Czech University of Life Sciences Prague

Faculty of Agrobiology, Food and Natural Resources

Department of Food Science

Nutritional Quality of Meat from Pig

Master´s thesis

Nisengwe Vanessa

Professor Andrea Serra and Ing.Havlík Jaroslav

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Declaration

I now declare that I have done this thesis entitled ‘Nutritional quality of meat from pig’, all texts in this thesis are original, and all the sources have been cited and acknowledged using complete references and according to Citation rules of the faculty of agrobiology.

In Prague 13th, April,2024

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Nisengwe vanessa

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Contents

List of the abbreviations used in the thesis.

WB	wheat bran
CB	corn bran
SBP	sugar beet pulp
OB	oat bran
SH	soybean hulls
RB	rice bran
VFA	volatile fatty acids
IDF	insoluble dietary fiber
SDF	soluble dietary fiber
RS	resistant starch
FCM	feeds conventional efficient
PUFA	polyunsaturated fatty acids
ADG	average daily grain
AP	amylopectin
WAT	white adipose tissue
BAT	brown adipose tissue
MUFA	monounsaturated fatty acids
UFA	unsaturated fatty acids
RDS	rapidly digestible
SDS	slowly digestible
FA	fatty acid
IMF	intramuscular fat
FAS	fatty acid synthesis
BCAA	branched-chain amino acid
GIT	gastrointestinal tract
DPA	docosahexaenoic
ICA	feeds conventional index
FCR	feed conversion ratio

PSE	pale, soft, exudative
DFD	dry, firm, dark

Abstract

Primary source of protein. Corn-based feed is now widely used in swine production, significantly impacting pork nutrition. However, highly digestible maize, specifically waxy corn, has never been investigated in finishing pigs for ham production. Waxy maize has been found to increase lipid metabolism, which could be a promising approach to improving pig fat deposition. Nonetheless, heavy gilts and barrows have different metabolic processes. Therefore, the present study aimed to assess the influence of a waxy corn diet and sex on growth performance, carcass traits, and meat quality among pigs. This study utilized twenty-four Large White × Pietrain pigs, with twelve gilts and twelve barrows equally assigned to two dietary treatments: the non-waxy (CON) and the waxy (WAX) corn diet. Experimental diets have been formulated based on the nutritional needs of finisher pigs to be isocaloric and to provide the same amount of starch. The trial lasted from 77.9 ± 5.2 of live body weight to slaughter at 173.1 ± 5.2 kg. Our study highlights that substituting non-waxy corn with waxy corn in the finishing diet of heavy pigs yields significant effects on ADG and F: G ($P<0.01$). Specifically, the WAX barrows exhibited better growth performance compared to those fed the CON diet or gilts on the WAX diet.

Furthermore, the WAX diet significantly enhanced hot carcass weight and yield. However, these parameters were also influenced by gender: barrows exhibit better carcass performance than gilts. Backfat thickness and lean meat percentage were influenced by diet, sex, and their interaction ($P<0.05$). The WAX barrows showed increased backfat thickness and reduced carcass lean meat percentage compared to CON barrows or WAX gilts. The meat quality traits such as colour, pH24h, and water-holding capacity were not negatively influenced by waxy corn diet or sex.

Additionally, both the intramuscular fat content of the *Longissimus dorsi* muscle and the composition of fatty acid classes remained unaffected by the diet. Nevertheless, barrows exhibited approximately 2 grams more intramuscular lipids than gilts. In Particular, a higher concentration of saturated fatty acids was detected in the intramuscular fat of barrows ($P<0.05$), while gilts demonstrated a higher concentration of PUFA ($P<0.01$). Finally, the diet influenced the content of several individual fatty acids in the n-3 and n-6 series. In conclusion, the waxy corn diet may effectively increase carcass adiposity in barrows without compromising meat quality or carcass depreciation.

Keywords: pork, amylose/amylopectin ratio, waxy corn feeds, carcass traits, meat quality.

1 Introduction

According to the United Nations, the global population currently stands at 8 billion people, with a projected increase of 11 billion people by 2100 (Roser & Ortiz-Ospina, n.d.; Wilmoth et al., 2022). Agriculture is critical to feeding the world's rising population through increased efficiency, sustainable practices, innovation, global trade, job opportunities, climate change adaptation, food security, poverty reduction, and nutritional need balance (Wilmoth et al., 2022). Livestock plays a vital role in protein provision since it is a valuable source of high-quality protein through the production of meat, milk, and other animal-based products that contribute to the nutritional needs of human populations around the world (Moore et al., n.d.; Randolph et al., 2007a). The studies conducted (Randolph et al., 2007b) have shown a general increase in pork consumption by 34% compared to beef (20%) and sheep (5%), as the promotion of pig over meat can be impacted by cultural, religious, economic, and environmental concerns, as well as production efficiency, health, and gastronomic tastes, with an emphasis on different dietary choices to suit nutritional demands to human's health (Stiftung et al., 2021).

According to Toldrá et al. (1996), pork nutrition Significantly influences the composition and quality of the meat, while protein and fat levels are frequently the focus. Modern pig breeding and feeding practices produce leaner meat and fat with more unsaturated fatty acids. The key factors influencing the composition of fatty acids include genetic origin, age, weight at slaughter, feed composition, and husbandry practices (van Erp et al., 2020). Carbs, including storage, can also influence the quality of meat. Carbohydrates can interact with other components in the diet, such as proteins and fats, and impact subsequent effects on meat quality. Because they comprise most of the energy used in the pork diet and affect digestion and the gastrointestinal tract's health in pigs and humans, carbohydrates play a crucial role in pig nutrition. Characterizing the roles of carbs and enhancing their use are essential since they comprise such a significant portion of the diet (Yin et al., 2004). Most of the starch ingested is digested into glucose by host enzymes, and starch serves as a substrate for microbial fermentation. Because of differences in chemical makeup and structural characteristics, dietary fibre from wheat bran (WB), corn bran (CB), sugar beet pulp (SBP), oat bran (OB), soybean hulls (SH), and rice bran (RB) influenced the composition of the

gut microbiome—intestine of the pig. The VFA produced by the gut microbiota to ferment dietary fibre is positively correlated to IDF (insoluble dietary fibre) content in fibre-rich ingredients, not SDF (soluble dietary fibre) (Zhao et al. 2020). The carbohydrates include non-monomer carbohydrates, divided into oligosaccharides and polysaccharides, with the dividing point being around ten monomer residues. The carbohydrate insoluble in 80% ethanol has historically been recognized as polysaccharides.

80–90% of all polysaccharides in the diets of pigs and humans are made up of starch, a mixture of the a-glucan polysaccharides amylose and amylopectin in proportions that depend on the botanical source (van Erp et al., 2020; Yin et al., 2004). The type and quantity of carbohydrates in a pig's diet can affect the levels of Omega-3 and Omega-6 fatty acids in pork meat due to the different lipid compounds. Diets high in Omega-3 sources encourage higher levels of Omega-3 in the meat, whereas diets high in grains and soy promote higher levels of Omega-6, affecting the meat's overall nutritional profile (van Erp et al., 2020; Yin et al., 2004).

2 Literature review

2.1 The corn in pig diet

Due to its high energy content, corn is a common and significant part of pig diets. It acts as a primary source of carbohydrates and gives pigs the energy they require for growth and upkeep. Pigs mainly obtain their energy from starch, which accounts for 40–55% of their dry matter diet. Host enzymes break down most of the starch consumed into glucose. Starch that doesn't break down acts as a microbial fermentation substrate. Despite the significant disparity in starch digestion among the starch components frequently found in pig feed, digested starch is believed to possess a constant nutritional value independent of the source. This amount is determined by its energy source for upkeep and productive operations, such as expansion, and the so-called net energy content (McGhee & Stein, 2023; van Erp et al., 2020).

Corn is an essential component of the pig diet because it is readily digested and provides a balanced spectrum of vital elements (Erp, 2019). It is composed of carbohydrates (70-75%), and starch represents 60-70 of total carbs. Starch is a polysaccharide comprising glucose units and is corn's primary energy source. Corn protein's content is approximately 8-10%. However, due to its relatively low lysine level, The most necessary amino acid in pigs, additional protein sources are often supplemented in pig diets. Corn fat content is approximately 3-5%, mainly consisting of unsaturated fatty acids, including linoleic and oleic acids. 2-4% of Fibers are contained in corn diet, including both soluble and insoluble forms (Sheena Kim, 2021).

According to Barlow et al. (2012), the effect of waxy corn silage on milk production, given corn silage and grain derived from a waxy corn hybrid till early lactation dairy cows and observed higher Milk and FCM outputs were compared to cows fed diets supplemented with corn silage. And grain produced from a regular corn hybrid.

The starch in waxy hybrids is primarily amylopectin, whereas starch in conventional hybrids is approximately 75% amylopectin and 25% amylose. Amylopectin is highly digestible in the rumen

(van Erp et al., 2020), which could make waxy corn more beneficial than average corn hybrids to ruminants.

In several pigs, corn is the primary energy source in diets worldwide, including the US. However, more information is needed to compare the growth rates of pigs raised on diets containing hybrid rye instead of corn (McGhee & Stein, 2023). According to research from Europe and Canada, the substitution of hybrid rye for barley in diets for growing and finishing pigs has not been shown to impair animal growth performance. However, in finishing pigs, replacing wheat with hybrid rye decreased average daily gain (ADG) and average daily feed intake (ADFI) (Smit et al., 2019).

