# THESIS

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**M.Sc. Animal Nutrition and Feed Safety Engineering**

# MEDIUM CHAIN FATTY ACIDS; PERFORMANCE AND DIGESTIBILITY IN WEANED PIGLETS

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## ABSTRACT OF THESIS

**MEDIUM CHAIN FATTY ACIDS; PERFORMANCE AND DIGESTIBILITY IN WEANED PIGLETS**

Ongoing issues in the swine sector, such as reduced antibiotics usage and stress during weaning, have sparked requests for novel feed additives to help pig producers. Medium-chain fatty acids (MCFAs) have emerged as a promising choice due to their unique chemical characteristics and various activities, which include improved growth performance. The most recent advancements in evaluating MCFAs as feed additives in pig diets are given, and their implications for a wide variety of production difficulties, such as growth performance, pathogenic infections, and gastrointestinal health, are discussed. This study investigates the potential of MCFAs as feed additives to improve pig gut health. The trial was conducted with 24 healthy cannulated weaned Topigs x DanDuroc hybrid barrows. The piglets' live weight (LW) was between 17 and 19 kg when they were surgically fitted with a simple T-cannula at the distal *ileum*. The implemented cannula was suitable for a maximum of 40-45 kg LW pig. This study aimed to prove the mode-of-action of a gut-health-improving product based on sustainable raw materials that enhances technical performance reduces the need for antibiotics, and reduces post-weaning mortality. The objectives of the current research were to investigate the possible differential response of MCFA produced from palm kernel oil and bio-based odd-chain MCFA. The average daily gain (kg) and feed conversion ratio (kg/kg) on the day 0 –7 (1 week gain) of the treated group was significantly different compared to the control group ($p<0.05$). The current study had no significant effect on nuclear cell viability on the 1$^{st}$, 2$^{nd,}$ and 3$^{rd}$ collection($p>0.05$) for apoptosis, necrosis, and live percentages. However, the 2$^{nd}$ collection showed a significant difference at the late apoptotic stage ($p<0.05$). It was also observed that MCF content and ratio had no significant effect ($p>0.05$) on the faeces and ileal digesta. In summary, using MCFA in nursery pig diets improves growth performance, does not significantly affect polymorph nuclear cell viability, and does not significantly alter content and ratio in faeces and ileal digesta. Looking ahead, we anticipate that MCFAs may become an important class of feed additives in pig production for gut health enhancement.

KEY WORDS

MCFAs, piglet, performance, digesta

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# 1 INTRODUCTION

## 1.1 Background of the study

The growing energy expense has generated a renewed interest in regulating the use of supplementary lipids in pigs. Nutritionists work to satisfy the needs of today's high-achieving pigs by increasing the dietary energy density through dietary lipids, which may be obtained from various sources. Most swine feeds are not formulated based on digestible lipids, and lipid digestibility data is often not included in formulation programs. However, because lipids aid in the absorption of energy from diets, lipid digestibility is sometimes tested in feed components (NRC, 2012). The impact of dietary fat on performance at different production phases has historically been the focus of attention in lipids for swine nutrition.

Nevertheless, for the pig to efficiently utilize the supplied fat, its digestive and absorption systems must function normally. Additionally, there is potential to improve the understanding of how fatty acids (FA) contribute to forming pigs' guts throughout their early diet. Fat digestibility is low in weanling pigs, but the ability to digest fat increases with age, particularly for animal fats compared with vegetable oils (Carter, 2010). Since monogastric animals directly use dietary FAs, the sow's milk and colostrum FA profiles represent her intake of nutrients, providing the possibility to alter the piglet's FA supply and the milk's FA composition. Piglets' milk replacers, pre-starter, and starter diets should thus be developed using the sow milk's lipid content and FA profile as a guide (Metzler-Zebeli, 2021). Over the past 30 years, only slight variations in the sow colostrum, as well as milk content of macronutrients, have been observed, irrespective of genetic modifications (e.g., development of hyper prolific sows); similarly, for the total lipid content, an increase from 6.5 to 7.5% has been reported (from the 1980s to 2010s) (Zhang et al., 2018). Certain research findings suggest that an inadequate intestinal energy supply may result from the reduced digestive and absorptive ability linked to a reduced feed intake following weaning. The intestinal mucosa of pigs experiencing development retardation has a decreased capacity for nutritional absorption and an abnormal energy status (Xiong et al., 2015; Qi et al., 2020), as in the case of pigs born prematurely as a result of high litter sizes from hyper prolific sow lines that have been shown to have compromised gut colonization (Kamal et al., 2019).

Suiryanrayna and Ramana (2015) have recently evaluated the effects of organic acids routinely provided to pigs. Due to their possible advantages for nutritional absorption and intestinal health in weaned piglets, medium-chain fatty acids (MCFAs) have drawn interest in swine nutrition. Additionally, the digestion of MCFAs in weaned piglets and their effects on the health and performance of piglets have been the subject of several research (Hanczakowska et al., 2013, 2017; Suiryanrayna and Ramana, 2015; Lauridsen, 2020).

MCFAs have the potential to enhance nutrient absorption, particularly during the early post-weaning stage, when pigs often have difficulty acclimating to solid diets. Research by Hanczakowska et al. (2013) showed that the supplementation of MCFAs improved absorption and digestibility, feed efficiency, and weight gain of animals. It was discovered that MCFAs were effectively metabolized by pigs, which enhanced both growth performance and energy utilization. MCFAs can positively impact the intestinal health of weaned pigs because of their well-known antibacterial qualities. In addition to enhancing general gut health, they could lessen the frequency of diarrhea (Thormar, 2011; Gebhardt et al., 2020).

Certain fatty acid sources, food composition, and piglet age can all affect the ideal amount of MCFAs in a piglet's diet. Per Rojas and Stein (2016), achieving the correct balance is essential to maximizing the advantages.

### 1.2 Classification of Dietary Fats

Based on historical categorization, lipids are mostly "fats" and "oils"; in general, "oils" are lipids derived from plants and are liquid at room temperature, whereas "fats" are lipids derived from animals and are typically solid. These categories contain some significant exclusions, such as "poultry oil." Lipids comprise several hydrophobic compounds, including triglycerides, phospholipids, sphingolipids, cholesterol and ester derivatives, and fat-soluble vitamins. They also include the single fatty acids (C6 to C32), their derivatives, and their bioactive derived metabolites (Zárate et al., 2017). Boskou (2015) states that triglycerides comprise around 98% of the entire weight of the fats and oils used in animal feeding, with minor molecules making up the remaining 2% of the total lipid content.

