OPTIMIZING THE USE OF HYBRID RYE IN SWINE DIETS

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ABSTRACT

A newly developed hybrid rye, promising for swine due to lower ergot alkaloid levels and higher yield, was evaluated for its nutritional value and any improvements via grain processing and enzyme supplementation. The first experiment determined the effects of particle size on digestible energy (DE), metabolizable energy (ME), and net energy (NE) contents of hybrid rye in growing pigs. Finely ground rye grain showed greater (P < 0.05) DE and ME contents than coarsely ground rye, with a tendency for higher NE (P = 0.084). The second experiment evaluated the effects of particle size and multi-carbohydrase (MC) supplementation on nutrient digestibility in hybrid rye. Diets containing finely ground hybrid rye had higher (P < 0.05) apparent total tract digestibility (ATTD) of neutral detergent fiber (NDF) and ether extract (EE). Multi-carbohydrase supplementation increased (P < 0.05) ATTD of dry matter (DM), gross energy (GE), crude protein (CP), NDF, and EE, and improved (P < 0.05) the standardized ileal digestibility (SID) of CP and most amino acids (AA). The third experiment investigated the effects of particle size and phytase supplementation on phosphorus (P) digestibility in hybrid rye. The ATTD and standardized total tract digestibility (STTD) of P were greater (P < 0.05) for diets containing finely ground grain than those containing coarsely ground grain. There were linear (P < 0.05) and quadratic (P < 0.05) improvement in the STTD of P with increasing phytase levels. The fourth study determined the effects of MC supplementation on growth performance, nutrient digestibility, short-chain fatty acids (SFCA) production, and gut microbiota composition in weaned piglets fed wheat-based or hybrid rye-based diets. Pigs fed wheat-based diets had higher (P < 0.05) average daily gain (ADG) and average daily feed intake (ADFI), with no differences (P > 0.10) in gain to feed ratio (G:F). Multi-carbohydrase supplementation of hybrid rye diets improved (P < 0.05) the ATTD of NDF and increased acetic acid, propionic acid, and total SCFA production, as well as the relative abundance of Bifidobacterium and Lactobacillus. In conclusion, grain processing and enzyme supplementation could significantly enhance the nutritional value of hybrid rye for pigs.

DEDICATION

This thesis is dedicated to my dear wife (Yinlu Wang) and our lovely daughter (Viola Song), and to my parents Haizhu Song and Huiying Shan

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FOREWORD

The thesis adheres to the format specified by the *Journal of Animal Science* and consists of four manuscripts, each corresponding to a separate chapter. Manuscript I was published in A*nimal Feed Science and Technology*, while Manuscript III was published in *Journal of Animal Science*. Manuscript II was submitted to the *Journal of Animal Physiology and Animal Nutrition*. Manuscript IV is under preparation.

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LIST OF ABBREVIATIONS

AA	Amino acids
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
AID	Apparent ileal digestibility
Ala	Alanine
Arg	Arginine
Asp	Asparagine
ATTD	Apparent total tract digestibility
BCFA	Branched chain fatty acids
BW	Body weight
Ca	Calcium
CO_2	Carbon dioxide
CP	Crude protein
Cys	Cysteine
d	Day
DDGS	Distiller's dried grains with solubles
DE	Digestible energy
D_{gw}	Geometric mean diameter
DM	Dry matter
DMI	Dry matter intake
EAL	Endogenous loss of amino acids

EE	Ether extract
FAOSTAT	Food and Agriculture Organization of the United Nations
FHP	Fasting heat production
G:F	Gain to feed ratio
GE	Gross energy
GIT	Gastrointestinal tract
Glu	Glutamine
Gly	Glycine
h	Hour
HI	Heat increment
His	Histidine
HP	Heat production
IC	Indirect calorimetry
Ile	Isoleucine
kcal	Kilocalorie
kg	Kilogram
Leu	Leucine
Lys	Lysine
MC	Multi-carbohydrase
ME	Metabolizable energy
Met	Methionine
MT	Metric ton
N	Nitrogen

NDF	Neutral detergent fiber
NE	Net energy
NRC	National Research Council
NSP	Non-starch polysaccharides
O_2	Oxygen
P	Phosphorus
Phe	Phenylalanine
Pro	Proline
RE	Retained energy
REL	Retained energy as lipid
REP	Retained energy as protein
RQ	Respiratory quotient
SBM	Soybean meal
SCFA	Short-chain fatty acids
Ser	Serine
SID	Standardized ileal digestibility
SEM	Standard error of the mean
S_{gw}	Geometric standard deviation
STTD	Standardized total tract digestibility
TDF	Total dietary fiber
Thr	Threonine
TiO_2	Titanium dioxide
Tyr	Tyrosine

Val	Valine
VFA	Volatile fatty acids

CHAPTER ONE: GENERAL INTRODUCTION

Rye (*Secale cereale* L.) is a cereal grain that belongs to the grass family Poaceae and is predominantly cultivated in Europe and North America (Bushuk, 2001). Known for its resilience, rye can produce high yields even under challenging environmental conditions such as cold weather, drought, and poor soil quality, which makes it a vital crop in areas where other cereal grains like wheat and barley may not be suitable for cultivation (Geiger and Miedaner, 2009; Jürgens et al., 2012). In addition to its use in making bread and whisky, rye can also serve as a valuable feed ingredient for livestock (Bengtsson et al., 1992).

Although rye is not commonly used as a feed ingredient in the swine industry due to its high levels of antinutritional factors and susceptibility to ergot contamination, which can lead to health issues in animals (Antoniou et al., 1981; Boros et al., 1993; Dänicke and Diers, 2013; Coufal-Majewski et al., 2016; Waret-Szkuta et al., 2019), recent advancements have led to the development of a new hybrid of fall rye. This new hybrid rye exhibits reduced levels of antinutritional factors such as trypsin inhibitors and alkylresorcinols and shows less vulnerability to ergot contamination (Schwarz et al., 2015; Schwarz et al., 2016; Smit et al., 2019). Hybrid rye possesses a robust root system that aids in its ability to survive the winter season. Compared to traditional rye varieties and other cereals like wheat and barley, hybrid rye is hardier and has a greater yield even under conditions of frost, drought, and sandy soils (McGhee and Stein, 2019; Hackauf et al., 2022). Given these advantageous traits, hybrid rye could become a promising crop in regions with harsh and cold winters.

Similar to other cereals such as wheat and barley, rye is a starch-rich grain and contains more protein than corn (Cervantes-Pahm et al., 2013; Cervantes-Pahm et al., 2014). Rye also has a higher proportion of fermentable fibers, which could be beneficial for the gastrointestinal health of pigs (Le Gall et al., 2009; Le Gall et al., 2010; Bach Knudsen et al., 2016; Bach Knudsen et al., 2017; Vuholm et al., 2017). Nevertheless, the high non-starch polysaccharides (NSP) content in rye could compromise nutrient and energy digestibility in pigs, leading to nutrient waste and environmental concerns (Nørgaard et al., 2016; Wilke et al., 2021; Wilke and Kamphues, 2023). Consequently, there is an urgent need to explore approaches that can reduce the antinutritional factors to make rye a more efficient feed ingredient for pigs.