Regarding the amylose/amylopectin ratio in corn starch, it typically has an approximate ratio of 1:3. This means that amylose makes up roughly 20-30% of the total starch content, while amylopectin constitutes around 70-80% (van Erp et al., 2020; Yan et al., 2023). The α-(1,4)-linked glucose units that make up the amylose in starch create tight helical geometries, which make the bonds relatively inaccessible to amylases. By comparison, amylopectin has a small amount of α-(1,6) connected glucose units, resulting in the molecule's branches being more vulnerable to enzyme breakage. This suggests that cereals higher in amylose are less digestible than those with a higher amylopectin content due to differences in their molecular configurations (Kim et al., 2005; van Erp et al., 2020).

2.2 The starch digestion

Starch is made of amylose and amylopectin, which are both types of polysaccharides. Molecules, where amylose contains linear polymers of α-D-glucose units linked by α-1,4 glycosidic linkages and amylopectin contains branched polymers of α-D-glucose units linked by α-1,4 and α-1,6 glycosidic linkages (Luo et al., 2015; van Erp et al., 2020).

Waxy corn is a particular variety of maize with a distinctive starch composition high in amylopectin and low in amylose. Amylopectin is a branched glucose polymer with intricate branching patterns, whereas amylose is a linear glucose polymer with a spiral shape.

Approximately 95% of the starch in waxy maize is amylopectin, giving it unique qualities like improved gelatinization and durability (Escobar-Puentes et al., 2020).

Starch granules can be characterized by various structural and hierarchical levels, starting from the molecular connections that form the macromolecules amylose (AM) and amylopectin (AP). These macromolecules subsequently organize into nano-lamellar and semi-crystalline structures, eventually creating concentric shells and blocklets and culminating in the granular structure (Liang et al., 2023).

The arrangement of chain segments within the starch granule can be elucidated by the backbone model, as described by Jayarathna et al. (2023). Amylopectin (AP) is suggested to include long flexible chains, so-called B2- and B3-chains, from which short-branched building blocks of A and B chains stretch. While lengthy AP chains mainly comprise the backbone, short AP chains stretch from branched "building blocks" to construct double helices. Distinct starches from botanical sources often have different structural characteristics, which affects how easily they can be digested. Potato starch, for instance, has stronger enzymatic resistance than cereal starches due to its B-type crystalline structure (Liang et al., 2023).

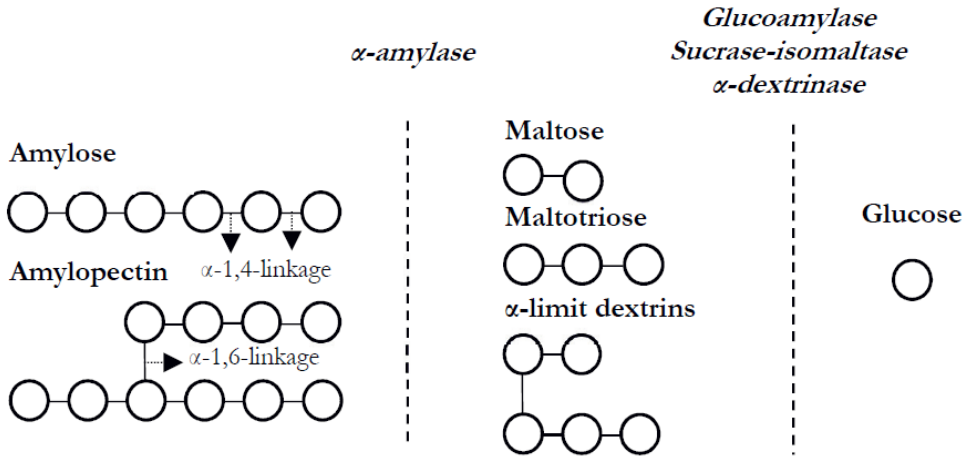

Figure 1: Enzymatic hydrolysis of amylose and amylopectin into glucose. Each circle represents a glucose molecule (van Erp et al., 2020).

In pigs, pancreatic amylase mostly breaks down the polysaccharide starch, which comprises the amino acid amylose and amylopectin. As demonstrated in humans, salivary amylase is thought to play a minor role in starch digestion. This is explained by the brief duration of feed retention in the mouth and the quick inactivation of salivary amylase in the stomach's acidic environment. The primary end products of α-amylase's starch digestion are maltose and maltotriose. The 1,6-linkages, which are primarily found in amylopectin and to a lesser level (1%) in amylose, cannot be hydrolyzed by amylase, and it is also unable to break down the α-1,4-linkage (van Erp et al., 2020).

According to Luo et al. (2015) and Nayik et al. (2023), amylopectin is fast digested due to the abundant presence of sites for enzymatic hydrolysis on its branching structure, while amylose is known to be slowly digested by α-amylase found in human or monogastric animal duodenum due to its different structure. Not-digested starch acts as a microbial fermentation substrate in the hindgut. It has been established that a variety of bacteria in the hindgut of monogastric animals are capable of in vitro amylose or amylopectin degradation. Only a tiny number of Bifidobacterium and Clostridium species can degrade amylose (Escobar-Puentes et al., 2020; Purwani et al., 2012) while some Bacteroides, Bifidobacterium, Eubacterium, Clostridium, and Propionibacterium species can use amylopectin and other soluble starches (Purwani et al., 2012).

The rate of digestion influences the site of starch degradation in the small intestine, whereas the extent dictates how much-undigested starch is accessible for microbial fermentation. In vitro, starch is incubated with pancreatic amylase and amyl glucosidase to measure the rate and degree of starch digestion. According to this method, starch is divided into three groups based on the rate at which glucose is released from 0 to 20 minutes, 20 to 120 minutes, and after 120 minutes, respectively: rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS). The RDS, SDS, and RS fractions for unprocessed feed components used in pig feed range from 9 to 40%, 15 to 60%, and 5 to 76%, respectively. Resistant starch can be divided into four categories: retrograded starch (RS3), chemically modified starch (RS4), and physically inaccessible starch, such as partially milled grains and seeds (RS1). Since starch disappearance from the intestinal lumen is a common way to quantify starch digestibility in vivo, this method must distinguish

between fermented and digested starch. The maximal amount of starch hydrolysis by host enzymes is frequently represented by ileal starch disappearance since fermentation in the upper gastrointestinal tract is considered insignificant.

Total postprandial net portal glucose appearance reflects the most significant degradation of starch, whereas the amount of time needed for net portal glucose concentrations to peak reflects the combined influence of passage rate and starch digestion rate. However, the emergence of net portal glucose is simply an approximation of the digestion of starch because some of the absorbed glucose may be used by the intestine or fermented by bacteria. According to how much they raise postprandial blood glucose levels, meals' digestible rates of carbohydrates are ranked relative to one another in humans using the glycemic index. Starch is generally believed to be entirely digested in the small intestine; sources of starch that are quickly absorbed have a glycemic index close to one. The glycemic index of sources of slowly absorbed starch is much below one; part of the starch is undigested and used as a substrate for microbial fermentation. Bacteria ferment all starch; starch resistant to enzymatic hydrolysis has a zero glycemic index.

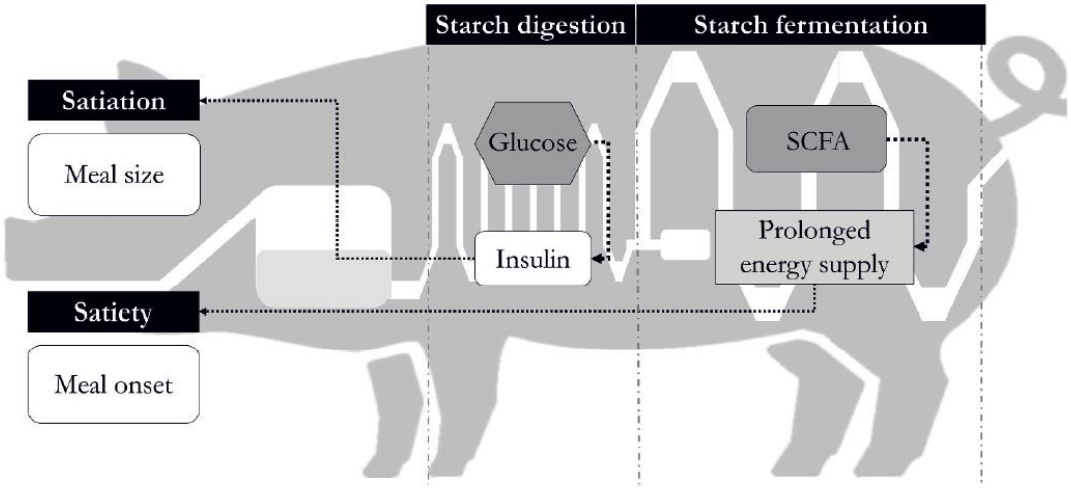

Figure 2: Effect of starch digestion (van Erp et al., 2020).

2.3 The influence of different starch digestibility on pig performance

According to van Erp et al. (2020), pig performance, including growth, feed efficiency, gut health, and nutrient utilization, is significantly impacted by the digestibility of starch in diets. Improved growth rates, effective nutrient use, and lower feed costs are all influenced by low and high starch digestibility. Proper diet formulation and processing methods are critical to maximize starch digestibility, which has favourable economic and environmental effects on pig production.