Fatty acids play a crucial role in the nutrition and health of pigs. They are essential components of the pig's diet and involve various physiological processes. Fatty acids are a dense source of energy in the pig's diet. They provide more energy per unit weight compared to carbohydrates and proteins. FAs constitute the main components of phospholipids, triglycerides, and cholesterol esters. FAs are acidic, monocarboxylic linear chains of variable length. For example, butyric acid

(C4:0), palmitic acid (C16:0), and arachidic acid (C20:0) contain 4, 16, or 20 carbon atoms in their chains, respectively. Short-chain fatty acids (SCFA) are fatty acids with up to 5 carbon atoms, medium-chain fatty acids (MCFA) have 6 to 12, long-chain fatty acids (LCFA) 13 to 21, and very long-chain fatty acids (VLCFA) are FAs with more than 22 carbon atoms. (Agostoni and Bruzzese, 1992). They can be further subdivided into saturated (no double bond), monounsaturated (one double bond), and polyunsaturated (two or more double bonds). FAs are important for the absorption of fat-soluble vitamins (A, D, E, and K) and other nutrients, such as calcium and selenium, in the digestive system of pigs (Upadhaya et al., 2016).

**Table 1**. Fatty acid profile (% of total fatty acids) of fats and oils which have high saturated fatty acid content

| | Dietary Vegetable Oils[1] | | | Lard |
|---|---|---|---|---|
| **Fatty Acid** | **Coconut Oil** | **Palm Oil** | **Harlan Animal Feed[2]** | **Prepared from Animal Fat[3]** |
| C6:0, caproic | 0.4 | | | |
| C8:0, caprylic | 7.3 | | | |
| C10:0, capric | 6.5 | | | |
| C12:0, lauric | 49.2 | 0.3 | | |
| C14:0, myristic | 18.9 | 1.3 | 1.5 | 1.3 |
| C16:0, palmitic | 8.9 | 43.4 | 24.8 | 20.7 |
| C18:0, stearic | 3.0 | 4.8 | 12.3 | 10.9 |
| C18:1, oleic | 7.5 | 40.0 | 38.7 | 39.1 |
| C18:2, linoleic | 1.8 | 10.5 | 10.0 | 19.6 |
| C18:3, linolenic | 0.1 | 0.3 | 0.1 | 1.2 |
| MCFA (satd) | 63.3 | 0.3 | 0.0 | 0.0 |
| LCFA (satd) | 30.8 | 49.4 | 38.6 | 32.9 |

[1]Codex Alimentarius Stan 210 (2013)

[2]Teklad Custom Research Diets, Harlan Laboratories.

[3]Rohman et al. 2012.

## 1.3 Dietary Fat Inclusion and Its Effect

According to Ratnayake and Galli (2009), the energy value of fats and oils in feed is determined by a variety of factors, including the length of the carbon chain, the specific organisation of saturated and unsaturated fatty acids in the glycerol molecule, the structure of free fatty acids, the structure of the feed, the amounts and types of triglycerides added to feed, and the intestinal flora, as well as the animal’s species, sex and age. Swine diets usually include up to 5% fat. Adding 3 to 4% fat in the nursery is mostly utilized to enhance the pelleting process of first diets that include high lactose levels. 1 to 5% fat is added to grower-finisher diets to enhance growth performance; feed efficiency is typically improved by about 2% and average daily gain by about 1% for every 1% of fat added. 3–5% fat is commonly used to improve dietary energy density during lactation. Diets during pregnancy usually do not include additional fat. When fat content exceeds 5% in a ration, it typically causes handling problems such as feeder bridging and mixer caking, while in pelleted diets, it results in lower-quality pellets (Carter, 2010). Excessive amounts of added fat in diets make them more likely to go rancid after long-term storage or exposure to high temperatures. The most cost-effective amount for fat inclusion should be determined by an economic analysis that considers the market price and the impact of small energy changes on production indicators. A manufacturing tool called the Net Energy Model has been created to help determine the energy level of the diet throughout the grow-finish phase (Carter, 2010).

Dietary fat is essential for meeting the energy requirements of growing pigs and reproducing sows (Patience et al., 2015). Fatty acids such as Omega-3 FAs have anti-inflammatory properties that can support the pig's immune response, helping reduce the risk of diseases (Wijtten et al., 2011). Previous authors suggested that the reduction of non-favorable microbial populations, such as Escherichia coli, and the increase of beneficial microorganisms may be attributed to the ability of fatty acids to regulate intestinal pH or diffuse into the bacterial cells (Hanczakowska et al., 2016; Jackman et al., 2020). Quessenel and Farmer (2019) concluded that the dietary fatty acid composition positively impacted the concentration of immunoglobulins in colostrum and milk. This result may be related to the immunomodulatory actions of the FA.

The dietary fatty acid composition of the diet can be manipulated to influence the quality of pork meat to meet consumer preferences for healthier and more nutritious pork products (Daza et al., 2020).

# 2 LITERATURE REVIEW

## 2.1 Short-chain fatty acids

Short-chain fatty acids(SCFAs) are a subset of saturated fatty acids with six or fewer carbon molecules. SCFAs are byproducts of dietary fiber and resistant starch fermentation, and they have been shown to have a variety of favorable impacts on pig energy metabolism (Den Besten et al., 2013). Short-chain fatty acids are rapidly absorbed through the digestive system, potentially increasing nutrient utilization and overall digestive efficiency (Stiegler, P., et al., 2013).

SCFAs play an important function in swine nutrition and have implications for many areas of pig health. SCFAs have been found in studies to enhance lipid and glucose metabolism in pigs, protect against high-fat diet-induced obesity, and reduce fat deposition in pigs through modulating associated hormones and genes (Jiao et al., 2020; Zhou et al., 2021). SCFAs have been found to positively impact gut development and microbial succession in piglets, particularly during the early growth stages. The gut bacteria produce SCFAs by the fermentation of dietary fiber and resistant starch, which is directly linked to bioamine levels in the gut (Qi et al., 2021).