Feed processing is an essential part of modern animal husbandry, playing a critical role in optimizing the efficiency of animal production. Among various feed processing techniques, grinding is one of the most important steps in feed manufacturing process in which particle size of feed ingredients is reduced (Vukmirović et al., 2017). Particle size reduction by grinding can improve feed efficiency by increasing the surface area, allowing digestive enzymes to access nutrients and break down the fiber matrix more easily, making essential nutrients more digestible (Wondra et al., 1995; Laurinen et al., 2000; Kim et al., 2016; Kiarie and Mills, 2019). Furthermore, finer feed tends to be more appetizing to animals, encouraging greater consumption and ultimately improving growth performance (Amerah et al., 2007; Nemechek et al., 2016). However, too fine particle size is associated with an increased risk of gastric ulcers in pigs (Ayles et al., 1996; Kiarie and Mills, 2019).

The addition of enzyme supplements to animal feeds marks a significant innovation in the field of animal nutrition. Specifically, enzymes like multi-carbohydrases (MC) and phytase have been extensively researched for their potential to improve the nutritional value of feed ingredients, thereby contributing to improved animal performance, economic efficiency, and environmental sustainability (Woyengo and Nyachoti, 2011; Ndou et al., 2015; Gallardo et al., 2017; Zeng et al., 2018). A typical MC is a mixture of different carbohydrases such as cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, and amylase, etc. (Meng et al., 2005; Slominski, 2011). Numerous studies have investigated the effects of MC supplementation on improving the nutritional value of ingredients and diets, as well as their role in nutrient digestibility and growth performance in monogastric animals (Meng et al., 2005; Adeola et al., 2011; Slominski, 2011; Cozannet et al., 2017; Liu et al., 2021). In cereal grains and oilseeds, phosphorus (P) mainly exists in the form of phytate, which pigs are unable to digest due to the lack of the relevant digestive enzymes. This results in a large amount of P in feed ingredients being unutilized by pigs and thus excreted to environment as a waste (Humer et al., 2015). Phytase is specialized in its ability to break down phytate, which not only makes P available for absorption but may also help release other bound nutrients such as calcium (Ca) and amino acids (AA), significantly improving the nutritional value of feed ingredients (Selle and Ravindran, 2008; Velayudhan et al., 2015; Cowieson et al., 2017; Zouaoui et al., 2018). Nevertheless, there are also some challenges involved in effective enzyme supplementation, such as enzyme stability throughout the feed production process to ensure that they remain active within the animal's gastrointestinal tract. Due to this, a careful decision-making is required when it comes to selecting the type of enzyme, the dosage, and the formulation (Adeola and Cowieson, 2011).

Feed processing and enzyme addition have been recognised as useful methods for enhancing digestive utilization of nutrients in NSP-rich feedstuffs (Zijlstra et al., 2010; De Vries et al., 2012). Utilizing both these techniques together has gained attention as a sustainable strategy for enhancing pig and poultry farming systems (Schedle, 2016). Therefore, the main objectives of this research were to evaluate the effects of feed processing and enzyme supplementation on improving the nutritional value of hybrid rye fed to pigs. To achieve the main objectives, the subsequent specific objectives were devised:

i) To determine the digestible energy (DE), metabolizable energy (ME), and net energy (NE) of hybrid rye and to investigate the effects of particle size and variety on energy contents of hybrid rye fed to growing pigs.

ii) To determine the effects of hybrid rye particle size and MC supplementation on the standardized ileal digestibility (SID) of AA in hybrid rye fed to growing pigs.

iii) To determine the effects of hybrid rye particle size and different phytase levels on the apparent total tract digestibility (ATTD) and the standardized total tract digestibility (STTD) of P in hybrid rye fed to growing pigs.

iv) To investigate the effects of MC supplementation on growth performance, nutrient digestibility, short-chain fatty acids (SCFA) production, and gut health of weaned piglets fed hybrid rye diets.

CHAPTER TWO: LITERATURE REVIEW

2.1 INTRODUCTION

Rye (*Secale cereale* L.) is a member of the Poaceae grass family and recognized for its robustness and significant role in global agriculture (Jardine et al., 2019). Rye thrives in challenging conditions, including poor soils and harsh climates, making it a key crop in many regions worldwide (Geiger and Miedaner, 2009). According to the report of the Food and Agriculture Organization of the United Nations (FAOSTAT), about 11.27 million metric tons (MT) of rye grain is harvested around the world, predominantly in Europe, with Germany, Poland, and Russian being major contributors (FAOSTAT, 2020). While Europe remains the principal producer, there has been a consistent growth in rye production in North America, specifically in Canada and the USA, in recent years (Korzun et al., 2021). The cultivation of rye in Canada is primarily concentrated in the provinces of Alberta, Saskatchewan, and Manitoba, where the climate and soil conditions are particularly suited to this crop. In these regions, rye plays a crucial role as a winter cover crop, contributing significantly to soil health improvement and the promotion of sustainable farming practices (Koudahe et al., 2022). In the United States, rye is a minor grain compared to wheat and corn but serves as an important winter cover crop in the Midwest and Northeast since it demonstrates strong winter resilience in these areas and is capable of producing substantial biomass, which is advantageous for the subsequent spring planting of other crops (Wallander et al., 2021).

Rye, traditionally recognized for its role in the production of bread, whisky, and biogas, has diverse applications in both food and non-food sectors (Geiger and Miedaner, 2009). It shares several nutritional similarities with wheat, making it a valuable grain in human and animal diets. As Bengtsson et al. (1992) noted, rye's nutritional profile makes it a viable feed ingredient for livestock, offering an alternative to commonly used grains like corn and wheat. However, the use of rye in animal feed, especially for pigs, has been historically limited due to its high vulnerability to ergot fungus (*Claviceps purpurea*) contamination (Haarmann et al., 2009). Ergot, commonly found in rye, produces toxic alkaloids that can severely impact the health of animals (Schumann et al., 2008; Scott, 2009; Klotz, 2015). These alkaloids are known to cause vasoconstriction, neurotoxic effects, and reproductive problems, leading to significant health risks for animals and affecting farm productivity (Agriopoulou, 2021). As a result, the challenges posed by ergot contamination have led to the need for stringent management practices and often deter the use of rye in animal feeds (Coufal-Majewski, 2016). Also, the high content of non-starch polysaccharides (NSP) in rye can negatively impact nutrient and energy digestibility in animals, potentially causing nutrient loss and raising environmental concerns (Nørgaard et al., 2016).

