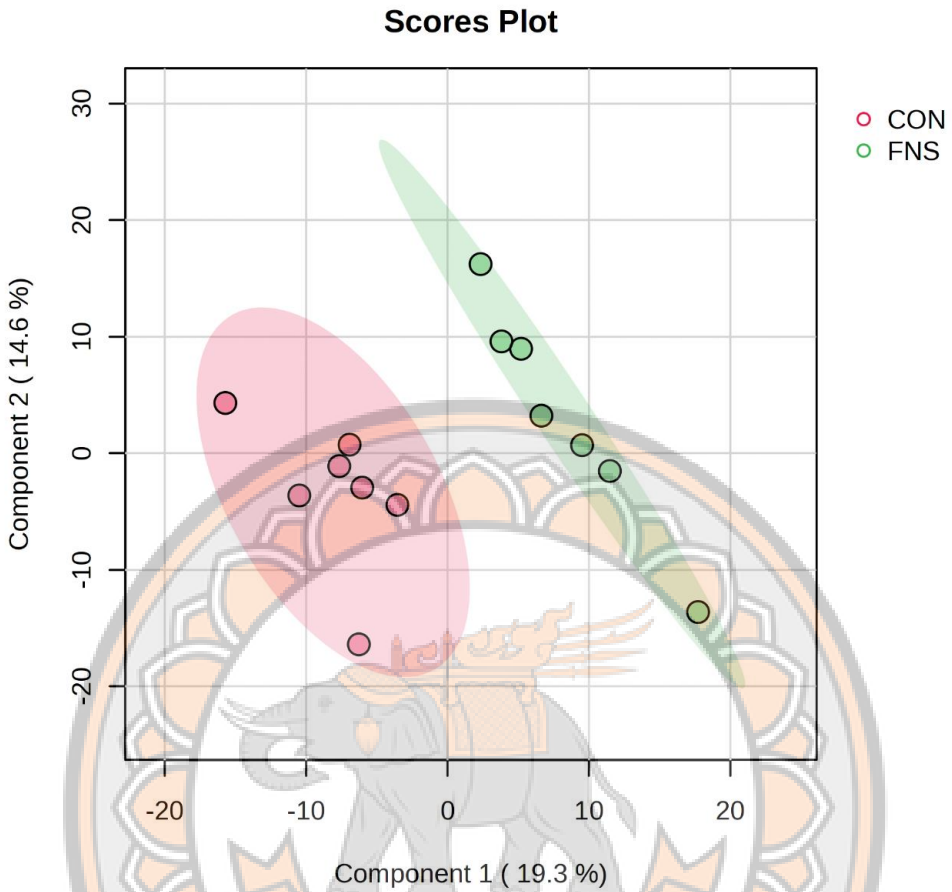

Figure 11 Scores plot between the selected PCs. FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

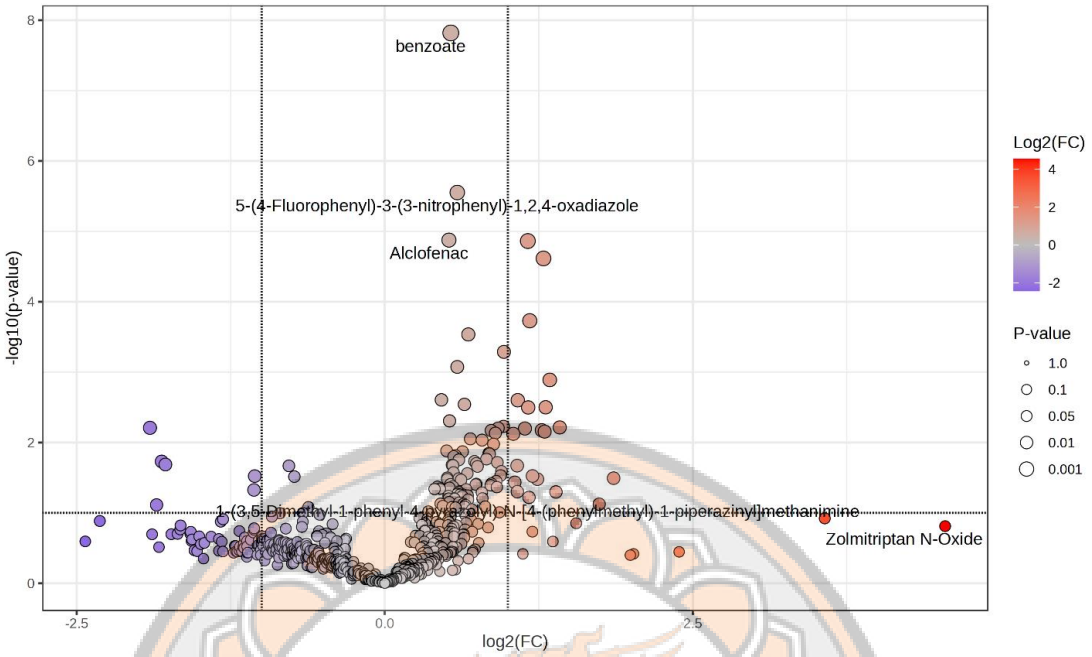

Figure 12 Important features selected by volcano plot with fold change threshold (x) and t-tests threshold (y) . FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