## 2.2 Medium Chain Fatty Acids

Medium-chain fatty acids are contained in triacylglycerol (TG) in general foods. MCFAs are markedly different from long-chain fatty acids regarding physical properties, digestion/absorption, biodegradation, and body fat accumulation. Saturated fatty acids with chain lengths varying from six to twelve carbon atoms are medium-chain fatty acids or MCFAs. According to St-Onge and Jones (2002), the main sources of medium-chain fatty acids include dairy products like butter, coconut oil, and palm kernel oil. Unlike long-chain fatty acids (LCFAs), medium-chain fatty acids are absorbed and metabolized differently, rendering them special, according to St-Onge and Jones (2002). MCFAs are quickly absorbed into the portal vein and transported to the liver, which can be efficiently oxidized and converted into energy. In addition, they enhance the intake of calcium and amino acids to support the production of intracellular protein (Bertevello et al., 2012). It should be mentioned, nonetheless, that the strong smell of no esterified FFA, or free MCFA, may deter feed intake due to its robust, goat-like scent. Using MCTG, in conjunction with lipases, to improve the enzymatic release of MCFA in situ in the foregut could help overcome this obstacle (Lauridsen, 2020). The effects of MCFA on growth performance greatly depend on MCFA type, purity, and inclusion rate in the diet (Gebhardt et al., 2017).

### 2.2.1 Classification and Effect of Medium Chain Fatty Acids

The main constituents of MCFAs are lauric acid (C12), capric acid (C10), caprylic acid (C8), and caproic acid (C6). According to Reiner (2018), each of these MCFAs has distinct qualities and potential health advantages.

Caproic acid, also known as hexanoic acid, is a six-carbon MCFA, and its potential advantages in swine diets have been investigated. Research indicates that caproic acid supplementation and other organic acids can enhance pigs' growth performance, nutrient digestibility, and microbiota counts (Nguyen et al., 2020).

Caprylic acid, an eight-carbon MCFA, has demonstrated antibacterial properties, which can aid in controlling gut pathogens in swine and poultry (Skrivanova et al., 2008). Capric acid, a ten-carbon MCFA, has been found to have protective effects against cyclophosphamide-induced small intestinal dysfunction in pigs. Furthermore, it has been demonstrated that in pigs treated with cyclophosphamide, capric acid decreases the generation of inflammatory cytokines, reduces oxidative stress, and increases the expression of genes linked to oxidative stress (Lee and Kang, 2017).

Capric acid has been found to have similar antimicrobial properties as caprylic acid, making it beneficial for improving gut health in poultry and swine (Zentek et al., 2011). It has also been discovered that caprylic and capric acids are useful in lowering the amount and contagiousness of porcine epidemic diarrhea virus (PEDV) (Cochrane et at., 2019).

Lauric acid is a twelve-carbon medium-chain fatty acid (MCFA) that is highly digestible and has a high energy density, thereby, it can be added to swine diets to increase energy density and improve diet palatability (Carter, 2010). Lauric acid has gained attention for its potential role in enhancing the immune system and improving growth performance in various species (Saki et al., 2020). Furthermore, it has been shown that lauric acid possesses antibacterial properties, suggesting that it might take the role of antibiotics in feeds to avert post-weaning diarrhea and boost pig production in general (Yang et al., 2020). Weaned pigs' intestinal health and cell regeneration have been demonstrated to be enhanced by lauric acid (Zeng et al., 2022). However, porcine epidemic diarrhea virus (PEDV) was shown to be infectious in pigs fed lauric acid-containing feed; therefore, it is crucial to remember that the right MCFA choice will depend on the pathogen or pathogens being targeted (Cochrane et al., 2020).

MCFAs may also have a positive impact on the immune system. An investigation by Li et al. (2015) on the effects of MCFAs on the immune response of weaned pigs found that MCFAs enhanced immune function, potentially reducing the need for antibiotics. They can also help reduce the risk of pig intestinal infections. A study was conducted to investigate the impact of MCFAs on gut health and found that MCFAs can help control pathogens in the pig's gastrointestinal tract, leading to improved health and performance (Van Immerseel et al., 2016). Research conducted by Zhao et al. (2015) demonstrated that the dietary inclusion of MCFAs improved the growth performance of weaned piglets. The researchers found that pigs fed diets containing MCFAs had better average daily gain (ADG) and feed conversion ratio (FCR) than those on a standard diet. Additionally, research by Woyengo et al. (2017) revealed that MCFAs might boost pigs' small intestines' capacity to absorb nutrients, promoting nutrient utilization and growth.

The current results by Jiao et al. (2023) showed that dietary MCFAs and *Bacillus* in combination improved piglet intestinal barrier function by changing the intestinal microbiota and metabolic function, and thus alleviated diarrhea in the early weaning stage and improved growth performance throughout the trial period. MCFAs were also efficient in increasing piglet feed efficiency and antioxidant capacity. Furthermore, dietary MCFAs may help prevent fat buildup, according to research on how they affect lipid deposition and metabolism in developing pigs (Liu et al., 2016). Other studies also concluded that MCFAs increase villus height and up-regulate the expression of tight junctions in the ileum, which results in lower diarrhea incidence (Hanczakowska, et al. 2017; Lei et al. 2017, Liu et al., 2021) (Table 2 and Figure 1).

**Table 2**. Mucosal epithelium structure of the ileum (based on 6 piglets per group)

| Ileum morphology | Group I | Group II | Group III | Group IV | Group V | SEM |
|---|---|---|---|---|---|---|
| | control | (PF) | (PF+$C_8$) | (PF+$C_{10}$) | (PF+$C_8$+$C_{10}$) | |
| No | 271 | 257 | 157 | 297 | 150 | – |
| Villus height, mm | $255^{Aa}$ | $264^{Aa}$ | $272^{ABb}$ | $292^{Bc}$ | $272^{ABb}$ | 2.453 |
| Villus width, mm | $116^{Bb}$ | $122^{BCb}$ | $127^{Cc}$ | $129^{Cc}$ | $109^{Aa}$ | 0.913 |
| No | 161 | 168 | 107 | 85 | 92 | – |
| Crypt depth, mm | $287^{Aa}$ | $391^{Cc}$ | $328^{Bb}$ | $293^{Aa}$ | $305^{ABa}$ | 3.52 |
| Villus height/Crypt depth | 0.888 | 0.675 | 0.829 | 0.996 | 0.892 | – |

a,b, mean values in the same row with different letters indicate significant differences at $P < 0.01$ (A, B) or $P < 0.05$ (a, b) (Hanczakowska, et al. 2013)