Recent advances in plant breeding have led to the development of a new hybrid rye (KWS Lochow GmbH, Bergen, Germany) that shows promise in mitigating the risks associated with ergot contamination. This development, as detailed by Miedaner and Geiger (2015), involves the integration of the Rfp1 and Rfp2 pollen restorer genes into hybrid rye. These genes enhance the efficiency of pollen shedding, which in turn reduces the likelihood of ergot infecting the unfertilized seed heads. This improvement in pollen shedding is crucial, as ergot primarily infects rye through the unfertilized ovary of the flower. By ensuring more efficient fertilization, the chances of ergot fungus finding a suitable host are significantly reduced. Hybrid rye not only displays resistance to ergot contamination but also has reduced quantities of specific antinutritional components, like trypsin inhibitors (Stein et al., 2016). When comparing the total ergot alkaloid content, hybrid rye contains a significantly lower amount at 392 ppb, whereas traditional rye has a much higher concentration of 2,295 ppb (Table 2.1). Trypsin inhibitors can interfere with protein digestion, while ergot alkaloids have been associated with negative health effects (Gilani et al., 2005; Palliyeguru et al., 2011). The reduction of these compounds in hybrid rye enhances its nutritional value and digestibility, making it a more suitable ingredient for animal feed. Agronomically, hybrid rye demonstrates remarkable adaptability and robustness, thriving well on less fertile soils. This adaptability is especially beneficial for growing rye in areas where soil conditions are not ideal for other small grains such as wheat, barley, and oats. By being able to grow in these suboptimal conditions, hybrid rye can effectively utilize land that might otherwise be underutilized, contributing to more sustainable land use and agricultural practices (Igos et al., 2016). Moreover, hybrid rye shows greater potential for high-yield performance, which is critical in meeting the growing global demand for food and feed. Its high-yield potential and low maintenance requirements, owing to its adaptability to poorer soils and resistance to common pests and diseases, can lead to reduced input costs and higher profitability (Thiessen Martens et al., 2015). This economic aspect is vital for the long-term sustainability of farming communities, particularly in regions where agriculture is a primary livelihood (Herbstritt et al., 2022). Another key attribute of hybrid rye is its resilience to environmental stresses, such as low temperatures and drought (Hübner et al., 2013). This resilience is increasingly important in the context of climate change and makes hybrid rye a valuable crop for sustainable agriculture, capable of maintaining yields under harsh conditions. Given all these characteristics, hybrid rye emerges as an attractive alternative cereal grain for swine diets. Its enhanced nutritional profile, coupled with lower antinutritional components, makes it a more effective and healthier feed ingredient. The inclusion of hybrid rye in swine diets could lead to improved animal health and productivity, contributing to more efficient and sustainable livestock farming practices.

Table 2.1 Comparison of ergot alkaloid concentration between traditional rye and hybrid rye (ppb, dry matter basis)[1]

Ergot alkaloid	Hybrid rye	Traditional rye
Ergosine	70	88
Ergotamine	25	712
Ergocornine	31	162
Ergocryptine	138	424
Ergocristine	47	<5
Ergosinine	28	43
Ergotaminine	<20	395
Ergocorinine	<20	119
Ergocryptinine	32	351
Total	392	2,295

[1]Adapted from Blaney et al. (2009) and Rusche et al. (2020).

2.2 CHEMICAL COMPOSITION OF HYBRID RYE

2.2.1 Chemical composition of hybrid rye

Hybrid rye is an important source of energy and protein in animal diets. Like other cereals, it is mainly composed of carbohydrates, proteins, fats, and a variety of micronutrients. As indicated in Table 2.2, hybrid rye, barley, corn, and wheat, display unique nutritional characteristics. In hybrid rye, carbohydrates predominantly exist as starch, comprising 55.7% of its content (as-fed basis), thus serving as an important energy source. The total dietary fiber (TDF) in hybrid rye, amounting to 16.8%, is less than in barley (19.0%) but exceeds the levels in corn (10.3%) and wheat (11.3%). Both insoluble and soluble TDF, as well as neutral detergent fiber (NDF) and acid detergent fiber (ADF) in hybrid rye, exceed those in corn and wheat, but are lower than the levels found in barley. The crude protein (CP) level in hybrid rye stands at 9.3%, which is lower than that of barley and wheat, and it surpasses that of corn, which is at 7.6%. The fat content in hybrid rye is notably low. Additionally, rye grain is noted for its enrichment with a variety of phytochemicals and antioxidants, such as phenolic acids, lignans, and phytosterols, which contribute to its health advantages (Glitsø and Bach Knudsen, 1999; Glitsø et al., 2000; Heiniö et al., 2008). A significant portion of these phytochemicals is concentrated in the bran layer of the rye kernel (Liukkonen et al., 2003, Nyström et al., 2008).

Table 2.2 Chemical composition of hybrid rye, barley, corn, and wheat (%, as-fed basis)[1]

Item	Hybrid rye	Barley	Corn	Wheat
DM	88.66	92.62	87.72	89.98
CP	9.34	10.56	7.62	10.06
EE	1.22	1.79	3.61	1.71
Ash	1.56	2.55	1.35	1.67
TDF	16.75	18.99	10.27	11.31
Insoluble TDF	14.50	16.44	10.27	10.56
Soluble TDF	2.25	2.55	-	0.75
NDF	17.66	19.75	8.80	10.74
ADF	3.04	5.78	2.31	2.79
Starch	55.73	51.74	57.98	57.07

[1]Adapted from McGhee and Stein (2019; 2020).

2.2.2 Factors that influence the hybrid rye's chemical composition

The chemical composition of hybrid rye, which influences its nutritional value for animals, is impacted by various factors including the genotype of the rye, environmental conditions, and agricultural practices (Hansen et al., 2003; Hansen et al., 2004; Laidig et al., 2017). One of the main concerns in using rye grains in animal feed is the presence of antinutritional factors. These include alkylresorcinols, NSP, and trypsin inhibitors, which are predominantly found in the outer layers of the grain (Chuppava et al., 2020). The presence of these compounds can reduce the digestibility of nutrients, hinder animal growth, and potentially cause health issues. Variations in hybrid rye strains lead to differences in the amounts of certain antinutritional substances, including alkylresorcinols and water-soluble and viscous arabinoxylans often referred to as pentosans

(Jürgens et al., 2012). Also, the synthesis of antinutritional compounds in rye grains is greatly influenced by climatic conditions (Grabiński et al., 2021). For example, stressful environmental conditions, especially low rainfall and temperature variations have been linked to increased levels of these antinutritional factors.

There are primarily two agricultural technologies used for producing rye grains, intensive and integrated (Filson, 2005). Intensive technology, characterized by the extensive use of industrial production methods, mineral fertilizers, and chemical plant protection agents, tends to yield higher rye grain output (Kumar et al., 2022). However, this method poses several environmental risks. On the other hand, integrated technology involves moderate use of production resources and targeted pest control treatments (Deguine et al., 2021). It was reported that rye grains cultivated using intensive methods have higher concentrations of alkylresorcinols and water-soluble pentosans compared to those produced through integrated cultivation (Grabiński et al., 2021).

2.3 CARBOHYDRATES SOURCE

Carbohydrates are categorized based on their molecular size and level of polymerization, and further divided by the count and type of monosaccharide units they contain (Cummings and Stephen, 2007). This classification encompasses monosaccharides, disaccharides, oligosaccharides, starch, and non-starch polysaccharides. Usually, a cereal grain consists of three primary structural components: the germ (which plays a crucial role in the formation and growth of the new sprout), the endosperm (serving as a source of nutrients during seed germination and early seedling growth), and the bran (made up of several layers that protect the grain) (Evers et al., 1999). The carbohydrate percentage in rye grain ranges from 71 to 87%, with this variation attributed to differences in composition among various strains (Szentmiklóssy et al., 2023). Starch stands out as the most prominent carbohydrate in hybrid rye, which is the main source of energy.