Starch in pig diets comes from various botanical sources, affecting the gastrointestinal transition rate of starch digestion and, in turn, how quickly glucose appears in the portal circulation. It has been shown that variations in the kinetics of starch digestion impact pig performance. When compared to pigs fed diets high in rapidly digestible starch (RDS), for instance, pigs fed diets high in non-digestible starch (resistant starch, R.S.) or slowly digestible starch (SDS) had more extended meal and inter-meal intervals and lower energy losses by activity-related heat production. Additionally, when pigs and poultry are fed restrictively, asynchrony between glucose and amino acid arrival rates in the blood harms how much protein is used (Martens et al., 2019).

The performance of pigs' growth is said to benefit from delayed starch digestion. This is accounted for by the slower response to postprandial insulin release with slowly versus quickly digestible carbohydrates. Such a response enhances protein and fat synthesis due to insulin's role in facilitating glucose absorption in insulin-responsive muscle and adipose tissues (van Erp et al., 2020). In addition, the release of non-esterified fatty acids from adipose tissues and glucose from glycogen is suppressed. Since slowly digested starch is thought to improve animal performance, changes in these metabolic pathways because of changes in blood insulin levels are likely less pronounced with slowly digestible starch than with rapidly digestible starch. The degree of starch digestion may impact pig performance because fermented carbohydrates produce less energy than digested carbohydrates. Therefore, animals are hypothesized to exhibit poorer performance when ileal starch digestion is low (Martens et al., 2019; Yang et al., 2015).

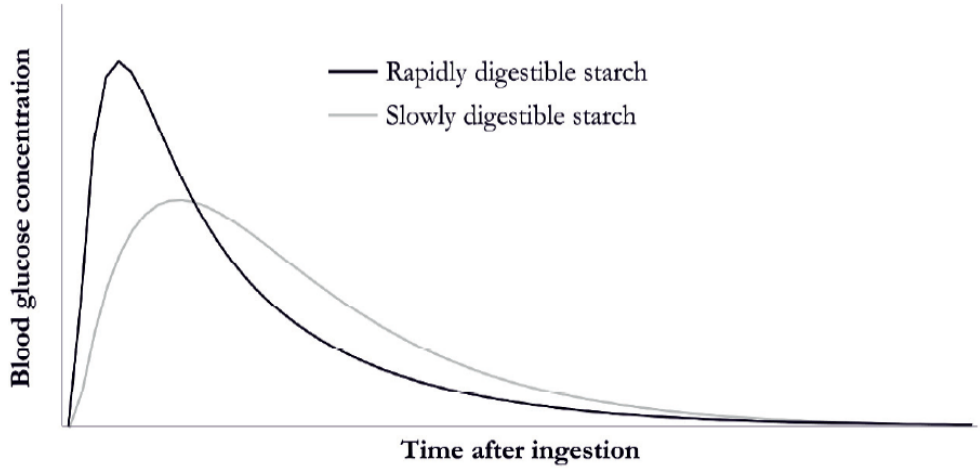

Figure 3: Response in postprandial blood glucose concentrations after ingestion of rapidly and slowly digestible starch.

According to the rate and extent of their enzymatic digestion, starches have been categorized by Bolhuis et al. (2008) and Doti et al. (2014) as rapidly digestible starch (RDS), or slowly digestible starch (SDS), and resistant starch (R.S.), which is starch that is not broken down by α-amylase Brush boundary enzymes in the small intestine. Variations in the proportions of these fractions impact postprandial glucose availability and insulin blood concentration, which in turn affect energy expenditure and protein and lipid metabolism. Therefore, the kind of primary source may impact growth features and the amount of fat in meat, and this effect would depend on how quickly glucose is released during digestion or how blood glucose concentrations alter. The gastrointestinal tract is also related to the feeding pattern since high postprandial glucose level induces long-lasting effects on satiety.

In pig diets, maize and barley are frequently utilized as starch sources, but adding legumes like beans or peas as a protein source also suggests an input of starch. Broken rice has also been used as pig feed, but only for piglets due to its compatibility with human diets. While rice's small particle size permits quick digestion, maize's digestion is somewhat shielded by the endosperm protein matrix. Peas have a certain degree of resistance to amylase digestion due to the crystal structure of their starch, high amylose content, and cell structures encapsulating the starch granules (Doti et al., 2014).

As a by-product of the manufacture of rapeseed oil, rapeseed meal (RSM) is a product of rapeseed oil that mainly consists of minerals, Fiber, and protein(Shuai et al., 2023). According to Dingyuan & Jianjun (2010), RSM has a super mix of essential amino acids and is particularly rich in sulfur amino acids. However, due to its high-fat content, RSM's nutrient digestibility in monogastric animals is low, and the content of anti-nutritional elements such as Fiber, phytic acid, and glucosinolates, loss of appetite, a decrease of dietary net energy and impaired functions of the thyroid and kidney were observed after RSM ingestion because of the crude Fiber and glucosinolates (Khajali & Slominski, 2012; Shuai et al., 2023; Torres-Pitarch et al., 2014)

There are two main methods for enhancing the RSM's nutritional value. The first is microbial fermentation, which is among the most effective methods to get rid of the anti-nutritional elements (including Fibers, phytic acids, and glucosinolates) and boost the nutritional value of RSM (Dingyuan & Jianjun, 20210). Pre-treating the RSM with industrial enzymes is another strategy. For example, pre-treating RSM with enzymes that break down Fiber considerably boosted the concentration of glucose and fructose, increasing nutrient availability in RSM (Shuai et al., 2023; Torres-Pitarch et al., 2014).

According to Shuai et al. (2023), several extracellular hydrolytic enzymes, such as cellulase and pectinase, may not be present in the bacterial strains employed in microbial fermentation, which could result in an insufficient elimination of the anti-nutritional components. To address this, enzymes and microorganisms are combined to enhance the quality of RSM fermentation. Specifically, co-fermentation of RSM with enzymes and microbes resulted in an 81.7% increase in the concentration of small-molecular proteins and a 30.06% reduction in glucosinolate content (Shuai et al., 2023). The vitro digestibility of crude protein (C.P.) and dry matter (D.M.) was enhanced by 23% and 20%, respectively, by fermented rapeseed meal (FRSM) treated with Fiber-degrading enzymes and Lactobacilli(Zhu et al., 2021). When FRSM was introduced into pigs' diets for the first time in 1976, research in the years that followed showed that it improved animal performance when compared to RSM. Recent research has also shown that FRSM had a beneficial impact on intestine absorption, such as raising the villus height to crypt depth ratio (V: C ratio) and digestive enzyme activity, which enhanced piglets' performance and the ability to absorb

nutrients (van Erp, R. J.J.de Vries, S.van Kempen, T. A.T.G.Den Hartog, L. A.Gerrits, W. J.J.; Shuai et al., 2023; Zhu et al., 2021).

Intestinal shape and the activity of digestive enzymes are essential markers of an animal's ability to digest food, and the action of feeding FRSM on digestive enzymes, nutrient transporters, intestinal integrity, and intestinal morphology may be the cause of any positive benefits on nutrient digestibility and intestinal health (Shuai et al., 2023).

Intramuscular fat (IMF) content directly impacts the flavour, juiciness, and tenderness of pork. Pork quality, particularly IMF, has declined because of long-term breeding techniques meant to increase commercial pigs' growth performance and lean percentage. Apart from genotype, various environmental factors, such as diet composition and nutritional levels, influence IMF content (Zheng et al., 2023). Nutritional intervention has become essential in the complex regulation of meat quality. According to Zheng et al. (2023), fat deposition dominates protein accumulation during the finishing phase. As a result, increasing dietary energy density is necessary to meet the demand for lipid deposition for finishing pigs.

Numerous attempts were made to examine the effects of energy levels in diets based on corn-soybean meals on IMF content. The elevating the dietary energy density led to an increase in IMF deposition via upregulating the expression levels of fatty acid synthase (FAS), fat acids binding protein (FABP), acetyl CoA carboxylase (ACC), and peroxisome proliferator-activated receptor (Lombardi et al., 2020). However, a shorter fattening period was associated with a higher subcutaneous fat content and a smaller percentage of lean meat when dietary energy levels were raised without altering the mix of amino acids (Zheng et al., 2023).

While suitably reducing the lysine (Lys): energy ratio suppressed the high-energy diet-induced increase in backfat thickness and total fat in pigs, high-energy meals with a reduced Lys: energy ratio increased IMF content. The effects of dietary wheat content on meat quality and the efficiency of lowering the Lys: energy ratio to raise IMF content when partially replacing corn with wheat are still debatable. However, reducing the Lys: energy ratio is anticipated to influence body metabolism. The Lys will undoubtedly impact meat quality: energy ratio-induced change in nutrient metabolism in finishing pigs (Zheng et al., 2023).

2.4 The lipid metabolism

A pig's body mechanisms for producing, dissolving, storing, and utilizing fats are called lipid metabolism. These procedures are essential for manufacturing hormones, cell structure, and energy. Pigs can produce their own triglycerides, which they can store surplus energy and release when needed. They also regulate cholesterol, transport lipids in the blood, and metabolize dietary fats. Essential fatty acids are obtained from their diet. In pig farming, optimal lipid metabolism is critical for growth, meat quality, and overall health.

2.4.1 Lipid synthesis

It has been established that 99% of lipid synthesis in pigs occurs in adipose tissue, with the remaining 1% occurring in the liver. Subcutaneous adipose tissues serve as the starting point for synthesis, which subsequently moves on to intermuscular adipose tissues and, lastly, to intramuscular tissues. It must be noted that this fluctuates depending on the animal's age. Therefore, besides the lipids presumed to be present in the diet, endogenous lipids, or those produced internally, are also present in animal meat (J. Ma et al., 2022; Yan et al., 2023b).