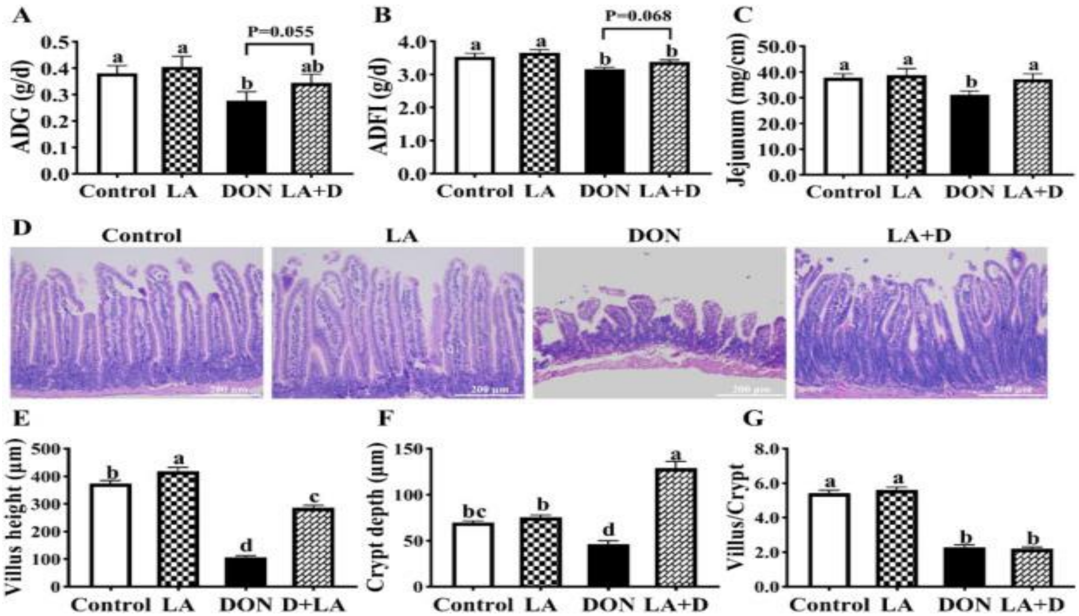


**Figure 1.** The villus height treated with LA was increased, the crypt depth was slightly decreased, and the villus height/crypt depth ratio was improved. These results indicated that supplementation with LA improved growth performance and intestinal morphology (Liu et al., 2021)

These studies collectively suggest that medium-chain fatty acids can positively impact pig nutrition, including growth promotion, antimicrobial properties, improved energy utilization, enhanced immune function, and better nutrient absorption.

## 2.3 Long Chain Fatty Acids

Since dietary fatty acids are an important energy source, long-chain fatty acids (LCFAs) are vital to piglets' diets. Notwithstanding the complexity of LCFA digestion and absorption, these FAs have bioactive effects on inflammatory responses through their incorporation into membranes. To serve as a barrier, epithelium must possess both immunological and antibacterial qualities. Acute and chronic inflammatory immune responses are important for intestinal health and strength, and they can be reduced by dietary n-3 PUFA, particularly the LCFA found in fish oil (Lauridsen, 2020; Metzler-Zebeli, 2021).

Alpha-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6) are two important fatty acids that are mostly present and are utilized in pig diets (NRC, 2012; Lauridsen, 2020). LCFA supplementation has been shown to greatly improve the growth performance of grower and finisher pigs, primarily by increasing the energy density regardless of the basal diet and saturation

(Li et al., 2019). Also, in the study by Huber et al. (2018), dietary n-6 to n-3 reduction had a positive effect on parameters like growth performance during the early nursery period and immunity (measured by a reduction in hyperdermal IgG response and attenuation of acute-phase protein production) of weaned piglets upon challenge. Research has examined how dietary n-3 long-chain fatty acids affect the microbial variety and composition of sows' faeces, colostrum, milk, and the faeces of suckling piglets, and it was concluded that dietary n-3 LCFA had a positive impact on the microbiome of suckling piglets' faeces by increasing microbial diversity and some beneficial bacteria populations (Llauradó-Calero et al., 2022). However, blends of fat sources, particularly those with a high FFA concentration, may reduce gut integrity by increasing the risk of inflammation and oxidative stress (Zhu et al., 2012). The long-chain n-3 PUFA highly present in fish oil can interfere with the activation of the toll-like receptor 4 (TLR4), thereby ameliorating the inflammatory signal (Childs et al., 2019). Oxidation of dietary polyunsaturated fatty acids may, on the other hand, impair the function of the intestinal epithelium (Lauridsen, 2020).

### 2.4 Ileal Digestibility

Because of advances in understanding active lipids and the availability of agricultural coproducts with high-fat contents, lipid research in swine diets has risen during the last decade. However, lipid research is required to assess the standardized ileal digestibility of fat sources in pigs, particularly nursery piglets (NRC, 2012). The small intestine plays an important role in the terminal digestion and absorption of nutrients during postnatal growth in animals. Because lipids are poorly soluble in the aqueous environment of the small intestine, their digestion and absorption require sequential steps in the small intestine (i.e., emulsification, enzymatic hydrolysis, micelle formation, transport through the unstirred water layer, and absorption into the enterocytes) (Bauer et al., 2005). During crucial stages like the transition from the prenatal to the postnatal environment and from nursing to weaning, the gut adjusts to significant changes in nutrient supply and profiles; therefore, there is a need to emphasize how the piglet's digestive ability and the makeup of the dietary fatty acids affect the lipids' digestion, absorption, and metabolism.

Various factors, including the form of dietary fat, dietary protein levels, animal factors, and the composition of dietary fatty acids influence the ileal digestibility of fatty acids in pigs. The apparent ileal digestibility of fatty acids can be increased by including high dietary protein levels because undigested protein might stabilize micelle formation. However, increasing dietary protein may also decrease the apparent digestibility of lipids because the binding of free fatty acids to amino acids may reduce their availability for absorption. The form of dietary fat, such as liquid or intact form, and the concentration of dietary NDF can also affect the ileal and total tract endogenous losses of fat. Lipid digestibility is thus more correctly defined as ileal digestibility rather than total tract digestibility (NRC, 2012). Figure 2 below illustrates the steps in the metabolism of fat and oil (Dayrit, 2014).