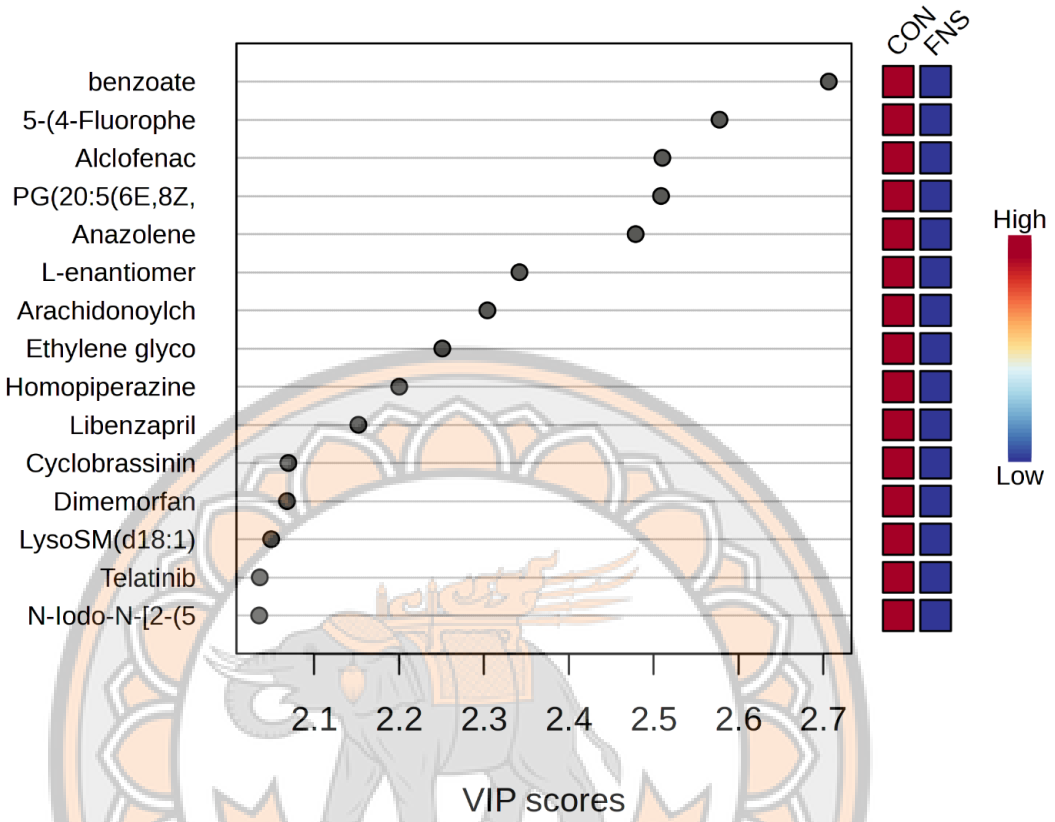

Figure 13 Important features identified by PLS-DA. The colored boxes on the right indicate. The relative concentrations of the corresponding metabolite in each group under study. FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