The pig has pockets of adipose tissue. According to Yan et al. (2023b), it comes in a variety of colours, including adipose tissue, which can be white (WAT), brown (BAT), or beige. By releasing bioenergetic substrates through lipolysis and storing extra nutrients in lipid droplets, WAT functions as an energy storage site and plays an essential function in glucose and lipid balance. Additionally, WAT can generate a variety of adipokines and carry out several functional tasks via endocrine and paracrine signaling.

Adipose tissue plays a crucial role in the balance of glucose and lipids, but its contribution to systemic protein and amino acid metabolism needs to be better understood. Adipose tissue may digest significant amounts of branched-chain amino acids (BCAAs), according to in vitro and ex vivo research (Herman et al., 2010; Yan et al., 2023b). BCAA metabolism enzymes have

regulation in the adipose tissue of mice that have their adipose tissue over-expressed with GLUT4. As a result of higher amounts of circulating BCAA, there is a decrease in BCAA oxidation rates in adipose tissue but not muscle. We show that transplanting healthy adipose tissue into animals with general peripheral BCAA metabolic defects lowers circulating BCAA levels by 30% (fasting) to 50% (fed state), confirming the ability of adipose tissue to control circulating BCAA levels in vivo (Herman et al., 2010). This is demonstrated by the fact that fatty acid synthase, glucose-6-phosphate dehydrogenase, acetyl-CoA carboxylase, citrate lyase, and malic enzyme are all found in high concentrations in adipose tissue (S. Ma et al., 2023; Yan et al., 2023b).

Approximately 80% of the total lipids are produced through the synthesis of saturated and monounsaturated fatty acids, the most representative of which are palmitic acid C16:0 and oleic acid C18:1. This process begins with the synthesis of dietary glucose, which serves as the primary precursor of fatty acids (Yan et al., 2023b). The dietary composition, such as reducing carbohydrates in favour of more protein, can decrease lipid synthesis (Drackley, 2000a; Yin et al., 2004).

Research on how the approach to daily ration delivery impacts lipid synthesis has yielded mixed results, with some studies noting significant effects while others observe none. For instance, it has been observed that restricting meals causes alterations at the synthesis level due to an increase in the activity of lipogenic enzymes (Yan et al., 2023a). Conversely, other studies have found that de novo synthesis and enzymatic activity decrease in pigs fed randomly without a specific nutritional plan (Song et al., 2021). Yet, further research indicates that de novo synthesis remains unaffected by varying the number of daily meals as long as consistent fat intake. These conflicting findings highlight the complexity of dietary impacts on lipid metabolism.

Additionally, sex is another factor influencing lipid synthesis, though consistent data on different feeding strategies and their effects across sexes are lacking. Pérez-Ciria et al. (2022) reported that castrated male pigs, when fed the same diet as sows, exhibited a higher rate of lipid synthesis. However, analyses of carcasses revealed that intact males had a lower lipogenesis rate than castrated males and sows, resulting in a leaner physique. This variance emphasizes the nuanced interplay between genetic, physiological, and dietary factors in lipid metabolism.

Finally, a more significant percentage of saturated fatty acids (C14, C16, and C18) and monounsaturated (C16:1 and C18:1) fatty acids were found in the back fat of lean and obese animals that had both been fed ad libitum (as requested) with the same diet. These are the by-products of the synthesis of lipids, and their increased quantity may be accounted for if de novo synthesis is the primary source of fat in obese animals (Aboagye et al., 2020; Wood et al., 2007). Al-Goblan et al. (2014) found a direct correlation between the amount of saturated fatty acids in subcutaneous fat and the level of obesity in pigs.

2.4.2 Lipid digestion

Lipids are being digested, and Triacylglycerols make up most of the lipids. Lipid substances break down in the stomach, where acid-stable lipases release some free fatty acids from dietary triacylglycerols. Because triacylglycerols are hydrophobic molecules that tend to collect, the stomach cannot efficiently cleave them, and lipases can only hydrolyze the triacylglycerols on the surface of the aggregates. Many triacylglycerols are inaccessible to enzymes due to the stomach's low surface area to volume ratio and limited surface area (Drackley, 2000b).

There are emulsification processes for lipids in the small intestine. As the partially digested material is forced into the comparatively narrow areas of the intestinal lumen by the small intestine muscles, the lipid clumps are first mechanically dispersed. The intestine also includes bile salts and acids, which act as detergents to separate the more giant micelles of lipids (Infantes-Garcia et al., 2023; Lipid_metab,2010). The pancreas produces several digestive enzymes, which are also found in the small intestine. These enzymes include various phospholipases, which release free fatty acids from phospholipids, Pancreatic cholesteryl ester hydrolase, and Pancreatic lipase, which releases free fat acids from the 1- and 3-positions of triacylglycerols. In contrast, cholesteryl esters release free cholesterol. l from cholesteryl esters and several other and many more phospholipases. As additional detergents, monoacylglycerols, partially hydrolyzed phospholipids, and free fatty acids help break the more enormous lipid complexes (Drackley, 2000b).

Free fatty acids, 2-monoacylglycerols, and bile Acids can diffuse from the intestinal lumen into the body once their micelles are small enough. The fatty acids are esterified within the body to create triacylglycerols once more. These triacylglycerols form chylomicrons, which serve as

triacylglycerols' serum transport particles when they interact with lipoproteins excreted by the intestines (Drackley, 2000b).

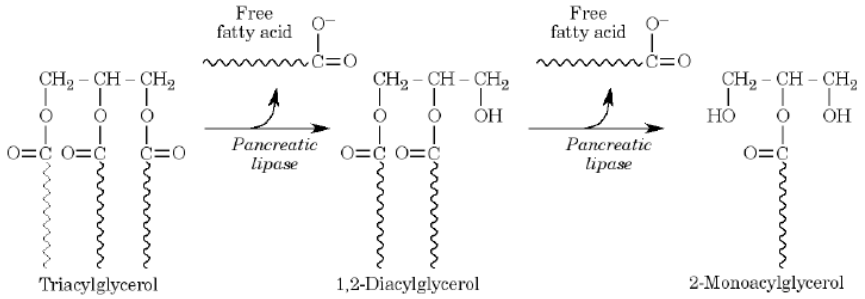

Figure 4: Digestion of a triacylglycerol

Dietary fibers may impact how lipids are digested in the gastrointestinal tract (GIT) model, primarily by adsorbing to and binding to specific species in the small intestine, increasing the viscosity of the digest, and inactivating digestive enzymes. Physicochemical and functional properties, such as adsorption property, surface activity, thickening effect, and emulsifying property, vary among dietary fibers with different molecular properties (such as molecular weight, electrical properties, and particle size) and different sources. As a result, diverse dietary fibers might affect lipid digestion behaviour in distinct ways (Yu et al., 2023).

2.4.3 Lipid Absorption and Deposition

The process enables the synthesis of triglycerides from fatty acids, resulting in molecules structurally different from the original ones. Short- and medium-chain fatty acids, instead of being esterified into triglycerides, are directly released into blood vessels, and transported to the liver while bound to albumin (S. Ma et al., 2023). In contrast, triglycerides containing long-chain fatty acids, newly synthesized phospholipids, and free cholesterol form micellar aggregates known as chylomicrons. These chylomicrons serve as a "transport mechanism", allowing triglycerides to move in an aqueous environment. They enter the general circulation through the subclavian vein and are released into the lymphatic vessels (Drackley, 2000b). Chylomicrons and fatty acids can then travel from this location through the circulatory system to a range of tissues, including adipose tissue and skeletal muscle, mammary glands, and heart tissue. Lipoprotein lipase is the enzyme in

charge of lysing triglycerides in each of these. The enzyme hydrolyses triglycerides, releasing fatty acids and glycerol into the tissues where they are utilized or stored depending on circumstances (Drackley, 2000b; Yan et al., 2023b). In conditions of low energy intake, or at least reduced energy intake, lipoprotein-lipases are more active at the level of muscle tissue; in contrast, in conditions of high-calorie intake, they are more active at the level of adipose tissue (Drackley, 2000b).

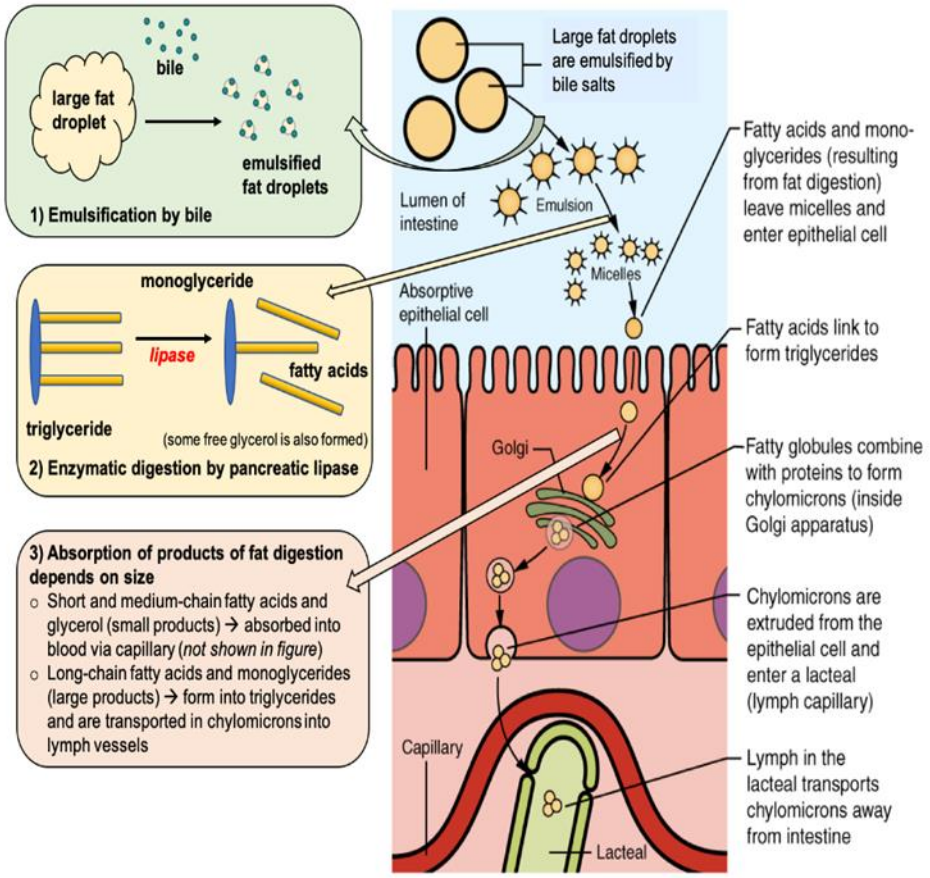

Figure 5: Lipid digestion and absorption in the small intestine(Callahan et al.,2020; Kerr et al., 2015)