Predominantly found in the endosperm, starch makes up roughly 60% of the grain's total mass (Hansen et al., 2004). Additionally, rye grain's dietary fiber is composed of NSP and lignin, which together account for about 14 to 21% of the grain on dry matter (DM) basis (Vithana Pathirannehelage and Joye, 2020).

Non-starch polysaccharides refer to all plant polysaccharides except for starch. Common NSP found in seeds and grains include arabinoxylans, β-glucans, cellulose, galactomannans, arabinogalactans, xyloglucans, and pectic polysaccharides (Choct, 1997). These NSP are categorized into two types, soluble and insoluble. Water-soluble NSP like arabinoxylans and β-glucans are antinutritional factors for pigs and chicken due to their high water-holding capacity (Habte-Tsion and Kumar, 2018). They can increase the viscosity of digesta, which slows down the diffusion of digestive enzymes and affects nutrient absorption and digestion (Wellock et al., 2008; Hooda et al., 2010). Many studies reported that supplying diets with high NSP content for pigs and chicken reduced the average daily gain (ADG), average daily feed intake (ADFI), and nutrient digestibility (Slominski and Campbell, 1990; Högberg and Lindberg, 2006; Zijlstra et al., 2010). The water-insoluble NSP like cellulose, hemicellulose, pectin, and lignin are often inactive in the digestive tract function and have minimal impact on nutrient absorption (Lattimer and Haub, 2010). However, insoluble fiber can increase digesta passage rate, reducing the time available for digestive enzymes to interact with dietary components (Wenk, 2001). For instance, studies have shown that insoluble fibers from sources like wheat bran, corn bran, and cellulose can shorten the transit time of digesta, leading to reduced nutrient digestibility (Owusu-Asiedu et al., 2006; Wilfart et al., 2007; He et al., 2018). Nonetheless, the insoluble NSP may be beneficial for supporting gut health in pigs and poultry because they can be fermented by the microbiota residing in the large intestine to produce short-chain fatty acids (SCFA), which can lower the pH of the colon and cecum, inhibit the growth of pathogenic bacteria, and prevent diarrhea (Scott et al., 2008; Molist et al., 2009).

As shown in Table 2.3, the most abundant NSP in hybrid rye are arabinoxylans, β-glucans, and cellulose. Monogastric animals cannot hydrolyze these NSP by their own digestive enzymes, but these substances can be broken down by the microbial population of the lower gastrointestinal tract (Boros et al., 1998). The concentration of arabinoxylans in hybrid rye ranges between 8 and 9% on a DM basis, and the concentration of β-glucans and cellulose in hybrid rye is about 2 and 1.5%, respectively, on a DM basis (Strang et al., 2016). The fructans concentration of rye is higher than in other cereal grains and can be fermented in the large intestine to produce SCFA such as butyrate, which may have benefits in improving pig gut health (Verspreet et al., 2015). The concentration of resistant starch in rye is less than 1% based on enzymatic isolation method, and the concentration of Klason lignin and acid detergent lignin, which are key components of dietary fiber in rye is about 1.5 and 0.8%, respectively on a DM basis (Rodehutscord et al., 2016; Buksa, 2018). Lignin is not a desirable component in pig diets because it cannot be digested by endogenous enzymes and fermented by the microbiota in the large intestine, affecting the nutrient digestibility in pigs (Wenk, 2001).

Table 2.3 Non-starch polysaccharides (NSP) contents of hybrid rye, wheat, and corn (%, as-fed basis)[1]

Item	Hybrid rye	Corn	Wheat
Arabinoxylans	7.2	2.0	2.8
β-glucans	1.9	0.1	1.0
Cellulose	1.0	0.8	1.8
NCP[2]	10.9	7.0	9.5
Total NSP	11.9	9.0	11.3

[1]Adapted from Bach Knudsen (1997) and Strang et al. (2016).
[2]NCP, non-cellulosic polysaccharides.

2.4 PROTEIN AND AMINO ACIDS SOURCE

The CP content of the cereal grains can differ based on various factors, including the grain's genotype and growing conditions. Compared to the old cultivars, the new hybrid rye has a lower CP content, with values ranging from 8.7 to 11.2% (as-fed basis) (Rodehutscord et al., 2016; Strang et al., 2016; McGhee and Stein, 2018). In rye grain, albumins form the majority of proteins, making up to 29-40% of total protein, while globulins comprise 8-11%, and the storage proteins, prolamins and glutelins, constitute 17-19% and 9-15% respectively (Németh and Tömösközi, 2021). Lysine is often the first limiting amino acid (AA) in grain-based diets (Liao et al., 2015). Studies have demonstrated that the level of lysine in the diet significantly affects the growth performance of pigs (Kim et al., 2011; Taylor et al., 2015). As shown in Table 2.4, hybrid rye has an average lysine level of 0.37%, which is higher than that in both wheat (0.35%) and corn (0.25%). On the other hand, the methionine level in hybrid rye is 0.16%, less than that found in wheat (0.22%) and corn (0.18%). The threonine concentration in hybrid rye averages 0.33%, which is greater than that of corn (0.28%) but not as high as that of wheat (0.35%). Furthermore, the tryptophan level in hybrid rye accounts for 0.10%, exceeding that of corn (0.06%) but falling short of wheat (0.14%). Overall, the total indispensable AA content of 3.58% in hybrid rye, surpasses that of corn (3.39%) and is lower than that of wheat (3.87%).

Table 2.4 Amino acids (AA) contents of hybrid rye, wheat, and corn (%, as-fed basis)

Item	Hybrid rye[1]	Corn[2]	Wheat[2]
Indispensable AA			
Arginine	0.52	0.37	0.52
Histidine	0.26	0.24	0.28
Isoleucine	0.30	0.28	0.34
Leucine	0.63	0.96	0.68
Lysine	0.37	0.25	0.35
Methionine	0.16	0.18	0.22
Phenylalanine	0.48	0.39	0.52
Threonine	0.33	0.28	0.35
Tryptophan	0.10	0.06	0.14
Valine	0.43	0.38	0.47
Total	3.58	3.39	3.87
Dispensable AA			
Alanine	0.41	0.60	0.42
Aspartic Acid	0.70	0.54	0.58
Cysteine	0.22	0.19	0.30
Glutamic Acid	2.45	1.48	2.92
Glycine	0.43	0.31	0.49
Proline	1.18	0.71	1.04
Serine	0.47	0.38	0.44
Tyrosine	-	0.26	0.30
Total	5.87	4.47	6.49

[1]Adapted from Strang et al. (2016).
[2]Adapted from NRC (2012).