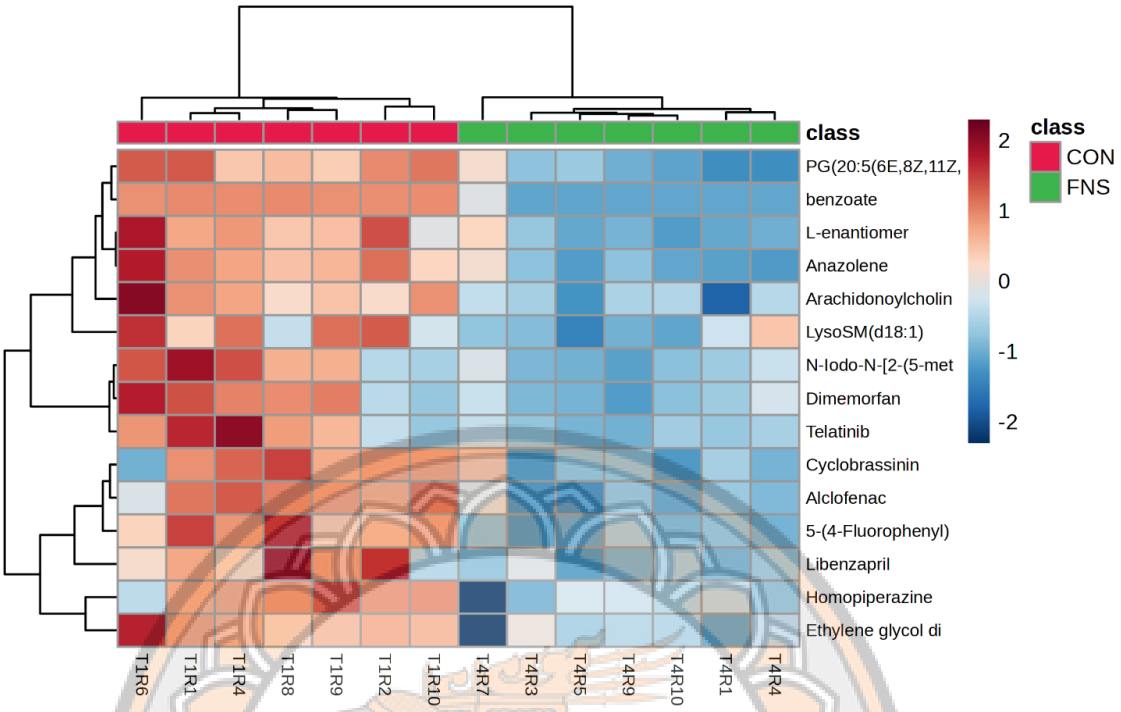

Figure 14 Clustering result shown as heatmap (distance measure using correlation, and clustering algorithm) FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

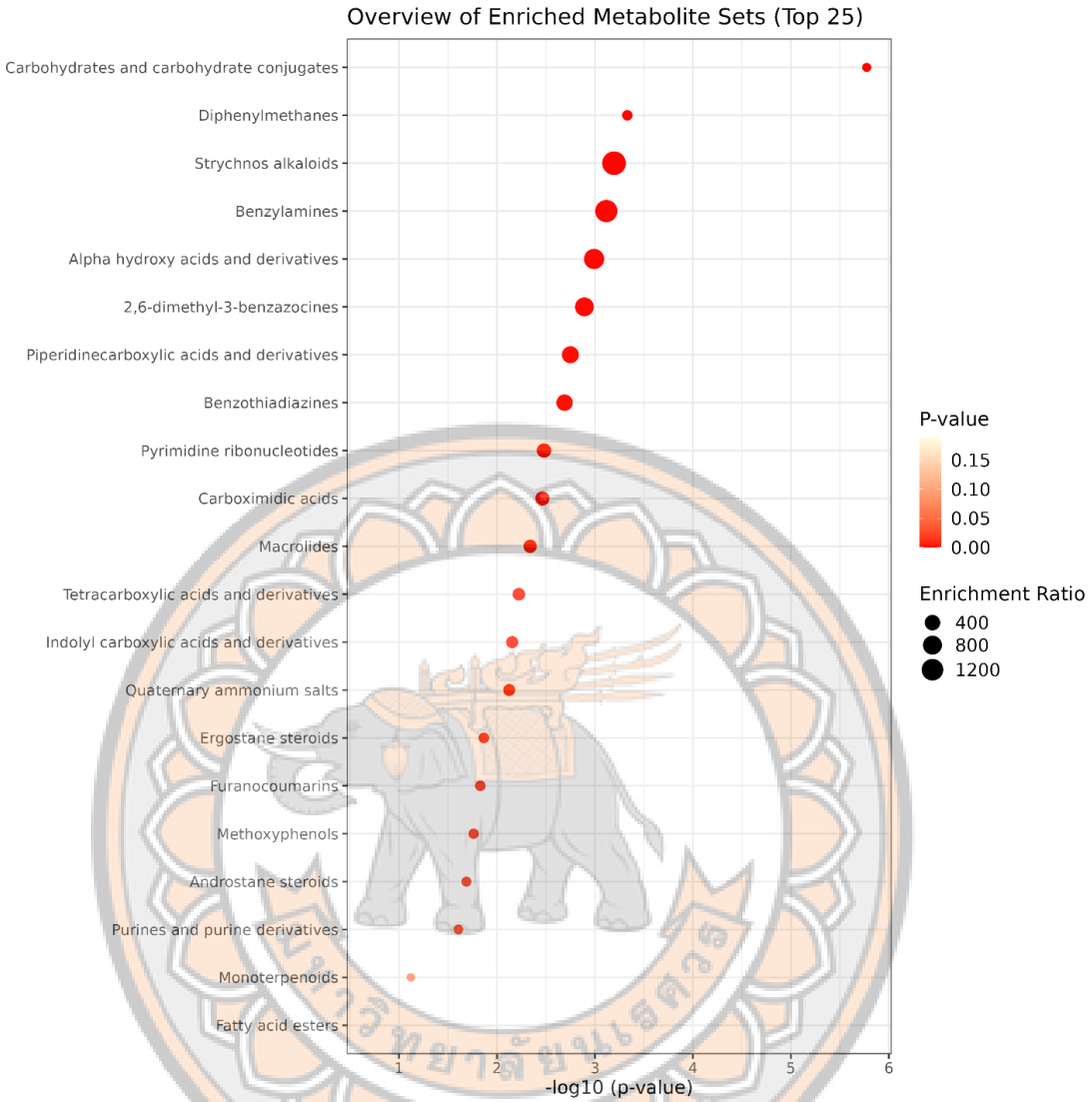

Figure 15 Enrichment analysis of sow colostrum fed functional nutrient products. FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