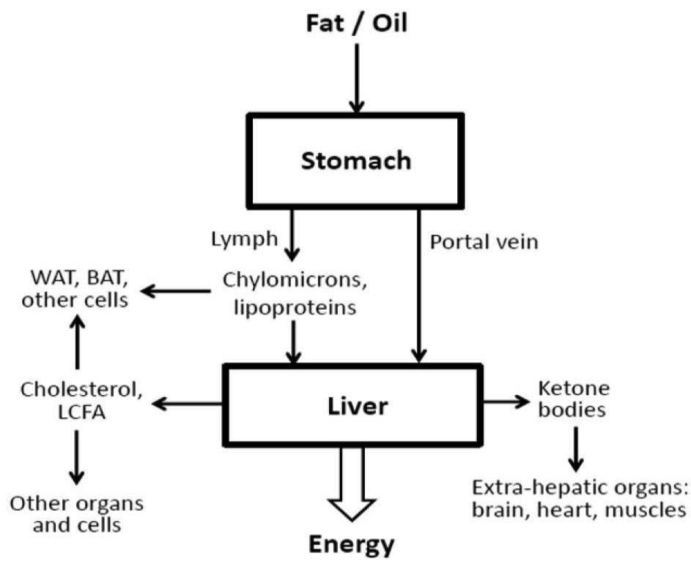


**Figure 2:** Overview of fatty acid metabolism. (WAT = white adipose tissue; BAT = brown adipose tissue).

### 2.4.1 Digestibility of Medium Chain Fatty Acids

Dietary lipid digestion, absorption, and metabolism are influenced by the FA composition of the dietary-derived lipids, namely the carbon chain length, saturation level, and FA location on the TG molecule. According to Ravindran et al. (2016), unsaturated dietary lipids are often simpler for pigs to digest than saturated lipids. This is because lipase can more readily each TG. In summary, the stomach performs emulsification, during which a partial breakdown of the TG is carried out as the first step in the digestion of dietary lipids. The stomach mucosa releases gastric lipase, whereas lingual lipase is secreted by the salivary gland. PL cannot be hydrolyzed by these enzymes, nonetheless. Due to the hydrolysis of the FA at the TG molecule's sn3-position, diacylglycerol and FA (such as non-ionized long-chain FA) are produced. Several studies have been conducted recently to assess the energy value and lipid digestibility of fat blends for young pigs, including different amounts of FFA (Kerr et al., 2016; Lindblom et al., 2017). The digestibility of MCFAs in the ileum is of interest, as these fatty acids have unique properties that make them potential beneficial additives in pig diets. By examining the digestibility of MCFAs, we can gain insights into their effects on pig performance, energy metabolism, immune function, and gut health.

Medium-chain fatty acids (MCFAs) undergo efficient absorption and utilization in the ileum of weaned pigs. Their unique structure allows them to be readily absorbed across the intestinal epithelium, bypassing the need for micelle formation and bile acid-mediated uptake. Once absorbed, MCFAs are transported through the portal vein to the liver, where they are metabolized into energy through β-oxidation (Manjarín et al., 2022). Results by Qi (2019) indicated a reduction in the expression of genes encoding enzyme, transporter, receptor, and their related pathways involved in the metabolism of the nutrients in the jejunal mucosa of growth retardation pigs, indicating that the digestion and absorption of nutrients are closely related to the weight gain of piglets. A research study by Cui et al. (2020) showed that the addition of medium-chain fatty acid glycerides to a low-protein diet could improve the utilization rate of amino acids and help piglets to synthesize protein as well as lower intestinal acidity, enhance digestive enzymes and improve energy status.

### 2.4.2 Flow Cytometric Analysis of Apoptosis and Necrosis

Cells die for various causes and are quickly removed by other cells that operate as morticians. Every day, many hundred billion cells die and are replaced by freshly produced cells. The kind of cell determines how they die (Nagata, 2018). According to Nagata (2018), cells infected with viruses or bacteria go through cell-autonomous necrosis or are destroyed by the immune system. Macrophages may not recognize these cells as entire, and necrotic cells emit components that can trigger the immune system. Inflammation produced by a bacterial or viral infection entails a huge generation of white blood cells, and when the infection subsides, these cells rapidly die. In the context of a certain dietary intervention or condition, the percentages of apoptotic, late apoptotic, necrotic, and viable cells in swine feeding might be indicative of the physiological status of the cells, and understanding the balance of these processes can provide insights into the impact of dietary factors or disease on the health and function of swine cells. Apoptosis has been linked to developing viral infectious illnesses in swine, including swine entero pathogenic coronaviruses and the classical swine fever virus. Apoptosis induction in pig cells by viruses such as the porcine delta corona virus (Xu et al., 2020; Sato et al., 2000; Galindo et al., 2012). There is evidence that anti-stress dietary techniques in pigs might alter apoptosis since some nutritional variables have been associated with cyto protection against oxidative stress-induced apoptosis (Wang et al., 2023)

While apoptosis and necrosis are important in pigs' cellular responses to infections and stresses, their dysregulation can have long-term consequences for pig health, immunological function, and production. Additional research into these processes is critical for understanding their influence on pig welfare and devising focused strategies to reduce negative outcomes.

## 2.5 Aim of The Study

This study aimed to determine the impact of MCFA produced from palm kernel oil and bio-based odd-chain MCFA on the performance and ileal digestibility of weaned piglets.

### 2.5.1 Specific objectives

1. Investigate performance trials (live weight, feed intake, average daily gain, feed conversion ratio)
2. Determine the content of Caproic (C6) and Lauric acid (C12) in the faeces and ileal digesta samples

# 3 MATERIAL AND METHODS

## 3.1 Material and methods

The regulatory license number of the trial was SO/31/01271-3/2022. Titan-dioxide use regulatory license number is SO/31/00944-3/2023.

## 3.2 Animals and housing

The trial was conducted with 24 healthy cannulated weaned Topigs x DanDuroc hybrid barrows. The piglets' live weight (LW) was between 17 and 19 kg when they were surgically fitted with a simple T-cannula at the distal *ileum*. The implemented cannula was suitable for a maximum of 40-45 kg LW pig. The surgery was done according to the method of Kik et al. (1988). The silicone cannula was surgically inserted 15-20 cm anterior to the ileo-cecal junction and extended through the body wall to the outside of the pig. A washer fitted onto the cannula barrel on the outside of the pig holds the cannula in place, and a cap prevented ileal fluids from leaking out of it. The recovery period was two weeks. During this time and the experiment, the piglets were placed in individual flat deck cages (85 x 85 cm). To avoid injuries, the walls of the cages were smooth plastic sheets, in which the free movement of the animals was not restricted at all, including during the sample collection period. The room temperature was set and controlled to follow the needs of nursery pigs.