2.4.4 Fatty acids

A fatty acid molecule (FA) is formed by an aliphatic hydrocarbon chain linked to a terminal carboxyl (-COOH) group. In nature, these chains consist of carbon atoms ranging from 4 to 22 (C4 to C22). Chains containing only single bonds are classified as saturated fatty acids (SFA), while those with at least one double bond are known as unsaturated fatty acids (UFA) (Aboagye et al., 2020; Ponnampalam et al., 2021). This final group of fatty acids is further broken down into two subgroups: monounsaturated fatty acids (MUFA), which are those with only one double bond in the chain, and polyunsaturated fatty acids (PUFA), which are those with multiple double bonds. UFA, which has both cis and trans isomerism. The first is the most prevalent type in nature, and in contrast to the trans, its hydrogens are located on the opposite sides of the chain at the double bond level (Mariamenatu & Abdu, 2021; Ponnampalam et al., 2021).

Beginning with counting the carbon atoms from the end of the methyl group, PUFAs can be divided into four families based on the position of the first double bond in the chain: n-3 (Omega3), n-6 (Omega6), n-7 (Omega7), and n-9 (Omega9) (IUPAC, 1978). The synthesis of those in the n-7 and n-9 families begins with C16:0 palmitic acid and C18:0 stearic acid, respectively (Mariamenatu & Abdu, 2021).

According to Duan et al. (2014), the fatty acids from these two families are regarded as non-essential since human bodies can produce them; in contrast, the fatty acids from the n-3 and n-6 families are regarded as necessary because they must be received through diet.

The unsaturated fats (or even those with a short chain) are liquid because of their lower melting points. Still, they are also the least stable in interactions with oxygen, meaning they oxidize more readily. In contrast, fats primarily constituted of SFA are present at room temperature in solid form. Since they serve as the building blocks for all other n-6 and n-3 PUFAs, linoleic acid (LA, C18:2 n-6) and -linolenic acid (ALA, C18:3 n-3) are the two most crucial necessary PUFAs.

The picture below shows that an n-3 FA cannot be transformed into an n-6 FA even if both families use identical elongation and desaturation enzymes (Duan et al., 2014; Mariamenatu & Abdu, 2021).

Polyunsaturated fat acids are critical in cell membrane construction, lipoprotein enzymatic processes, and lipid transport. Prostaglandins, thromboxane or eicosanoids, and leukotrienes are only a few examples of lipids with a hormonal role that are precursors to eicosanoids. These compounds assist in controlling several physiological processes, such as the immunological response, blood clotting, and venous pressure. Additionally, ALA and LA are sources of crucial fatty acids like eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), as well as other critical and significant fatty acids (Calder, 2004; Mariamenatu & Abdu, 2021). EPA is a precursor to series three prostaglandins, thromboxane, and series five leukotrienes and has a modulatory influence on the generation of eicosanoids starting from arachidonic acid. On the other hand, DHA is thought to be crucial for the health of the retina and the brain. According to international authorities, EPA and DHA intake should be between 200 and 650 mg daily (Aboagye et al., 2020; Wood et al., 2007).

A high PUFA content may enhance the consumer's health but may also indicate a technical issue with the product. Too much unsaturated fat lowers the melting point of the fat, lowering its consistency, making it difficult to process, and making it difficult to slice the cured meats made from it. Additionally, as was already indicated, they are more susceptible to the effects of oxygen interaction due to their double bonds, making them more oxidizable. Additionally, a product with a higher vulnerability to oxidation will have a shorter shelf life (Wood et al., 2007). Vitamin E enrichment of feed, an antioxidant, has been discovered to remedy this issue.

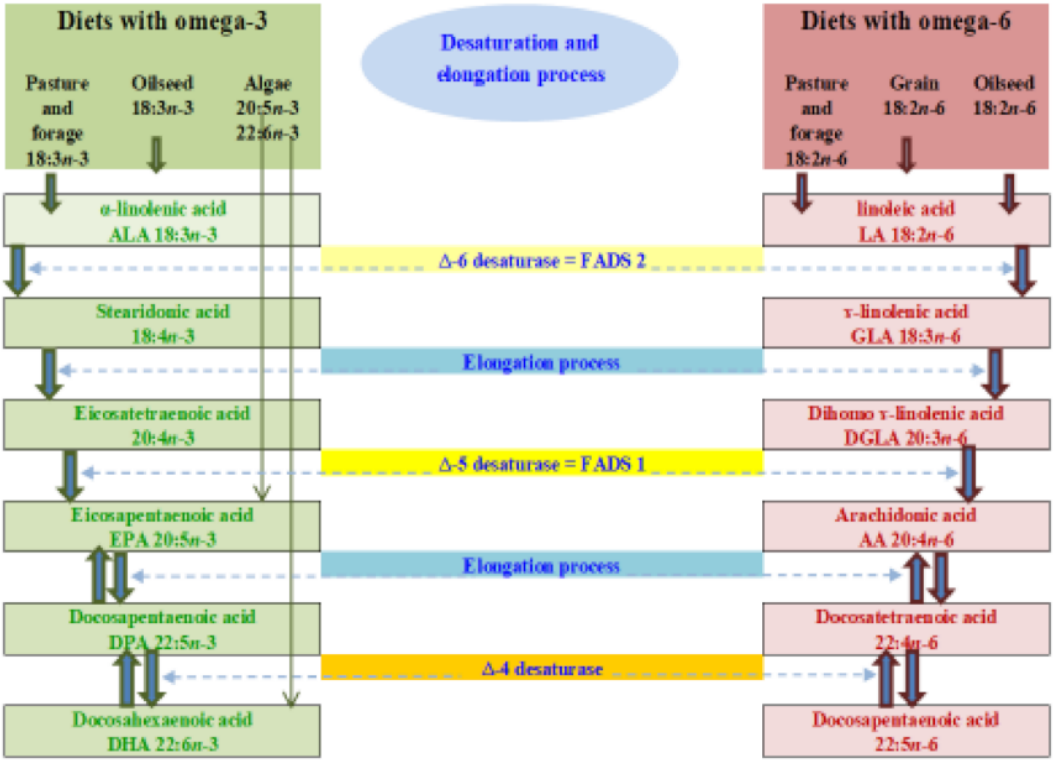

Figure 6: Dietary sources and biosynthesis of omega-3 and omega-6 fatty acids through enzymatic desaturation and elongation processes (Ponnampalam et al., 2021).

3 AIM OF THE THESIS

Considering what has been said previously, starches with different digestibility levels could also have different amounts of digestible, metabolizable, and net energy, consequently having a diversified effect on the performance of the fattening pig. Therefore, this study aimed to evaluate the influence of a waxy corn-based diet and sex on growth performance, carcass characteristics and meat quality of heavy pigs.

4 METHODOLOGY

4.1 Animals, Diets, and Experimental Design

The study was conducted at a commercial farm specializing in heavy pig production in Central Italy. It involved twenty-four healthy Large White × Pietrain pigs, evenly distributed into two dietary groups, with a balanced sex ratio (1:1; barrow: gilt) in each pen. The pigs were housed in pens measuring 6.5 m by 2.0 m, featuring a solid concrete floor and an adjoining slatted paddock of 2.0 m by 2.5 m. Each pen was outfitted with a 6.5 m trough and two duckbill drinkers located in the outdoor area.

The experimental diets were designed to meet the nutritional requirements of finisher pigs, ensuring isocaloric conditions and identical starch levels (according to the National Research Council-Nutrient Requirements of Swine, NRC, 2012). The diets were the same in composition, differing only in the type of corn starch used: one with low-amylopectin (CON) and the other with high-amylopectin (WAX) (Table 1). There were no differences in other nutritional components. Pigs received equal feed portions three times daily (7:00, 12:30, 17:30), with unrestricted access to water throughout the study.

The trial spanned from the start of the fattening phase, with pigs weighing 77.9 ± 5.2 kg until the end at a slaughter weight of 173.1 ± 5.2 kilograms. Monthly weigh-ins were conducted to monitor the Average Daily Gain (ADG) and the Feed to Gain ratio (F: G).

To calculate the mean daily gain (IMG) and the feed conversion index (ICA) of the two experimental groups, the animals were weighed monthly, and the quantity of feed consumed by each group was recorded.