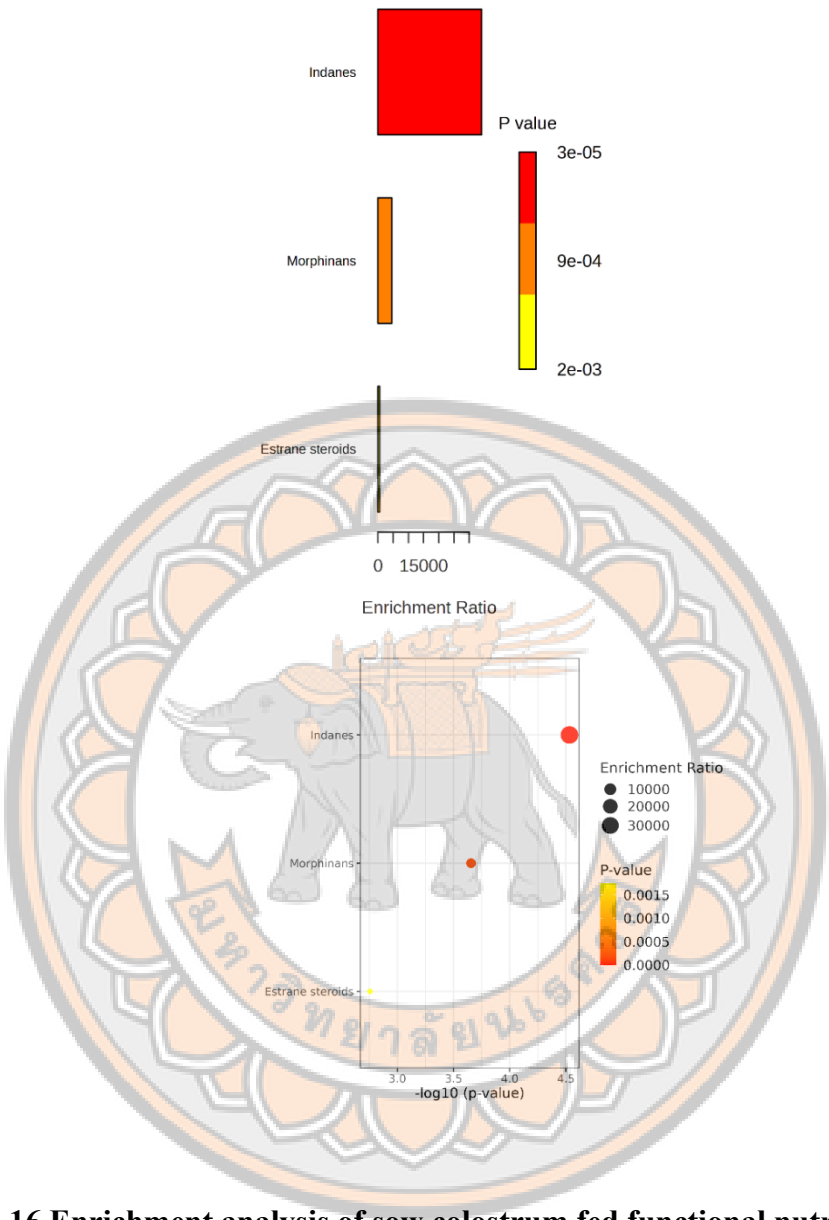

Figure 16 Enrichment analysis of sow colostrum fed functional nutrient products (upregulated metabolites in FNS) FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