## 3.3 Diet and treatments

Pigs were randomly assigned to one of 3 treatment groups, thus, 8 piglets were used in each treatment. The treatments were the following: Trt A pigs were fed with a basal diet containing no specific feed supplement or growth promoter, Trt B and C pigs received a basal diet supplemented with MCFA derived from palm-kernel oil (2 kg/T) and with sustainable MCFA (3 kg/T), respectively. The source of MCFA derived from palm kernel oil was a C8/C10/C12 mixture on a carrier. The sustainable MCFA was a mixture of C6/C9 (and some C4) on a carrier. The amount of MCFA on end feed was similar in both groups. The basal diet was a corn-soybean-wheat-based diet that had a nutrient content that was in accordance with the NRC's (2012) recommendations. The composition and nutrient content of the basal diet is presented in Table 3.

### 3.4 Feeding of the animals

The feed intake was limited during the trial to avoid problems related to digesta passage in the cannula and control the growth and live weight of the pigs. Water was offered *ad libitum* via automatic drinkers. A feeder and a drinking nipple were installed in each pen.

**Table 3**. Composition and calculated nutrient composition of the basal diet

| **Ingredients** | **0-7 day of trial g/kg** | **8-26 (28) day of trial g/kg** |
|---|---|---|
| Corn | 26.93 | 34.02 |
| Wheat | 77.85 | 60.00 |
| Barley | 62.50 | 40.00 |
| Wheat-middling | 10.00 | 13.20 |
| Soybean meal | 40.00 | 35.00 |
| MCP | 2.93 | 1.38 |
| Limestone | 2.49 | 2.70 |
| Lysine | 1.48 | 1.08 |
| Methionine | 0.59 | 0.30 |
| Threonine | 0.66 | 0.36 |
| Tryptophan | 0.09 | 0.02 |
| Premix 0.5% | 1.25 | 1.00 |
| NaCl | 1.41 | 1.14 |
| Cossette | 4.61 | 6.00 |
| Soybean protein | 7.50 | 0.00 |
| Sunflower oil | 9.71 | 3.80 |
| $TiO_2$ | 1.25 | 1.00 |
| MCFA (TrtA) | 0.00 | 0.00 |
| MCFA I (TrtB) | 0.50 | 0.40 |
| MCFA II (TrtC) | 0.75 | 0.60 |
| **Total:** | **1000.00** | **1000.00** |

| | | |
|---|---|---|
| **Nutrient content** | | |
| Dry matter | 888.66 | 889.62 |
| NEs*(MJ/kg) | 10.40 | 9.96 |
| Crude protein | 176.34 | 167.60 |
| Crude fat | 59.96 | 42.34 |
| Crude fibre | 36.58 | 42.32 |
| Lysine* | 12.70 | 11.70 |
| Methionine + Cysteine* | 7.80 | 6.77 |
| Calcium | 6.51 | 7.10 |
| Phosphorus (total) | 5.87 | 4.93 |
| Phosphorus (available) | 3.55 | 2.73 |
| Na | 2.00 | 2.01 |
| **Measured nutrient content (g/kg)** | | |
| Dry matter | 905 | 904 |
| Crude protein | 176 | 169 |
| Ether extract | 61 | 36 |
| Crude fibre | 41 | 41 |
| Ash | 56 | 55 |
| Calcium | 7.08 | 7.32 |
| Total phosphorus | 6.13 | 5.24 |

* : calculated value

** : **1 kg premix contain:** Vit. A: 2400000 IU, Vit. D3: 348000 IU, Vit. E: 6000 mg, Vit. K3: 198 mg, Vit. B1: 402 mg, Vit. B2: 1002 mg, Vit. B3: 10020 mg, Vit. B5: 4980 mg, Vit. B6:402 mg, Vit. B12: 7,8 mg, Biotin: 19,8mg, Folic acid: 102 mg, Cholin: 62792 mg, Fe: 45855 mg, Zn: 33600 mg, Mn: 26880 mg, Cu: 330000 mg, Co: 134 mg, I: 488 mg, Se: 67 mg.

## 3.5 Experimental methods, data recording

The study started with the arrival of the animals, the period, including a few days of adaptation, surgery, and recovery of the animals, was considered as the pre-trial period. The experimental period was considered in the timeframe when the trial feeds were offered. Live weight was recorded individually after a few days of adaptation and before the preparation of the animals via surgery. After that, pigs were weighed at weekly intervals with a precision of 0.1 kg. The amount of feed offered and left was recorded daily. Daily feed intake, weight gain and conversion ratio were calculated accordingly. The experiment started after a 2-week recovery period when experimental diets were started to be fed. On days 7 and 8 and on days 28 and 29, digesta was collected continuously for 12 h into clear plastic bags emptied hourly into a plastic container. Approximately 0.5 to 1 L of digesta was collected within each 12 h collection period. One part of those samples as stored at -20° C for volatile fatty acid analysis, and the other was freeze-dried for MCFA content determination.

Blood samples were collected in two vacutainer tubes with or without containing EDTA via jugular vein puncture on d0, d7, and d28 of the experiment.

### 3.6 Laboratory analysis

The diets' nutrient contents (dry matter, crude protein, crude fat, crude fibre, crude ash, Ca, P) were determined per the AOAC (1990) recommendations. The MCFA analyses (C6, C7, C8, C9, C10, C12) were done with GC-MS from feed and digesta samples.

The blood samples taken on days 0, 7, and 28 were centrifuged for further analysis. Blood samples were stored between 2°C and 8°C. Blood samples collected to vacutainer tubes with EDTA were used to determine polymorph nuclear cell viability via flow cytometry and annexin V FITC/propidium iodide labeling.

### 3.7 Statistical analysis

The experimental data were analyzed with one-way ANOVA (SAS OnDemand for Academics, 2023). In case of any significant treatment effects, the Tukey test checked the differences among the experimental groups (SAS OnDemeand for Academics, 2023).

# 4 RESULTS

## 4.1 Growth performance

The results of growth performance are summarized in Table 4. Statistical analysis showed that the treatment had no significant effects on live weight ($p>0.05$) for the entire trial period (day 0-26). However, the average daily gain (kg) on the day 0 –7 (1 week gain) of Treatment B was significantly higher than treatment A value by 10% ($p<0.05$). On the contrary, on days 8-14 and 15-26, the average daily gain of treated groups (B and C) was not significantly different compared to the control group (A) ($p>0.05$). The feed conversion ratio of treatment B was 0.07 kg/kg lower than treatment A on days 0-7 ($p<0.05$). On the contrary, the feed conversion ratio on days 8-14 and 15-26 of the treated groups were not significantly different compared to the control group ($p>0.05$). MCFAs have been evaluated as compounds to improve growth performance, as reviewed by Hanczakowska (2017).