Evaluating the bioavailability of AA in pigs' diets is crucial for determining the nutritional quality of feed ingredients. Formulating feed based on digestible AA enhances feed efficiency and reduces nutrient waste in the environment. Since AA are absorbed in the small intestine and affected by hindgut fermentation, ileal digestibility is a key measure for accurately assessing AA bioavailability. A widely utilized technique for this assessment is the partial collection method using an indigestible index compound such as chromic oxide and titanium dioxide (Kong and

Adeola, 2014). For swine, the preferred method of ileal digesta collection involves a simple T-cannula insertion, which is less invasive and avoids surgical interventions on the lower digestive tract (Stein et al., 2007). The AA at the terminal ileum include both dietary and endogenous sources, such as shedded epithelial cells and secretions from mucin and digestive enzymes (Nyachoti et al., 1997). Endogenous losses at the terminal ileum, comprising basal and specific losses, are significant. Basal endogenous losses are related to dry matter intake (DMI) but independent of feed type, while specific losses are induced by certain feed components like fiber and antinutritional factors (Stein et al., 2007). Apparent ileal digestibility (AID) tends to underestimate true nutrient digestibility as it does not consider endogenous nutrient losses. Consequently, standardized ileal digestibility (SID), which corrects AID for basal endogenous AA losses, is a more accurate measure for feed formulation. The SID values are more additive in mixed diets for swine and poultry than AID values, making them more suitable for diet formulation (Stein et al., 2005; Kong and Adeola, 2013).

Few studies have measured the CP and AA digestibility of hybrid rye in pigs. According to NRC (2012), the SID of CP in old cultivars of rye is 83%. However, the average SID of CP in three different genotypes of hybrid rye is about 77%, implying that the digestibility of CP in old cultivars of rye might be greater than that in hybrid rye (Table 2.5). Strang et al. (2016) have reported that the average SID of CP in eight different genotypes of hybrid rye is approximately 73%. The SID of lysine, methionine, tryptophan, and threonine in hybrid rye is about 63, 78, 76, and 66%, respectively, which is lower than that of corn and wheat (NRC, 2012). The lower AA digestibility in hybrid rye might be explained by the fact that it contains more NSP, which encapsulates more proteins in the matrix so that they cannot be digested by endogenous enzymes in pigs, and that more fiber content in rye may increase the endogenous losses of AA (Rosenfelder et al., 2013).

Table 2.5 Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in hybrid rye[1]

SID, %	Hybrid rye
CP	77.10
Indispensable AA	
Arginine	81.57
Histidine	75.53
Isoleucine	74.10
Leucine	74.83
Lysine	63.47
Methionine	78.03
Phenylalanine	78.27
Threonine	65.80
Tryptophan	75.77
Valine	70.77
Total	73.77
Dispensable AA	
Alanine	67.60
Aspartic Acid	69.60
Cysteine	75.17
Glutamic Acid	86.20
Glycine	73.27
Proline	75.73
Serine	73.73
Total	89.37

[1]Adapted from McGhee and Stein (2018).

2.5 MINERALS SOURCE

Phytic acid, known as myo-inositol-1,2,3,4,5,6-hexakisphosphate, is characterized by its structure consisting of six phosphate groups esterified to the hydroxyl groups of the cyclic alcohol myo-inositol (Pallauf and Rimbach, 1997). This compound is predominantly found in plant-based ingredients and is crucial in the diet of monogastric animals like pigs and poultry, accounting for 60 to 90% of the phosphorus (P) in these feedstuffs (Wu et al., 2009). However, due to very low phytase activity in their digestive systems, monogastric animals are inefficient at utilizing phytate-bound P. This inefficiency leads to the excretion of unabsorbed phytic acid-bound P, contributing to environmental pollution, particularly eutrophication in regions with dense animal farming (Kleinman et al., 2002). Additionally, the inability of pigs and poultry to digest phytic acid-bound P necessitates the use of costly inorganic P supplements in their diets, which significantly increase the feed costs (Létourneau-Montminy et al., 2011). Moreover, phytic acid can reduce nutrient digestibility and increase the endogenous secretion of nutrients because it is negatively charged at all pH conditions in the gastrointestinal system and binds positively charged molecules in diets and endogenous gastrointestinal tract secretions (Dersjant-Li et al., 2015). Numerous studies have reported the negative effects of phytic acid on nutrient digestibility and growth performance in swine and poultry (Liu et al., 2009; Onyango and Adeola, 2009; Woyengo et al., 2012; Zouaoui et al., 2018).

Table 2.6 illustrates that hybrid rye has a P concentration of 0.26%, which is more than that in corn but less than that of wheat. In these grains, most of the P is in the form of phytate. For instance, in hybrid rye, approximately 70% of the total P is bound to phytate. Hybrid rye, however, stands out as it contains considerably higher levels of intrinsic phytase (2,650 FTU/kg) compared to wheat (580 FTU/kg) and corn (less than 70 FTU/kg). Rodehutscord et al. (2016) also observed even higher phytase activities in hybrid rye, suggesting variability in phytase activity across different hybrid rye strains. Regarding calcium (Ca) content, it is generally low in cereal grains. Hybrid rye and wheat have similar Ca levels, both higher than that found in corn. Additionally, hybrid rye surpasses corn in terms of other macro minerals like potassium (K), magnesium (Mg), and sulfur (S). For micro minerals, hybrid rye's contents of copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are lower than those in wheat but exceed the levels found in corn.

Table 2.6 Mineral and phytate composition of hybrid rye, wheat, and corn (as-fed basis)[1]

Item	Hybrid rye	Corn	Wheat
Macro minerals, %			
Ca	0.03	0.01	0.03
K	0.44	0.36	0.43
Mg	0.10	0.08	0.13
Total P	0.26	0.23	0.36
Phytate	0.68	0.66	0.99
Phytate-bound P	0.19	0.19	0.28
Non-phytate P	0.07	0.04	0.08
Phytase, FTU/kg	2,650	<70	580
S	0.11	0.09	0.14
Micro minerals, mg/kg			
Cu	0.48	<0.10	1.12
Fe	28.20	17.30	37.80
Mn	16.90	3.64	37.70
Zn	23.30	18.20	27.80

[1]Adapted from McGhee and Stein (2019) and Archs Toledo et al. (2020)

The evaluation of P digestibility is commonly done through apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) metrics (Almeida and Stein, 2010). The ATTD for P is determined by analyzing the P present in both the feed and the feces. However, this method tends to give a lower estimate of true P digestibility since the P in feces comes from both the diet and the animal's body. One of the challenges with ATTD is the potential non-additivity of these values in mixed diets, leading to the use of standardized digestibility that accounts for the animal's endogenous basal P loss (González-Vega et al., 2015). To refine ATTD values found in existing literature, the NRC (2012) applied a correction using a basal endogenous P loss rate of 190 mg/kg DMI, thereby deriving the STTD values for different feed components.

Hybrid rye and conventional rye both have high levels of phytase. The average phytase content is 2,650 FTU/kg in hybrid rye and 7,716 FTU/kg in conventional rye (Slominski, 2007;

McGhee and Stein, 2019; Archs Toledo et al., 2020). As shown in Table 2.7, the ATTD of P and STTD of P in hybrid rye were 46.1 and 53%, respectively. These percentages are higher than those found in barley, corn, sorghum, and wheat. Additionally, conventional rye showed a STTD of P at 50%, surpassing the values in corn, barley, and sorghum (NRC, 2012). The increased digestibility of P in rye grain may be attributed to its significantly higher intrinsic phytase activity compared to other cereals, which is thought to improve P digestibility (Rodehutscord et al., 2016).