Table 1. Composition and nutritional values of treatment diets for finisher pigs

Items	CON	WAX
Ingredients. as-fed basis (%)		
Non-waxy corn	44	
Waxy corn		44
Barley	19.5	19.5
Puffed rice	9	9
Wheat bran	10	10
Extruded soya meal 48%	10	10
Pea	5	5
Premix[1]	2.5	2.5
Total	100	100
Chemical composition		
Dry matter (DM). %	88.15	88.24
Starch. % DM	48.33	47.92
Crude protein. % DM	16.84	17.08
Ether extract. % DM	3.84	3.76
Crude fiber. % DM	5.48	5.55
Crude Ash. % DM	4.52	4.58
Gross energy (kcal/kg)	3876.47	3881.25

[1] Premix provided the following per kg of the diet: Cu 15.10 mg. Fe 150 mg. Se 0.30 mg. Zn 90 mg. Mn 61 mg. vitamin D 386 IU. vitamin A 9100 IU. vitamin E 135 IU. vitamin K 2.24 mg. Vitamin B_6 1.40 mg. Calcium pantothenate 19.70 mg. niacin 32.20 mg. vitamin B_{12} 0.028 mg. NaCl 4.10 g. $CaHPO_4$ 6.50 g. $CaCO_3$ 10.80 g.

4.2 Sample collection.

At the time of slaughter, the hot carcass weight was immediately noted, and the carcass yield was then calculated. pH and temperature measurements were taken from the Semimembranosus muscle on the right side of the carcasses 45 minutes post-slaughter. Measurements of muscle and fat thickness were performed using a caliper at the 4th lumbar vertebra, and the percentage of lean meat was calculated using the formula provided in Circolare Mipaaf 2420/2014:

y = (15.31+(0.51 (PC 2.20462)) - (31.277 (X1 0.03937)) + (3.813 (X2 0.03937)))/PC * 100
where:
y = estimated % of lean meat in the carcass.
PC = warm carcass weight.
X1 = thickness of back fat including rind (mm).
X2 = thickness of Longissimus dorsi muscle (mm).

Depending on the % of lean meat obtained, the carcasses were placed in the respective SEUROP class (Regulation (EC) No 1234/2007).

Further analysis on a section of Longissimus dorsi muscle, taken between the second and third cervical vertebrae, included assessments of i) color; ii) water retention; iii) chemical composition; iv) fatty acid composition; and v) cholesterol content. Meat color was evaluated in the CIE L* a* b* space using a Konica Minolta spectrophotometer CM-700d, with hue angle (H*) and chroma (C*) calculated respectively as $H^* = \tan^{-1}(b^*/a^*)$ in degrees, and $C^* = \sqrt[2]{a^{*2} + b^{*2}}$. Water holding capacity (WHC) was estimated by centrifuging 1 g of muscle at 1,500 x g for 4 minutes and determining residual water after oven-drying the sample at 70 °C overnight. Moisture, ash, protein, and fat content were determined following AOAC (2006) methods, with total lipids (TL) extracted using a chloroform/methanol 2:1 mixture as per Boselli et al. (2005), and fatty acid composition as detailed by Tinagli et al. (2023).

4.3 Statistical processing

The data were processed using JMP 17 PRO software using the following statistical model:

1. $y_{ij} = \mu + D_i + S_j + D_i * Sj + \varepsilon_{ij}$
2. $y_{ijk} = \mu + D_i + S_j + D_i * S_j + Ak [D_i] + \varepsilon ijk$
3. $y_{ijz} = \mu + D_i + S_j + D_i * S_j + P_z + \varepsilon_{ijz}$
4. who ^{2} – test

where:

y_{ijkz} = analyzed variables.

μ = mean.

D_i = fixed effect of the i-th diet (CON and WAX).

S_j = fixed effect of the j-th sex (male, female).

P_z = fixed effect of the z-th live weight at slaughter

A_k = random effect of the k-th animal (26 animals).

ε_{ijkz} = random error.

In particular, model 1 was used to analyze the weight at the beginning of the trial and after 4 months; model 2 for ICA, IMG; model 3 for the qualitative parameters of the carcasses (table 3), for the technological quality (table 4) and nutritional quality (table 5; table 6; table 7) of the meat; model 4 for age at slaughter.

The effects are considered statistically different when the P value is less than 0.05. For parameters that showed significance, a post-hoc analysis was done using contrasts on the least squares means.

5 Results and Discussion

5.1 Performances

Due to its peculiar starch composition, consisting of almost entirely amylopectin, waxy corn exhibits enhanced digestibility compared to non-waxy corn. This leads to higher available energy for pigs, both digestible and metabolizable energy (D. Ma et al., 2019).

The results of our study, shown in table 2, indicate that males of the WAX group exhibited better growth performance compared to both the males of the CON group and females of the same WAX diet. Specifically, WAX males had the highest average daily gain (ADG) and the lowest feed conversion ratio (FCR).

Table 2. Rearing performance in the fattening stage

	FEMALE		MALE		EX	p-Value		
	CON	WAX	CON	WAX		Diet	Sex	Diet * Sex
IMG (kg/ day)	0.723	0.657^{y}	0.639^{b}	0.771^{ax}	0.034	0.324	0.654	0.008
ICA (kg ration/kg IMG)	3.84	4.27	4.39^{y}	3.58^{b}	0.24	0.412	0.758	0.018

[a, b] different letters within the sex correspond to significantly different diet values.

[x, y] different letters within the diet correspond to significantly different sex values.

Data are significant when $P<0.05$.

Table 3 shows the results of the parameters useful for defining the quality of pig carcasses. Among these, the weight of the warm carcass, as well as the yield, and the percentage of lean meat are variables highly influenced by the live weight at slaughter. Therefore, to reduce the variability due to slaughter weight, the latter was included as a fixed factor in the statistical model considered.

The results show an influence of the diet on the hot weight of the carcass which therefore also affects the yield. The pigs of the WAX group have a warm weight of 140.72 kg against 138.87 kg

of CON pigs (p=0.008) and respectively 81.31% against 80.21% of yield (p=0.010). However, these parameters are also influenced by sex, where males have a hot weight (140.72 vs 138.87; p=0.011) and yield (81.28 vs 80.24; p=0.016) higher than that of females. In particular, it is observed that these differences are mainly due to a greater thickness of back fat in males of the WAX group (p=0.006). On the other hand, there was no difference between diet and/or sex for the thickness of the Longissimus dorsi muscle.

The product classification of the carcasses was also evaluated. This provides for the classification of carcasses in one of the SEUROP classes according to their percentage of lean meat. From the following experimental test, it emerged that the carcasses of the WAX males have a lean meat percentage of 54.38%, while that of the CON males, as well as that of the WAX females, are both equal to 54.78% (p=0.035; p=0.022). Our results show that, although the percentage of lean meat is statistically significant for both effects considered and their interaction, the difference found, equal to a few decimal points, is not sufficient to place the carcasses in two different SEUROP classes. However, this type of evaluation is purely commercial and is poorly indicative of the quality of the carcasses. It is in fact used to standardize the product classes and define the sales prices.

The diet does not seem to influence the pH and the temperature of the thigh at 45 minutes post-mortem, as well as the weight of the cerata.

Table 3. Carcass quality

	FEMALE		MALE		EX	p-Value		
	CON	WAX	CON	WAX		Diet	Sex	Diet * Sex
Hot carcass weight (kg)	138.14	139.59	139.59	141.85	0.76	0.008	0.011	0.580
Carcass yield (%)	79.80	80.68	80.63	81.94	0.46	0.010	0.016	0.619
Battleship weight (kg)	4.74	4.92	4.78	4.72	0.17	0.666	0.582	0.478
Thickness fat back (cm)	3.91	3.73 ^{y}	3.75^{b}	4.60 ^{ax}	0.18	0.036	0.031	0.006
Longissimus dorsi muscle thickness (cm)	5.83	5.94	6.22	5.65	0.38	0.473	0.872	0.343

Lean meat carcass (%)	54.73	54.78x	54.78 [y]	54.38 [by]	0.09	0.022	0.035	0.015
SEUROP class	HU	HU	HU	HU				
Length intestine large intestine (cm)	581.94	530.74	671.83	473.56	63.15	0.027	0.765	0.231
Thigh pH at 45 min	6.63	6.72	6.52	6.56	0.10	0.442	0.137	0.837
Thigh temperature at 45 min (°C)	39.53	38.83	39.23	39.32	0.32	0.275	0.722	0.169

[a, b] different letters within the sex correspond to significantly different diet values.

[x, y] different letters within the diet correspond to significantly different sex values.

Data are significant when $P<0.05$.

5.2 Quality of meat

Colour, water retention (WHC), and pH were determined on Longissimus dorsi muscle samples taken at the slaughterhouse. None of the parameters analyzed showed significant differences in diet or within sex (Table 4).

Colour is one of the main criteria that guide the final consumer in purchasing meat. However, for the difference in colour between two samples of the same cut of meat to be perceptible to the naked eye, it is not enough to evaluate the individual parameters of L*, a*, and b*. Still, instead, the δE * must be determined. The δE * is calculated according to the following formula $\delta E * = [(\delta L *)2 + (\delta a *)2 + (\delta b *)2] 0.5$, and if its value exceeds 3, then the colour difference is perceived by the human eye. In the case under examination, the δE * was calculated between the sexes in the same diet (F-CON vs M-CON; F-WAX vs M-WAX) and that between diets within each sex (F-CON vs F -WAX; M-CON vs M-WAX). Among the 4 cases analyzed, the value of δE * was higher than three only between males and females on the WAX diet (δE *=3.04). This result could be because, in absolute terms, the difference in a* values between males and females on the WAX diet is essential, although far from significant. In general, the meat of WAX males is darker and more intensely red than that of females fed the same diet.