**Table 4**. Effect of dietary treatments on the growth performance of pigs

| **Parameters/Treatments** | **unit** | **A** | **B** | **C** | **RMSE** | **P-value** |
|---|---|---|---|---|---|---|
| live weight on day 0 | kg | 24.3 | 23.6 | 24.2 | 1.30 | 0.5359 |
| live weight on day 7 | | 27.8 | 27.5 | 27.9 | 1.26 | 0.8259 |
| live weight on day 14 | | 32.8 | 31.7 | 32.1 | 1.45 | 0.3069 |
| live weight on day 26 | | 38.4 | 37.9 | 38.3 | 1.47 | 0.7447 |
| average daily gain on day 0-7 | kg/d | 0.50$^{b}$ | 0.55$^{a}$ | 0.53$^{ab}$ | 0.04 | ***0.0246*** |
| average daily gain on day 8-14 | | 0.71 | 0.59 | 0.61 | 0.13 | 0.1438 |
| average daily gain on day 15-26 | | 0.47 | 0.52 | 0.50 | 0.11 | 0.6684 |
| feed intake on day 0-7 | kg/d | 0.82 | 0.82 | 0.82 | | |
| feed intake on day 8-14 | | 0.90 | 0.90 | 0.90 | | |
| feed intake on day 15-26 | | 0.90 | 0.90 | 0.90 | | |
| feed conversion ratio on day 0-7 | kg/kg | 1.65$^{a}$ | 1.49$^{b}$ | 1.56$^{ab}$ | 0.11 | ***0.0218*** |
| feed conversion ratio on day 8-14 | | 1.30 | 1.61 | 1.53 | 0.32 | 0.1492 |
| feed conversion ratio on day 15-26 | | 2.40 | 2.13 | 2.12 | 0.66 | 0.238 |

P-value=level of significance (5%), RMSE=root mean square error

A=MCFA, B= MCFA I, C= MCFA II

## 4.2 Polymorph nuclear cell viability

In Table 5, this study shows no significant effect on nuclear cell viability on the 1st, 2nd and 3rd collections ($p>0.05$) for apoptosis, necrosis, and live percentages. However, the 2nd collection showed a significant difference at the late apoptotic stage ($p<0.05$). The late apoptotic value of treatment C was 58.6% lower than treatment B's.

**Table 5**. Effect of dietary treatments on polymorph nuclear cell viability at different time points of the trial

| | **Trt** | **Apoptotic (%)** | **Late apoptotic (%)** | **Necrotic (%)** | **Live (%)** | **Sum (%)** |
|---|---|---|---|---|---|---|
| **day 0** (1st collection) | **A** | 8.24 | 0.66 | 0.0335 | 91.08 | 100.01 |
| | **B** | 10.32 | 1.42 | 0.0610 | 89.08 | 99.58 |
| | **C** | 10.51 | 0.18 | 0.0266 | 88.99 | 99.63 |
| | **RMSE** | 4.33 | 1.35 | 0.0444 | 5.31 | |
| | **P-value** | 0.5248 | 0.2203 | 0.3126 | 0.6785 | |
| **day 7** (2nd collection) | **A** | 12.87 | 0.77[ab] | 0.0070 | 86.36 | 100.00 |
| | **B** | 11.78 | 1.45[a] | 0.0047 | 86.76 | 99.99 |
| | **C** | 11.57 | 0.60[b] | 0.0061 | 87.84 | 100.02 |
| | **RMSE** | 4.81 | ***0.64*** | 0.0062 | 5.21 | |
| | **P-value** | 0.8465 | ***0.0394*** | 0.7672 | 0.8435 | |
| **day 26** (3rd collection) | **A** | 38.15 | 0.29 | 0.0031 | 61.55 | 99.99 |
| | **B** | 33.79 | 0.29 | 0.0037 | 69.20 | 99.06 |
| | **C** | 36.70 | 0.35 | 0.0065 | 67.13 | 99.52 |
| | **RMSE** | 6.63 | 0.15 | 0.0050 | 10.70 | |
| | **P-value** | 0.4523 | 0.6550 | 0.4022 | 0.3534 | |

RMSE=root mean square error, P-value=level of significance

### 4.3 Medium chain fatty acid content and ratio in faeces and ileal digesta

The result for MCFA content and ratio in faeces and ileal digesta were not significantly different (p > 0.05) in both samples on days 7 and 26 respectively. MCFA content and ratio were observed to have no significant effect (p>0.05) on the faeces and ileal digesta.

**Table 6**. Effect of dietary treatments on the medium-chain fatty acid content and ratio in faeces and ileal digesta on day 7 of the trial

| | | | TREATMENTS | | | | |
|---|---|---|---|---|---|---|---|
| **Sample** | **Fatty acid** | **unit** | **A** | **B** | **C** | **RMSE** | **P-value** |
| **faeces** | **Caproic (C6)** | **mg/g** | 0.1320 | 0.1038 | 0.0840 | 0.0647 | 0.3473 |
| | **Caproic** | **%** | 0.3409 | 0.2683 | 0.2115 | 0.1645 | 0.3089 |
| | **Lauric (C12)** | **mg/g** | 0.1210 | 0.1167 | 0.1107 | 0.0234 | 0.6797 |
| | **Lauric** | **%** | 0.3186 | 0.3078 | 0.2778 | 0.0684 | 0.4776 |
| **ileum chyme** | **Lauric (C12)** | **mg/g** | 0.0173 | 0.0213 | 0.0183 | 0.0058 | 0.3606 |
| | **Lauric** | **%** | 0.0842 | 0.1122 | 0.0950 | 0.0269 | 0.1357 |

P-value=level of significance (5%). RMSE=root mean square error

**Table 7**. Effect of dietary treatments on the medium chain fatty acid content and ratio in faeces and ileal digesta on day 26 of the trial

| | | | TREATMENTS | | | | |
|---|---|---|---|---|---|---|---|
| **Sample** | **Fatty acid** | **unit** | **A** | **B** | **C** | **RMSE** | **P-value** |
| **faeces** | **Caproic (C6)** | mg/g | 0.1200 | 0.0868 | 0.1222 | 0.5695 | 0.0737 |
| | **Caproic** | % | 0.2900 | 0.2272 | 0.2859 | 0.7351 | 0.1779 |
| | **Lauric (C12)** | mg/g | 0.1136 | 0.1068 | 0.1180 | 0.0285 | 0.7323 |
| | **Lauric** | % | 0.2730 | 0.2741 | 0.2709 | 0.0619 | 0.9943 |
| **ileum chyme** | **Lauric (C12)** | mg/g | 0.0222 | 0.0275 | 0.0244 | 0.0075 | 0.3785 |
| | **Lauric** | % | 0.1172 | 0.1324 | 0.1230 | 0.0297 | 0.5928 |