Table 2.7 Apparent (ATTD) and standardized total tract digestibility (STTD) of phosphorus (P) in hybrid rye, wheat, barley, corn, and sorghum

Item	Hybrid rye	Barley	Corn	Sorghum	Wheat
ATTD of P, %	46.1	37.0	16.3	9.5	31.0
STTD of P, %	53.0	44.6	24.9	17.0	36.6

[1]Adapted from McGhee and Stein (2019) and Archs Toledo et al. (2020)

2.6 ENERGY SOURCE

Feed costs constitute the largest portion of total expenses in swine production, with energy being the most expensive component of a pig's diet (Noblet and van Milgen, 2013). Energy, essential yet not a direct nutrient, plays a critical role in pig diets for maintaining basic body functions, growth, reproduction, physical activity, immune function, and fat deposition, ensuring overall health and productivity (Kil et al., 2013). Additionally, the energy content of the diet significantly influences pigs' voluntary feed intake, as they tend to eat until their energy needs are met (Nyachoti et al., 2004). Therefore, comprehending the energy levels in pig diets is crucial for forecasting their feed consumption and ensuring their optimal health and productivity.

Not all the gross energy (GE) consumed by pigs is retained, as energy losses occur through feces, urine, gas, and heat. There are various energy systems to account for these losses during the process of energy utilization: digestible energy (DE), metabolizable energy (ME), and net energy (NE) (Velayudhan et al., 2015). The GE represents the total energy in feed ingredients, determined by the complete combustion of organic materials using bomb calorimetry, but it is not typically used in diet formulation due to its lack of information on energy availability for pigs. The DE is the GE minus fecal energy losses, accounting for the pig's digestion process, but not considering energy from gas and heat produced in the hindgut. The ME is DE minus the energy lost in urine and gas, though losses from gas (mostly methane) are often omitted in ME calculations due to their minimal amount in piglets and growing pigs and hard to measure (Le Goff and Noblet, 2001). The NE, defined as ME minus the heat increment (HI) from various physiological processes, is seen as the most accurate system for assessing available energy, since it includes all forms of energy that are not usable by pigs (Heo et al., 2014). Additionally, DE and ME may underestimate energy values in high-lipid and starch ingredients and overestimate for high-protein and fiber ingredients due to varying amounts of HI associated with these ingredients (Noblet and van Milgen, 2004). Therefore, compared with DE and ME, NE is a more precise measure for determining energy availability for pigs.

Table 2.8 shows that the DE, ME, and estimated NE values for hybrid rye are 3,633, 3,479, and 2,730 kcal/kg on a DM basis, respectively. These values are comparable to those of older rye cultivars, differing by less than 100 kcal/kg (NRC 2012). However, there is no published information on the NE value of hybrid rye for pigs. The ME value of hybrid rye is similar to that of barley, but it is lower than that found in corn and wheat (McGhee and Stein, 2020).

Table 2.8 Energy contents of hybrid rye, barley, corn, and wheat

Item	Hybrid rye	Barley	Corn	Wheat
DE, kcal/kg	3,277	3,208	3,384	3,416
DE, kcal/kg DM	3,633	3,464	3,858	3,796
ME, kcal/kg	3,139	3,095	3,274	3,276
ME, kcal/kg DM	3,479	3,342	3,732	3,641
NE, kcal/kg DM[2]	2,730	2,563	2,976	2,875

[1]Adapted from McGhee and Stein (2020)
[2]The NE in each ingredient was calculated using the following prediction equation: NE = (0.700 × DE) + (1.61 × EE) + (0.48 × starch) − (0.91 × CP) − (0.87 × ADF)

2.7 MEANS TO IMPROVE THE NUTRTIONAL VALUE OF HYBRID RYE FOR SWINE: EXOGENOUS ENZYMES

Cereal grains contain a high concentration of NSP, primarily arabinoxylan, along with cellulose, glucans, and other polysaccharides. However, NSP are poorly hydrolyzed by pigs as they lack specific endogenous enzymes for their degradation (Jha and Berrocoso, 2015). Exogenous enzymes such as carbohydrases, phytases, and multi-enzyme preparations, are extensively utilized as feed additives in diets for monogastric animals. The use of exogenous enzymes is crucial because many cereal grains, such as rye and sorghum, contain substantial amounts of antinutritional factors that greatly reduce their feed efficiency and restrict their use in animal nutrition. By incorporating these specific enzymes, it becomes possible to significantly improve the digestibility and nutritional value of these cereal grains, making them more suitable and beneficial for non-ruminant animal consumption.

2.7.1 Carbohydrases

Carbohydrases, which are a group of enzymes, play a significant role in breaking down complex carbohydrates into simpler sugars, thus optimizing the utilization of NSP such as arabinoxylans and β-glucans in pig feed. These enzymes, such as xylanase, glucanase, cellulase, mannanases, pectinase, galactanase, and amylase, target the hydrolysis of plant cell wall components such as xylan, cellulose, and β-glucan (Aranda-Aguirre et al., 2021). The use of carbohydrases in swine nutrition offers multiple advantages: they hydrolyze specific chemical bonds not adequately broken down by the animal's endogenous enzymes, increase the availability of starches, AA, and minerals by breaking down cell walls, reduce antinutritional factors like soluble NSP, facilitate the breakdown of insoluble NSP for improved hindgut fermentation thereby increasing energy absorption, and supplement essential enzymes in young animals with inadequate endogenous enzyme production (Kiarie et al., 2013). By hydrolyzing NSP, carbohydrases improve the digestibility of nutrients, making them more accessible to the gut microflora, which in turn benefits the animal's gastrointestinal health (De Lange et al., 2010). The application of NSP-degrading enzymes is especially beneficial in high-fiber diets for swine, as it improves efficient production due to the typically low fiber digestibility in fiber-rich ingredients (Ferrandis Vila et al., 2018). Furthermore, these enzymes are exceptionally valuable for young pigs, who may have limited digestive capacity and are adversely affected by high fiber levels, thus aiding in better growth and development (Hogberg and Lindberg, 2006).

Xylanase, a carbohydrase enzyme, plays a crucial role in enhancing pig diets at various production stages. Xylanase for commercial use in animal feed are primarily produced from bacteria such as *Bacillus* spp. and filamentous fungi such as *Aspergillus* and *Trichoderma* spp. (Paloheimo et al., 2010; Bhardwaj et al., 2019). Xylanase targets arabinoxylan, a primary NSP in cereal grains such as rye commonly found in animal diets. Xylanase not only enhances pigs' growth, intestinal health, and nutrient absorption but also aids in breaking down xylan into shorter molecules and dismantling the cell wall matrix. This action effectively reduces digesta viscosity, thereby releasing trapped nutrients and improving their digestibility by making them more accessible to digestive enzymes within a short transit time (Moita et al., 2022). Apart from countering the negative effects of arabinoxylan, xylanase benefits pig diets by promoting gut health (Duarte et al., 2019; Petry et al., 2020). This is achieved through the generation of more fermentable compounds from NSP, including xylooligosaccharides. Such compounds play a crucial role in improving both the diversity and the population of beneficial gut bacteria in the intestinal microbiota. For example, Tiwari et al. (2018) reported that xylanases supplementation increased the levels of acetate, propionate, and total volatile fatty acids (VFA) in vitro, while also improving the integrity of the intestinal barrier in vivo in pigs.