PH is an important parameter that defines the technological quality of meat. In particular, the pH at 45 minutes and the pH at 24 hours post-mortem is used to identify two meat problems: PSE

(pale, soft, exudative) and DFD (dry, firm, dark). In the first case, within 45 min post-mortem, the pH of the meat falls below 5.8, and at the same time, the temperature of the carcass rises; in the second case, at 24 hours post-mortem, the acidification of the meat does not take place correctly, and the pH is never lower than 6.0. In the current experiment, the pH at 45 min appears to have normal parameters, as well as the temperature, indicating no influence from the diet, while the pH measured at 5 hours was already lower than 6.0. Due to technical problems, it was not possible to record the pH at 24 hours; therefore, we used the 5-hour measurements as predictors of the 24-hour situation.

Finally, water retention was not significant for all the effects considered.

Table 4. Water retention, pH and color of the *Longissimus dorsi muscle*

	FEMALE		MALE		EX	p-Value		
	CON	WAX	CON	WAX		Diet	Sex	Diet Sex
Brightness (L*)	37.94	35.91	37.35	35.58	10.73	0.194	0.745	0.928
Yellow index (a*)	11.22	12.12	1.47pm	15.01	1.92	0.474	0.130	0.848
Red index (b*)	10.51	10.29	12.08	11.18	0.98	0.516	0.154	0.690
Tint (arctan b*/a*) (°)	43.97	40.50	42.24	39.51	2.75	0.206	0.565	0.877
Chrome$\sqrt{a^2 + b^2)}$	15.45	15.92	18.18	19.18	1.97	0.672	0.088	0.876
Water holding capacity - WHC (%)	69.87	69.59	68.78	69.02	1.38	0.983	0.484	0.828
pH at 5 hours	5.91	5.69	5.71	6.00	0.24	0.873	0.797	0.234

[a, b] different letters within the sex correspond to significantly different diet values.

[x, y] different letters within the diet correspond to significantly different sex values.

Data are significant when $P<0.05$.

The chemical-centesimal composition (table 5) and the fatty acid composition expressed in three different ways (tables 6, 7, and 8) were also evaluated on the same meat sample. The percentage (g of fatty acid/100g of total fatty acids) expresses the effective concentration of each single fatty acid after eliminating the main effects that can influence it: the composition of the lipid fraction in terms of percentage of phospholipids and triglycerides and the total quantity of intramuscular fat of the considered matrix. The parameters expressed in g fatty acid/100 g of total lipids are instead influenced by the composition of the lipid component, while those expressed as g of fatty acid/100 g of muscle by the amount of total lipids present in the meat matrix.

The results shown in Table 5 show an effect of diet on the percentage of dry matter (DM). In particular, WAX meat has an SS of 30.45% against 28.37% of CON (p=0.02). This data suggests a higher concentration of WAX meat components, although no statistical difference is found when they are analyzed individually. However, intramuscular fat content is strongly influenced by sex, but not by diet. Specifically, male Longissimus dorsi muscles had about 2 grams more than females, 7.28% and 5.41%, respectively (p=0.035).

Table 5. Chemical composition of meat (g/100 g of *Longissimus dorsi)*

	FEMALE		MALE		EX	p-Value		
	CON	WAX	CON	WAX		Diet	Sex	Diet * Sex
Dry substance	27.49	29.77	29.25	31.14	1.03	0.024	0.093	0.846
Lipids	4.54	6.29	7.10	7.46	0.95	0.193	0.035	0.446
Protein	20.85	21.24	20.90	20.22	0.61	0.780	0.374	0.362
Ashes	1.09	1.09	1.10	1.06	0.02	0.264	0.716	0.464

[a, b] different letters within the sex correspond to significantly different diet values.

[x, y] different letters within the diet correspond to significantly different sex values.

Data are significant when $P<0.05$.

The total lipids of a muscle are given by the sum of triglycerides, di-glycerides, mono-glycerides, and phospholipids. If on the one hand, triglycerides represent the energy reserves of the muscle, accumulated in intracellular lipid droplets, on the other hand, phospholipids are molecules with a structural function, incorporated into the cell membrane. In the presence of a highly energetic diet, the excess energy is stored in the form of triglyceride, thus increasing the intramuscular quantities, without however affecting the phospholipid quantity. Generally, phospholipids constitute only a small fraction of total lipids and about half of the fatty acids that compose it belong to the polyunsaturated class (PUFA). Triglycerides, on the other hand, represent about 70-80% of total lipids and are almost entirely made up of saturated (SFA) and monounsaturated (MUFA) fatty acids.

The study in question showed that the percentage content of SFA is strongly influenced by diet, respectively 37.507% and 35.517% for WAX and CON ($p=0.008$). The concentration of UFAs (unsaturated fatty acids, represented by the sum of MUFAs and PUFAs) is also affected by the diet, although the same effect has not been highlighted on the single classes of unsaturated fats. The UFAs are equal to 64.423% in the CON group and 62.454% in the WAX group ($p=0.013$).

Conversely, considering the effect of gender, a higher concentration of PUFAs is highlighted in females compared to males, respectively 14.186% and 11.903% ($p=0.003$), while there are no effects on the concentration of SFA, UFA, and MUFA. However, overall, the results suggest that the significance found is not due to a greater accumulation of PUFA in females, but probably to a dilution effect in males. In other words, males showed a greater amount of intramuscular fat, understood as a higher content of triglycerides, which therefore indirectly lowered the percentage of PUFAs.

Finally, evaluating the effects of diet and sex on the composition of fatty acids per 100 grams of muscle (Table 8), we find high significance for almost all the parameters considered. This result is due to the higher content of total lipids in pigs fed with the WAX diet ($p=0.002$), especially in males ($p=0.037$).

Table 6. Fatty acid composition (g fatty acid/100 g total fatty acids)

	FEMALE		MALE		EX	p-Value		
	CON	WAX	CON	WAX		Diet	Sex	Diet * Sex
Fatty acids								
C12	0.085	0.080	0.081	0.084	0.005	0.864	0.992	0.451
C14	1.085	1,045y	1.012b	1.265 ax	0.049	0.016	0.096	0.005
C15	0.037	0.044	0.039	0.036	0.004	0.543	0.257	0.167
C16	21.853	22.619	22.189	23.422	0.509	0.023	0.220	0.634
C16-1c9	2,781	2.981	2,877	2,599	0.293	0.872	0.573	0.397
C17	0.197	0.198	0.196	0.240	0.022	0.262	0.269	0.359
C18	11.664	12.743	10.377	12.409	0.938	0.050	0.344	0.598
C18-1t9	0.093	0.095	0.100	0.103	0.004	0.445	0.030	0.846
C18-1t11	0.044	0.063	0.046	0.053	0.011	0.148	0.652	0.538
C18-1t12	0.047	0.053	0.049	0.049	0.004	0.316	0.790	0.406
C18-1c9	43.525	43.085	43.299	41.796	1.070	0.296	0.462	0.636
C18-1c11	4.208	4.261	4.243	3.759	0.276	0.351	0.334	0.314
C18-2n6	10.681	10.916	9.735	8.971	0.537	0.554	0.006	0.335
C20	0.109	0.099	0.116	0.163	0.015	0.164	0.017	0.064
C18-3n6	0.034	0.029	0.032	0.029	0.006	0.473	0.860	0.872
C20-1c11	0.969	1.041	1.017	0.979	0.058	0.737	0.889	0.325
C18-3n3	0.396	0.454	0.372	0.383	0.026	0.119	0.045	0.339
C20-2n6	0.476	0.522	0.445	0.452	0.024	0.191	0.024	0.393
C20-3n6	0.219	0.216	0.201	0.134	0.016	0.017	0.002	0.053
C20-3n3	0.067[b]	0.108 ax	0.072 [y]	0.016 by	0.015	0.584	0.009	0.007
C20-4n6	1.334	1.191	1.186	0.564	0.147	0.005	0.007	0.101
C22-4n6	0.321	0.319	0.290	0.206	0.021	0.026	0.001	0.057
C22-5n3	0.173	0.155	0.149	0.103	0.017	0.032	0.016	0.381

C22-6n3	0.056	0.044	0.038	0.023	0.007	0.029	0.004	0.866
Classes								
SFA	35.767	37.388	35.266	37,626	0.802	0.008	0.858	0.635
UFA	64.185	62.394	64.665	62.514	0.863	0.013	0.704	0.829
PUFA	14.011	14.362	12.800	11.006	0.761	0.261	0.003	0.151
MUFA	52.413	52.144	51.718	50.459	1.466	0.348	0.586	0.747
PUFA n6	13.102	13.231	11.942	10.368	0.712	0.231	0.004	0.219
PUFA n3	0.734	0.799	0.653	0.530	0.049	0.489	0.001	0.058
n6/n3	18.046	16.831	18.470	19.221	0.862	0.693	0.043	0.159
trans 18-1 total	0.318	0.330	0.314	0.353	0.029	0.288	0.711	0.612
U/S = UFA/SFA	1.796	1.677	1.840	1.659	0.064	0.012	0.821	0.625
P/S	0.395	0.343	0.379	0.299	0.030	0.012	0.264	0.626
P/S2	0.602	0.638	0.550	0.423	0.049	0.266	0.005	0.092
C18:0/C18:2	1.132	1.288	1.082	1.350	0.125	0.045	0.954	0.640
SA/C18:2n-6	3.173	2.928	3,449	4.336	0.332	0.252	0.009	0.087
n6 HPUFA/n3 HPUFA	7.320	6.908	7.927	8.794	0.586	0.641	0.023	0.260

[a, b] different letters within the sex correspond to significantly different diet values.