P-value=level of significance (5%). RMSE=root mean square error

# 5 DISCUSSION AND RECOMMENDATION

## 5.1 Growth Performance

MCFA may impact growth performance through diverse means, including its usage as a readily accessible energy source. Nevertheless, continues to persist due to diversity in the MCFA content of various sources available to the industry and what inclusion level is essential for maximising performance (Hanczakowska, 2017). Hanczakowska (2017) has examined the impacts of MCFA inclusion in swine diets on growth performance, with many studies showing good effects on gain and feed efficiency. In the current experiment, the addition of MCFA resulted in an improvement in ADG at 7 days of trial compared with the control, and the feed conversion ratio was also improved when pigs were fed compared with the control. Hanczakowska et al. (2011b) observed an increase in ADG when 0.2% C8:0, 0.2% C10:0, and a combination of 0.1% C8:0 and 0.1% C10:0 were included in swine diets beginning at 7 days of age compared with control, and feed efficiency was also improved when pigs were fed 0.2% C8:0 compared with control. Hanczakowska et al. (2013) observed an increase in ADG when pigs were fed C8:0, C10:0, or a combination of C8:0 and C10:0 in diets containing propionic and formic acids compared with control-fed pigs; however, ADG did not significantly differ comparing pigs fed MCFA, propionic, and formic acids to propionic and formic acids alone. Kuang et al. (2015) found that replacing zinc oxide in piglet diets with MCFAs and organic acids improved feed intake and growth rates. Conversely, Zeng et al. demonstrated that at 28 days post-weaning, there was no significant difference in the ADG, ADFI, and gain-to-feed ratio between pigs fed lauric acid diets and those on the control diet. This may be due to differences in pig growth development and LA supplementation dosage (Zeng et al. 2022).

## 5.2 Polymorph nuclear cell viability

While most study results do not address the direct effect of MCFAs from palm kernel oil on polymorph nuclear cell viability, the antibacterial properties of MCFAs, notably lauric acid, imply that they may influence cell viability. MCFAs, notably lauric acid (C12), have been demonstrated to destabilize cell membranes and alter nutrient transport and energy metabolism in microorganisms, resulting in cell death (Dos Santos et al., 2022). In the current study, lauric acid showed no significant difference in cell death in the treated groups (B and C) as compared to the controlled group (A) on the $1^{st}$, $2^{nd}$ and $3^{rd}$ collections ($p>0.05$) for apoptosis, necrosis, and live percentages. However, the 2nd collection showed a significant difference at the late apoptotic stage ($p<0.05$). In a previous study by Fauser et al., (2014), lauric acid (C12:0) induced apoptosis. Further research addressing this relationship would be beneficial to fully understand the effects on polymorph nuclear cell viability although definitive apoptosis rates were not measured.

### 5.3 Medium chain fatty acid content and ratio in faeces and ileal digesta

Adding MCFAs to piglet diets has also been demonstrated by other researchers to have the ability to stabilise intestinal microbiota and promote piglet health after weaning. In particular, MCFAs have been found to have inhibitory effects on bacterial concentrations in the digesta and also result in lower diarrhea incidence (Gebhardt et al., 2017; Ruixia, et al. 2018; Lauridsen, 2020; Liu et al., 2021) At the end of the study, it was observed that MCF content and ratio had no significant effect ($p>0.05$) on the faeces and ileal digesta. However, the papers did not specify the precise effects of the medium-chain fatty acid (lauric acid) on the concentration and ratio in faeces and digesta. Further study may be required to establish the precise effects of lauric acid on piglet gastrointestinal flora.

# 6 CONCLUSION

In this experiment, the response of MCFA produced from palm-kernel oil and bio-based odd-chain MCFA diets improves average daily gain and feed conversion ratio of the 33-39 day-old piglets (0-7 trial days), does not affect polymorph nuclear cell viability, and does not significantly alter content and ratio in faeces and ileal digesta. Looking ahead, we anticipate that MCFAs may become an important class of feed additives in pig production since may induce a favorable effect under prolonged supplementation.

# 7 SUMMARY

In this study, we investigate the potential of MCFAs as feed additives to improve pig gut health. Performance and Ileal Digestibility trials were carried out for that purpose. The trial was conducted with 24 healthy cannulated weaned Topigs x DanDuroc hybrid barrows. Pigs were randomly assigned to one of 3 treatment groups, thus 8 piglets were used in each treatment. The piglets' live weight (LW) was between 17 and 19 kg when they were surgically fitted with a simple T-cannula at the distal *ileum*. The implemented cannula was suitable to a maximum of 40-45 kg LW pig. The experiment consisted of a 28-day-long performance trial.

During the trial, treatment A pigs were fed with a basal diet containing no specific feed supplement or growth promoter, Trt B and C pigs received basal diet supplemented with MCFA derived from palm-kernel oil (2 kg/T) and with sustainable MCFA (3 kg/T), respectively. The experimental data were analyzed with one-way ANOVA (SAS OnDemand for Academics, 2023). In case of any significant treatment effects, the differences among the experimental groups were checked by the Tukey test (SAS OnDemeand for Academics, 2023). It was observed that the average daily gain (kg) and feed conversion ratio (kg/kg) on the day 0 –7 (1 week gain) of Treatment B and C group was significantly different compared to Treatment A ($p<0.05$). The current study had no significant effect on nuclear cell viability on the 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ collection($p>0.05$) for apoptosis, necrosis, and live percentages. However, the 2$^{nd}$ collection showed a significant difference at the late apoptotic stage ($p<0.05$). Thus, the late apoptotic value of treatment C was 58.6% lower than treatment B's.

It was also observed that MCF content and ratio had no significant effect ($p>0.05$) on the faeces and ileal digesta.

In conclusion, using MCFA in nursery pig diets improves growth performance, does not significantly affect polymorph nuclear cell viability, and does not significantly alter content and ratio in faeces and ileal digesta. Looking ahead, we anticipate that MCFAs may become an important class of feed additives in pig production for gut health enhancement.

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