β-Glucanase, along with other carbohydrases like cellulase is also commonly incorporated into pig diets, which may have potential to enhance the nutritional value of hybrid rye for pigs. β-glucanase specifically targets β-glucans, a type of soluble NSP located in the cell walls of cereals such as barley, oats, and rye. By breaking down β-glucans, β-glucanase reduces the viscosity of the gastrointestinal contents in pigs, which improves the movement and mixing of feed in the gut (Lazaridou et al., 2007). This enzymatic action releases trapped nutrients, enhancing their availability and absorption, crucial for efficient nutrient utilization and overall growth in pigs. Furthermore, the decomposition of β-glucans by β-glucanase enhances gut health, as it reduces viscosity, minimizes the risk of digestive issues, and supports a healthier gut microbiome (Duarte et al., 2021). In pig production, β-glucanase is typically employed in conjunction with xylanase to address the complex nature of NSP in plant-based feeds (Tsai et al., 2017).

Cellulose, a prevalent plant polysaccharide, consists of glucose units linked by β-1,4-glycosidic bonds in a linear structure present in plant cell walls. It is a primary component of dietary fibers but can also have negative effects on monogastric animals used in food production due to its antinutritional properties. Cellulases, which include endoglucanases, exoglucanases, and β-glucosidase, are enzymes that break down cellulose and aid in its digestion by animals (Bhat et al., 2001). Commercial cellulases for monogastric species are typically derived from bacteria like *Bacillus subtilis* and fungi such as *Trichoderma* spp (Sadhu and Maiti, 2013). When added to pig diets, cellulase supplements, often part of enzyme blends, improve the digestibility of insoluble fibers, leading to improved utilization of energy and nutrients (Torres-Pitarch et al., 2019). Additionally, cellulase supplementation can regulate the population of intestinal microbes through the fermentation of cellulose breakdown products in the large intestine. This, in turn, positively influences the gut microbiota and may contribute to changes in gut barrier integrity and the immune response in host animals, with the production of VFA like acetate and butyrate playing a role (Zhang et al., 2017).

2.7.2 Phytases

Phytic acid, also known as phytate, is the primary form in which P is stored in cereal grains and oilseeds. The terms phytic acid and phytate are often used interchangeably in scientific literature, referring to the free acid form and its salt form, respectively (Cowieson et al., 2016). Monogastric animals, such as pigs and poultry, lack the necessary enzymes to break down phytate, rendering P in this form largely inaccessible to them. Historically, to fulfill the animals' P needs, diets had to be supplemented with inorganic P sources. This approach, however, led to diets high in indigestible P, resulting in substantial excretion of P in animal manure. Additionally, phytic acid acts as an antinutrient, hindering the absorption of various multivalent minerals and trace elements in monogastric animals (Cheryan and Rackis, 1980).

Phytase, a specific type of phosphomonoesterases, is crucial for gradually dephosphorylating phytate, the most widespread inositol phosphate in nature. Phytase is extensively utilized in animal feed supplements to enhance the digestion and uptake of nutrients that are typically hard to access in animal diets (Menezes-Blackburn et al., 2015). Numerous studies have demonstrated that adding microbial phytase to animal feeds significantly improved the utilization of P from phytate, thus reducing the need to supplement the feed with inorganic P (Augspurger et al., 2003; Zeng et al., 2016; Dersjant-Li et al., 2017). There are numerous variants of phytase, each with differing degrees of effectiveness. For example, phytase derived from *E. coli* bacteria has been shown to be more efficient in releasing P per unit of phytate than those derived from fungi (Adeola et al., 2004). Phytase supplementation has been proven to enhance growth performance in pigs at all stages of development, including weaning, growing, and finishing (Aranda-Aguirre et al., 2021). The inclusion of phytase in pig diets can improve the bioavailability of other minerals such as Ca, Zn, and Cu (Madrid et al., 2013). This improvement is due to phytase's ability to break down phytate, a substance in pig feed that normally binds to these minerals and limits their absorption. By reducing the binding effect of phytate, phytase makes these minerals more available, thereby enhancing their uptake in the digestive system of pigs.

In cereal grains, the highest intrinsic phytase activity is found in rye, followed by triticale, wheat, and barley (Slominski, 2007; Rodehutscord et al., 2016). Importantly, intrinsic phytase in plants is sensitive to high temperatures, so feeds processed at temperatures exceeding 65°C, like in pelleting or extrusion, generally lose this natural enzyme activity (Slominski, 2007; Dersjant-Li et al., 2015). However, this intrinsic phytase remains functional in the animal's digestive system when they consume unprocessed grains, thereby enhancing P digestibility. For example, it is reported that the intrinsic phytase in hybrid rye improved the ATTD and STTD of P in corn and soybean meal (Archs Toledo et al., 2020).

2.7.3 Multi-enzyme

Multi-enzyme supplements are a blend of various enzymes, including carbohydrases (like cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, and amylase), protease, phytase, and lipase, specifically formulated for animals. These supplements are superior to single enzyme supplements because they more efficiently break down cell walls and dissolve NSP due to their complex components (Juanpere et al., 2005; Meng et al., 2005; Olukosi et al., 2007). Nevertheless, understanding how enzymes synergistically work to break down their respective substrates and the mechanism of their combined action in animal diets is essential for maximizing productivity, especially when tailoring multi-enzyme blends for various cereal grains, given that the NSP profiles of these grains can vary (Adeola and Cowieson, 2011; Slominski, 2011).

Multi-carbohydrases (MC) can overcome the nutrient-binding effects of cell walls and reduce viscosity issues associated with certain NSP, such as arabinoxylans and β-glucans. This enhancement leads to better nutrient absorption and energy utilization in pigs, as confirmed by numerous studies. For example, Ayoade et al. (2012) discovered that a combination of enzymes (including pectinase, cellulase, mannanase, xylanase, glucanase, and galactanase) improved the average SID for indispensable and total AA increased by 3.4 and 3.8%, respectively, in pigs fed extruded full-fat soybeans. Zhang et al. (2020) found that carbohydrase mixture (including cellulase, xylanase, β-mannanase, α-galactosidase, β-glucanase, and pectinase) improved the ATTD of nutrients such as DM, CP and ash and energy digestibility in growing pigs fed the corn-based diet or wheat-based diet. Velayudhan et al. (2015) observed that multi-enzyme mixture (including cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, amylase, protease) improved the DE, ME, and NE content of the dry extruded-expelled soybean meal fed to growing pigs and the enzyme mixture at 0.05% of the diet improved NE values of DESBM by 4.9%. Huang et al. (2021) reported that adding a combination of MC (xylanase, β -glucanase, α-arabinofuranosidase) and phytase to a corn-wheat-soybean meal-based diet improved body weight gain, feed intake, feed efficiency, and the ATTD of nutrients such as CP, crude fat, ash, P, and Ca. Similarly, Omogbenigun et al. (2004) reported an increased ADG and feed efficiency in weaned pigs fed a diet enriched with a multi-enzyme blend, including corn-soybean meal with canola meal and wheat. Nonetheless, certain studies have shown that multi-enzyme did not markedly impact the growth and nutrient utilization in pigs. For instance, Kiarie et al. (2020) reported that a multi-enzyme supplement did not impact the SID of AA in roasted full-fat soybean and expelled-extruded SBM. The effectiveness of exogenous enzymes in enhancing nutrient digestibility depends on their activity and the availability of substrates (Emiola et al., 2009). Diets with high levels of NSP are more responsive to carbohydrase supplementation, whereas diets already high in nutritional value show less response to these enzymes (Adeola and Cowieson, 2011). Additionally, the efficacy of MC in pigs may vary based on the dosage used. Woyengo et al. (2018) reported that while a low dose (0.5 g/kg) of a multi-enzyme blend (xylanase, glucanase, cellulose, mannanase, invertase, protease, and amylase) did not affect the in vitro digestibility of DM in cold-pressed camelina cake, a higher dose (50 g/kg) significantly improved it, suggesting a dosage-dependent effect.