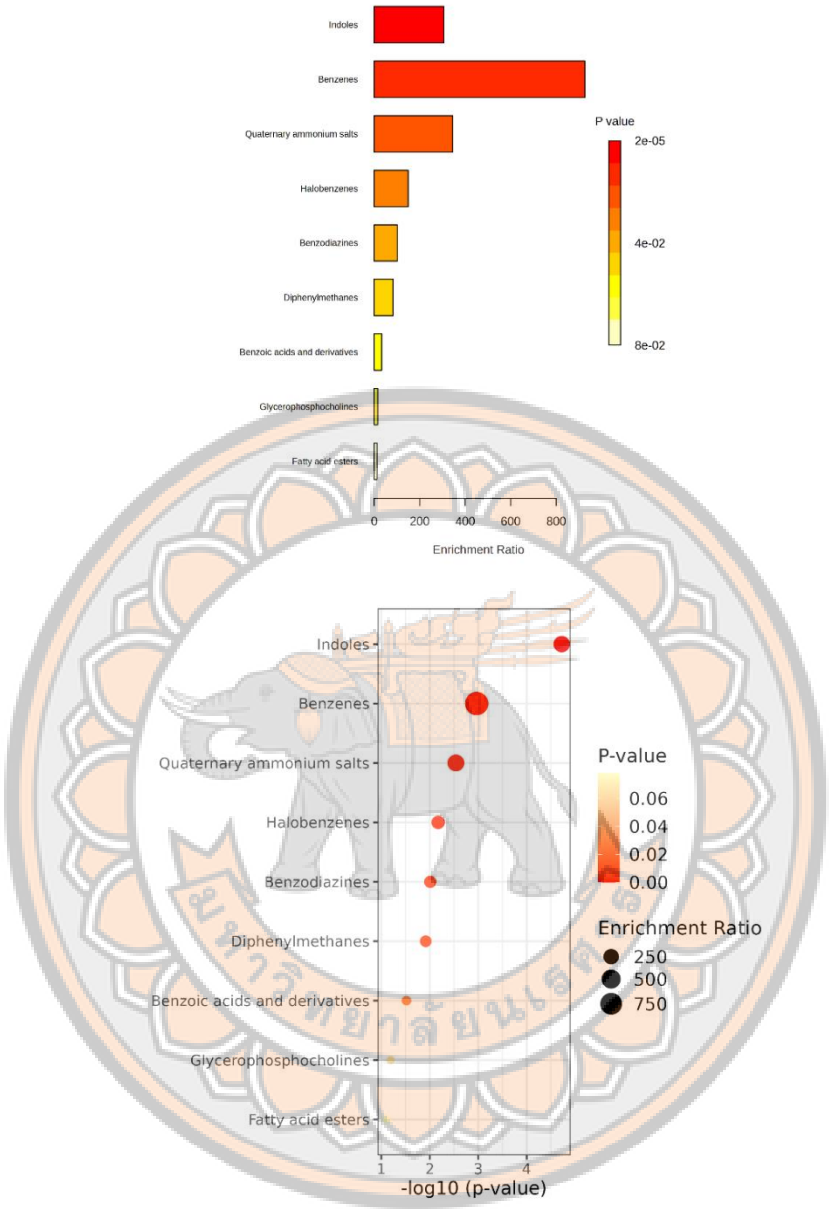

Figure 17 Enrichment analysis of sow colostrum fed functional nutrient products (downregulated metabolites in FNS). FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

Table 23 Chemical taxonomy of metabolites most strongly influencing discrimination by the partial least squares discriminate analysis (PLS-DA) that were upregulated and downregulated in colostrum of sow, following the conditions of VIP > 1.0 and |p-(corr)| 0.5

	Name	Chemical taxonomy	
		Super class	Sub class
Increased in FNS	Thiocyclam	Organoheterocyclic compounds	Trithianes
	Hydrogen phosphate	Organic oxygen compounds	Carbohydrates and carbohydrate conjugates
	D-Ribulose	Organic oxygen compounds	Carbohydrates and carbohydrate conjugates
	Phytantriol	Lipids and lipid-like molecules	Diterpenoids
	PGP(18:1)	Lipids and lipid-like molecules	Glycerophosphoglycerophosphates
	1-1-Aminoindan	Benzenoids	Indanes
Decreased in FNS	PG(42:10)	Lipids and lipid-like molecules	Glycerophosphoglycerols
	1-Naphthol	Benzenoids	1,1'-azonaphthalenes
	D-Allothreonine	Organic acids and derivatives	Amino acids, peptides, and analogues
	Libenzapril	Organic acids and derivatives	Amino acids, peptides, and analogues
	Dimemorfan	Alkaloids and derivatives	Morphinans
	Telatinib	Organoheterocyclic compounds	Pyridinecarboxylic acids and derivatives
	Melatonin	Organoheterocyclic compounds	Indoles
	DG(19:0)	Lipids and lipid-like molecules	Diradylglycerols
	2'-Nitroacetanilide	Benzenoids	Anilides
	Dimethylmethoxy chromanol	Organoheterocyclic compounds	1-benzopyrans
	Phenacyl alcohol	Organic oxygen compounds	Carbonyl compounds
	Encorafenib	Organoheterocyclic compounds	Pyrazoles
	Ulinastatin	Organoheterocyclic compounds	1,3-dioxanes
	Chlorprothixene sulfoxide	Organoheterocyclic compounds	1-benzothiopyrans
	2-amino-4,6-dinitrotoluene glucoside	Benzenoids	Toluenes

Name	Chemical taxonomy	
	Super class	Sub class
DG(18:1(9Z)	Lipids and lipid-like molecules	Diradylglycerols
2-4-Carbamimidoyl-benzoylamino	Benzenoids	Benzoic acids and derivatives
5-Pentyl-1,4-dioxan-2-one	Organoheterocyclic compounds	1,4-dioxanes
Phenylcyclohexyldiethylamine	Organonitrogen compounds	Amines
cis-Ferulic acid 4-glucuronide	Organic oxygen compounds	Carbohydrates and carbohydrate conjugates
4-Chloro-N-(1-pyridin-4-ylethyl)aniline	Organic nitrogen compounds	Amines