[x, y] different letters within the diet correspond to significantly different sex values.

Data are significant when $P<0.05$.

Table 7. Fatty acid composition (g fatty acid /100 g total lipids)

	FEMALE		MALE		EX	p-Value		
	CON	WAX	CON	WAX		Diet	Sex	Diet * Sex
Fatty acids								
C12	0.071	0.066	0.068	0.059	0.005	0.063	0.178	0.551
C14	0.932^{x}	0.842^{y}	0.781 ^{by}	1.052 ^{ax}	0.064	0.100	0.599	0.008
C15	0.032	0.036	0.030	0.030	0.003	0.436	0.130	0.464
C16	18.435	16.701	16.599	18.764	1.161	0.810	0.903	0.072
C16-1c9	2.404	2.406	2.213	2.168	0.269	0.921	0.363	0.927
C17	0.174	0.161	0.156	0.195	0.020	0.493	0.640	0.242
C18	9.307	10.119	8.741	10.381	1.037	0.141	0.868	0.677
C18-1t9	0.080	0.077	0.077	0.086	0.004	0.395	0.365	0.105
C18-1t11	0.037	0.051	0.035	0.045	0.009	0.120	0.617	0.826
C18-1t12	0.041	0.043	0.038	0.041	0.003	0.413	0.333	0.846
C18-1c9	36.282	36.559	34.297	34.151	1.647	0.963	0.145	0.893
C18-1c11	3.408	3.424	3.249	3.149	0.229	0.831	0.293	0.791
C18-2n6	9.126	8.805	7.483	7.438	0.463	0.635	0.001	0.754
C20	0.095	0.080y	0.090b	0.135 ^{ax}	0.014	0.207	0.058	0.039
C18-3n6	0.029	0.024	0.024	0.025	0.005	0.585	0.708	0.474
C20-1c11	0.836	0.844	0.782	0.803	0.053	0.740	0.307	0.891
C18-3n3	0.338	0.366	0.287	0.319	0.022	0.116	0.019	0.922
C20-2n6	0.408	0.422	0.342	0.372	0.021	0.215	0.005	0.701
C20-3n6	0.188	0.174	0.154	0.112	0.014	0.027	0.001	0.299
C20-3n3	0.059	0.088	0.054	0.012	0.013	0.612	0.005	0.016
C20-4n6	1.143	0.961	0.907	0.472	0.117	0.005	0.002	0.265
C22-4n6	0.276	0.258	0.223	0.171	0.019	0.035	0.000	0.347
C22-5n3	0.148	0.125	0.115	0.086	0.014	0.035	0.007	0.806
C22-6n3	0.047	0.036	0.029	0.019	0.005	0.023	0.001	0.843
Classes								

SFA	28.773	25.204 [y]	25.782	32.482[x]	2.369	0.429	0.303	0.034
UFA	57.211	55.572	51.193	50.320	2.658	0.570	0.024	0.880
PUFA	11.979	11,482	9.827	9.173	0.640	0.286	0.001	0.897
MUFA	45.147	44.015	41.294	41.100	2.271	0.725	0.098	0.828
PUFA n6	11.202	10.675	9.173	8.599	0.600	0.277	0.001	0.968
PUFA n3	0.628	0.649	0.512	0.436	0.042	0.427	0.000	0.236
n6/n3	18.009	16.509	18.095	19.472	0.804	0.926	0.040	0.073
trans 18-1 total	0.272	0.265	0.241	0.293	0.024	0.261	0.927	0.206
U/S = UFA/SFA	1.796	1.677	1.840	1,659	0.065	0.012	0.822	0.620
P/S	0.395	0.343	0.379	0.299	0.030	0.011	0.264	0.615
P/S2	0.602	0.638	0.551	0.423	0.049	0.262	0.005	0.090
C18:0/C18:2	1.040	1.273	1.076	1.364	0.141	0.026	0.603	0.838
SA/C18:2n-6	3.173	2.928	3.449	4,336	0.332	0.252	0.009	0.087
n6 HPUFA/n3								
HPUFA	7.312	6.814	7.621	8.981	0.551	0.351	0.017	0.090
Total	85.985	80.775	76.975	82.802	3.592	0.917	0.270	0.120

[a, b] different letters within the sex correspond to significantly different diet values.

[x, y] different letters within the diet correspond to significantly different sex values.

Data are significant when $P<0.05$.

Table 8. Fatty acid composition (g of fatty acid /100 g of muscle)

	FEMALE		MALE		EX	p-Value		
	CON	WAX	CON	WAX		Diet	Sex	Diet * Sex
Fatty acids								
C12	3,265	3,407	3.085	5.224	0.618	0.027	0.114	0.082
C15	1.488	1.822	1.327	2,536	0.316	0.008	0.318	0.157
C16	781.227	877.981 [y]	751,479b	1529.552 [ax]	157.270	0.003	0.026	0.026

C16-1c9	99.699	118.749	102.384	146.318	21.860	0.065	0.371	0.495
C17	7.272	8.301 [y]	5.886b	16.461 [ax]	2.311	0.007	0.104	0.042
C18	366.149	394.695 [y]	345.629b	870.520 [ax]	108.459	0.006	0.025	0.026
C18-1t9	3.391	4.068y	3.599b	7.700 [ax]	0.524	<.0001	0.000	0.002
C18-1t11	1.528	2.361	1.570	2.665	0.424	0.009	0.589	0.707
C18-1t12	1.916	2.421	1.711	3.453	0.402	0.003	0.245	0.119
C18-1c9	1559.428	1875,630	1569.022	2880.659	282,689	0.003	0.050	0.078
C18-1c11	150.988	172.412	150.425	216,756	26.845	0.039	0.294	0.320
C18-2n6	376.213	451.222 [y]	335.784[b]	620.583 [ax]	52.157	0.001	0.165	0.047
C20	4.053	4.410 [y]	3.983b	10.701 [ax]	1.217	0.002	0.008	0.013
C18-3n6	1.206	1.135	1.135	1.633	0.287	0.326	0.317	0.231
C20-1c11	34.880	43.297	35.879	67,583	6.888	0.002	0.046	0.089
C18-3n3	14.064	18.184 [y]	13.475[b]	26,850 [ax]	2.201	0.000	0.028	0.022
C20-2n6	16.915	21.884	15.575	30.795	2,867	0.001	0.140	0.073
C20-3n6	7.751	8.714	6.905	9.770	0.918	0.021	0.895	0.284
C20-3n3	2.500	4.168	2.504	1.339	0.979	0.780	0.135	0.161
C20-4n6	46.424	46.341	40.009	42.976	3.098	0.576	0.081	0.606
C22-4n6	11.312	12.978	9.977	14.843	1.306	0.007	0.814	0.209
C22-5n3	6.010	6.217	5.066	7.400	0.636	0.026	0.829	0.092
C22-6n3	2.029	1.779	1.361	1.699	0.369	0.886	0.250	0.407
Classes								
SFA extension	1206.870	1357.934 [y]	1160,102 [b]	2618.437 [ax]	276,183	0.002	0.020	0.022
UFA	2371.560	2834.546	2331.050	4262,892	403,482	0.002	0.060	0.069

PUFA	493,124	584,433	440,704	772,362	63,545	0.001	0.228	0.060
MUFA	1874,936	2246.419	1887,236	3486.152	342,807	0.003	0.047	0.073
PUFA n6	461,152	543,774	411,379	722.017	58.978	0.001	0.218	0.055
PUFA n3	25.916	32,729	22.919	38.610	4.029	0.003	0.680	0.256
n6/n3	18.009	16.509	18.095	19.472	0.804	0.926	0.040	0.073
trans 18-1 total								
U/S = UFA/SFA	1.796	1.677	1.840	1,659	0.065	0.012	0.822	0.620
P/S	0.395	0.343	0.379	0.299	0.030	0.011	0.264	0.615
P/S2	0.602	0.638	0.551	0.423	0.049	0.262	0.005	0.090
C18:0/C18:2	1.040	1.273	1.076	1,364	0.141	0.026	0.603	0.838
SA/C18:2n-6	3.173	2.928	3,449	4,336	0.332	0.252	0.009	0.087
n6 HPUFA/n3 HPUFA	7,312	6,814	7,621	8.981	0.551	0.351	0.017	0.090
Total	3578.348	4188.648[y]	3490,578[b]	6883.029[ax]	656,746	0.002	0.033	0.037

[a, b] different letters within the sex correspond to significantly different diet values.

[x, y] different letters within the diet correspond to significantly different sex values.

Data are significant when $P<0.05$.

6 CONCLUSION

Incorporating waxy corn into the diet for heavy pigs increases carcass fatness without compromising carcass value or adversely impacting meat quality. However, the impact of the waxy corn diet is significantly influenced by pig sex, with effective performance and increased carcass adiposity observed in barrows but not in gilts. Consequently, waxy corn emerges as a practical solution for enhancing the adiposity of heavy barrows intended for ham production.

7 APPENDIX

Figure 1: Pig farm

Figure 2: Rotovap.

Figure 3: Gas chromatography.

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