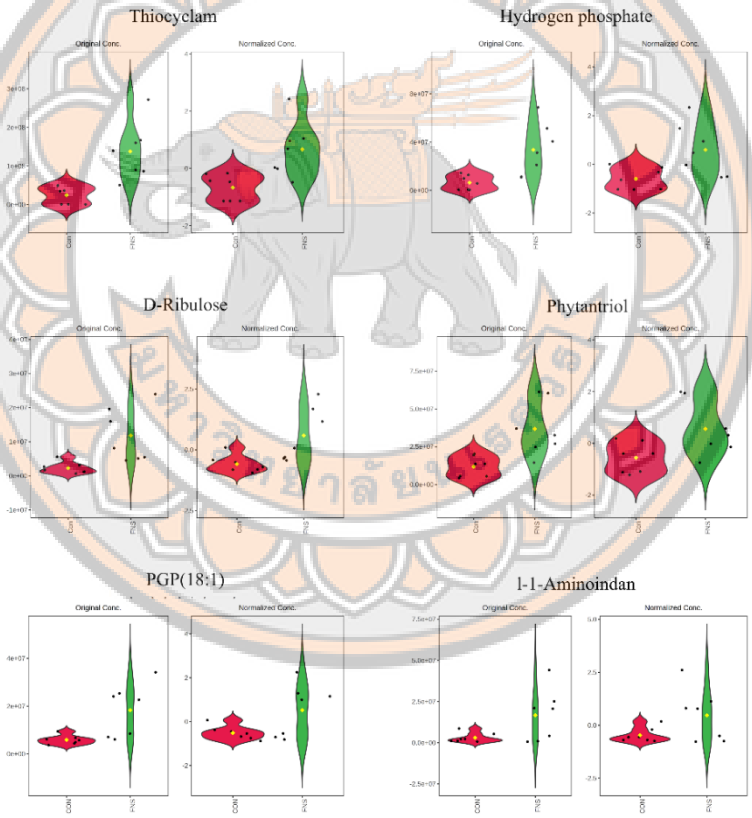

Figure 18 Fecal metabolites are most strongly influencing discrimination by partial least square discriminant analysis (PLS-DA) model of sow colostrum fed functional nutrient products. Upregulated metabolites in FNS. FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

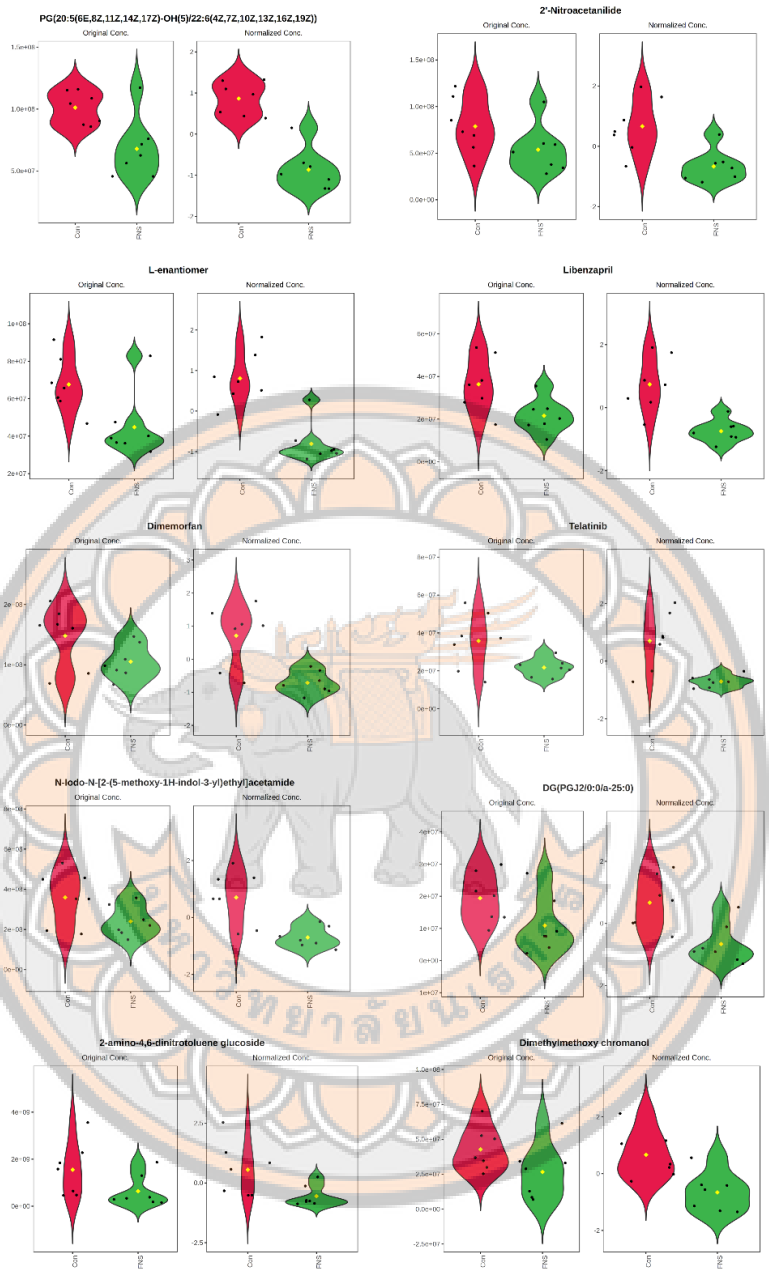

Figure 19 Fecal metabolites are most strongly influencing discrimination by partial least square discriminant analysis (PLS-DA) model of sow colostrum fed functional nutrient products. Downregulated metabolites in FNS. FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

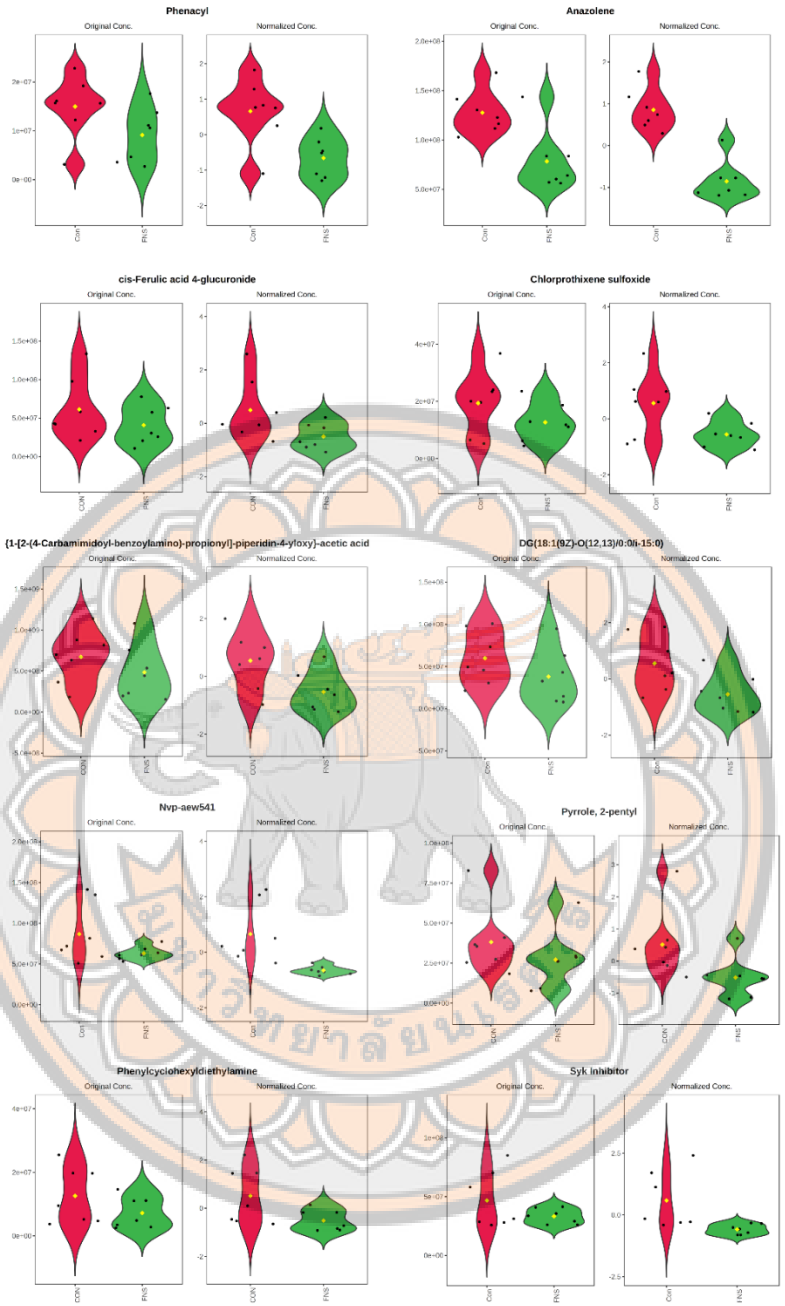

Figure 20 Fecal metabolites are most strongly influencing discrimination by partial least square discriminant analysis (PLS-DA) model of sow colostrum fed functional nutrient products. Downregulated metabolites in FNS. (Con.). FNS = Group supplemented with a high level of Functional nutrient for sow, 120 g/day contained: 74.91 g of miMCFA + 44.94 g of lignocellulose + 0.15 g of HKL137

CHAPTER VIII

SUMMARY CONCLUSIONS

Conclusions

This research aimed to explore the influence of functional nutrient supplementation (FNS), particularly the combination of microencapsulated medium-chain fatty acids (miMCFAs), lignocellulose, and heat-killed *Lactobacillus plantarum* L-137 (HK L-137), on sow productivity, colostrum composition, and piglet performance. The findings across the five studies provide valuable insights into the potential benefits of FNS in swine production, highlighting its ability to enhance colostrum quality and immune function, though its effects on traditional productivity metrics like litter size and piglet growth were more modest.

Study 1 focused on optimizing the microencapsulation conditions of MCFAs using spray drying and Response Surface Methodology (RSM). The study successfully identified optimal conditions that improved stability, encapsulation efficiency, and retention of essential fatty acids such as caprylic and lauric acids. The optimal conditions for MCFA microencapsulation involved preparing an emulsion with a NaCas:maltodextrin ratio of 1:4.98, a wall-to-core ratio of 70:30, and homogenization at 16,367.75 rpm, followed by spray drying at 200°C with a feed rate of 1.94 L/h, resulting in 83.13% encapsulation efficiency and 98.85% yield. The microencapsulated MCFAs produced under these optimized conditions were suitable for functional nutrient supplements in swine diets, providing a controlled release of bioactive compounds with enhanced stability. The optimal conditions for microencapsulation were established, resulting in high encapsulation efficiency (83.13%) and product yield (98.85%), with favorable moisture content and bulk density for long-term stability.

In Study 2, the effects of microencapsulated MCFAs (miMCFA) on sow and piglet performance were evaluated. The results demonstrated that miMCFA supplementation significantly improved colostrum production, piglet birth weight, and piglet survival rates, particularly in the groups receiving 50 g/day and 75 g/day of miMCFA. Additionally, sows supplemented with higher levels of miMCFA experienced less back-fat loss, indicating better maintenance of body condition during

lactation. These findings suggest that miMCFA supplementation can positively impact both sow health and piglet outcomes, making it a promising nutritional strategy in swine production.

Study 3 examined the combined effects of miMCFA, lignocellulose, and HK L-137 (100 g; 62.42 g of miMCFA + 37.45 g of lignocellulose + 0.13 g of HKL137) on sow performance, colostrum composition, and piglet immunity. The study found that supplementing with miMCFA and HK L-137 significantly increased immunoglobulin G (IgG) levels in colostrum, while lignocellulose improved nutrient absorption and fat content in colostrum. The combined supplementation of these three components during late of gestation 14 days before farrowing enhanced piglet growth and immune function, suggesting that such a multi-nutrient approach can provide more comprehensive benefits to both sows and piglets.

In Study 4, the effects of varying FNS supplementation levels on sow and piglet performance, colostrum composition, and IGF-1 gene expression were evaluated. Although FNS supplementation showed minimal influence on conventional productivity indicators such as litter size and piglet birth weight, it significantly improved colostrum composition particularly increasing fat content and IgG concentrations. Moreover, IGF-1 gene expression was upregulated in the FNS-supplemented groups, suggesting enhanced fetal growth potential during gestation. Overall, these results indicate that while FNS may not markedly change standard performance metrics, it can enhance piglet immune function and growth potential by improving colostrum quality and regulating IGF-1 expression.

Study 5 focused on the metabolomic analysis of colostrum in response to FNS. Using liquid chromatography-mass spectrometry (LC-MS), the study identified distinct metabolic profiles between the control and FNS groups. Key metabolites related to lipid metabolism, immune function, and stress response were upregulated in the FNS group, highlighting the potential of FNS to improve piglet health through enhanced bioactive components in colostrum. Although no significant differences were observed in traditional productivity metrics, the metabolomic data provided valuable insights into the biochemical pathways influenced by FNS, particularly those related to immune function and stress reduction.

Across all studies, the use of functional nutrients contained MCFAs, lignocellulose, and HK L-137 demonstrated their potential to improve key aspects of swine production. While the effects on litter size, piglet birth weight, and weaning weight were modest, the improvements in colostrum composition and immune function suggest that FNS could be a valuable tool for enhancing piglet survival and health. Moreover, the upregulation of IGF-1 and the distinct metabolic profiles observed in the FNS groups provide a foundation for further research into the long-term benefits of these functional nutrients.

Suggestions

1. Optimization of Supplementation Strategies: Future research should focus on optimizing the dosage and timing of FNS to maximize its benefits. While the current study showed positive effects on colostrum composition and immune function, further studies are needed to determine the most effective supplementation protocols for different production stages.

2. Combination with Other Dietary Interventions: To enhance the impact of FNS on sow and piglet performance, combining functional nutrients with other dietary interventions such as omega-3 fatty acid supplementation or specific prebiotics may yield more pronounced results.

3. Commercialization and Field Trials: The functional nutrient products developed in this study have the potential for commercialization. Field trials should be conducted to assess the real-world applicability of these supplements in various swine production systems, taking into account different environmental and management conditions.

4. Longitudinal Studies on Piglet Development: While this study focused on neonatal piglet performance, long-term studies are needed to assess the effects of FNS on piglet development beyond the weaning phase. This includes evaluating the impact of early-life supplementation on growth performance, immune function, and overall health throughout the pig's life cycle.

Sustainable Alternatives to Antibiotics: Given the growing concerns over antibiotic resistance, the findings of this study support the continued exploration of functional nutrients as a viable alternative to antibiotics in animal production. Future research should investigate the potential for FNS to reduce the incidence of disease and improve overall herd health, further reducing the need for antibiotics in swine production.

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