Multi-enzyme supplements also provide a significant benefit by converting cell wall polysaccharides into oligosaccharides or monosaccharides. These smaller molecules, produced through NSP hydrolysis, can be directly absorbed or further broken down by intestinal microflora into VFA, thereby enhancing hindgut fermentation and contributing to the development and health of the animal's digestive system (Kiarie et al., 2013). For instance, Kiarie et al. (2007) observed that supplementation with MC (including pectinase, cellulase, mannanase, xylanase, glucanase, and galactanase) led to increased lactate and lactobacilli levels in weaned pigs fed flaxseed-containing diets. Additionally, diets high in NSP may negatively affect gut health by altering digestive surface morphology. Koo et al. (2017) reported that dietary supplementation with multi-enzyme (cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, amylase, and protease) could improve villus height in the ileum and the villus height to crypt depth ratio in the duodenum. Furthermore, soluble NSP can lead to increased digesta viscosity, which may impede the absorption of nutrients and potentially encourage the growth of pathogenic bacteria. A more viscous digesta slows down the diffusion process, leading to the buildup of particulate matter that facilitates microbial adhesion. Such conditions can prolong the presence of microorganisms and enhance the proliferation of harmful bacteria. The use of MC can decrease the viscosity of digesta by breaking down soluble NSP either partially or completely, which can affect the composition and metabolic potential of bacterial populations (Kiarie et al., 2013).

2.8 MEANS TO IMPROVE THE NUTRTIONAL VALUE OF HYBRID RYE FOR SWINE: FEED PROCESSING

Feeding expenses constitute a substantial portion of the costs of raising pigs and poultry. To optimize nutrient utilization in feed ingredients and mitigate the effects of rising feed costs, several processing techniques are utilized. Common methods encompass mechanical processing like grinding, and hydrothermal processes, which include extrusion and pelleting (Rojas and Stein, 2017).

2.8.1 Grinding

Cereal grains are typically not ground before entering a feed mill, necessitating grinding to reduce their particle size. This process involves various types of mills, with the most common being hammer mills and roller mills (Rojas and Stein, 2015). Hammer mills have a series of hammers attached to a central shaft, enclosed in a metal case. The particle size is reduced through impact and attrition between the fast-moving hammers and the slower particles (Lyu et al., 2020). The mill has a screen that allows only the right-sized materials to pass. Roller mills, on the other hand, have parallel, cylindrical rollers rotating in opposite directions, crushing the feed between them. The choice of grinder depends on the desired particle size, grinding capacity, electric efficiency, and the type of ingredient (Hancock and Behnke, 2001). Hammer mills are simpler and cheaper to install than roller mills, but roller mills are more energy-efficient and provide a more consistent grind, although they are more complex and need more oversight. Recently, multi-stage grinding, which combines both mill types, has been introduced (Rojas and Stein, 2017). This approach aims to maximize efficiency and uniformity in particle size while minimizing electricity use. To determine the particle size of ground feed ingredients, the American Society of Agricultural Engineers has established a method (ASABE, 1995). This involves measuring the particle size distribution and calculating the average particle size. For this, 100 g of the feedstuff is placed on a series of sieves arranged from largest to smallest (U.S. sieve sizes #4 through #270, ending with a solid metal pan). These sieves are then shaken in a vibratory sieve shaker for 10 minutes. The quantity of feedstuff on each sieve is weighed to determine the particle size distribution and average size.

Reducing the particle size of cereal grains significantly affects their energy and nutrient digestibility in pigs. Smaller particle sizes increase the surface area exposed to digestive enzymes in the pig's gastrointestinal tract, thereby improving the breakdown and absorption of energy and nutrients. For instance, Huang et al. (2015) reported that finely ground corn increased the digestibility of GE and EE in weaned piglets. Montoya and Leterme (2011) found that reducing the particle size of field peas from 1,035 to 156 μm significantly increased the DE content from

14.0 to 16.1 MJ/kg DM. De Jong et al. (2016) demonstrated that finely grinding hard winter wheat with a hammer mill from 728 to 326μm enhanced the ATTD of GE in growing-finishing pigs. Owsley et al. (1981) observed that a finely ground sorghum diet improved the AID of starch and GE, as well as the DE and ME content, compared to medium and coarsely ground sorghum diets. The observed increase in DE and ME in these studies, corresponding to the reduction of particle size in feeds, is probably mainly due to enhanced ileal digestibility of starch. Additionally, particle size reduction can also aid in releasing nutrients from high-fiber diets by breaking down the fiber matrix. For example, Acosta et al. (2020) observed that decreasing the particle size of corn distiller's dried grains with solubles (DDGS) using a hammer mill or roller mill led to increased GE digestibility in growing pigs.

The reduction of particle size can enhance the digestibility of N and AA in cereal grains. Mavromichalis et al. (2000) found that the ATTD of DM and CP improved in growing pigs when wheat particle size decreased from 1,300 to 600 μm, and from 600 to 400 μm. Wondra et al. (1995) observed a 5% and 7% increase in the ATTD of DM and CP, respectively, when corn particle size was reduced from 1,200 to 400 μm in pigs. Acosta et al. (2019) reported that growing pigs showed greater ATTD of DM and CP with corn ground from 700 to 300 μm using a roller mill. Kim et al. (2009) showed that ATTD and AID of CP and SID of AA increased linearly in pigs when lupin particle size was reduced from 1,304 to 567 μm. Oryschak et al. (2002) found that reducing the particle size of a barley-field pea diet from 850 and 700 μm to 400 μm decreased total N excretion by 4 and 7%, respectively, in pigs. However, the impact of particle size on the digestibility of N and AA is not always consistent. Rojas and Stein (2015) reported no effect on the SID of CP or indispensable AA, except for tryptophan, when particle size was reduced from 865 to 339 μm. Medel et al. (2000) found that reducing barley particle size did not affect the ATTD of CP in weaned pigs. Regarding the digestibility of minerals, results vary as well. Oryschak et al. (2002) reported that reducing particle size of a barley-field pea diet from 700 to 400 µm decreased total P excretion by 12% and increased P digestibility by 15%. Conversely, Liu et al. (2012) found that reducing the particle size of DDGS from 818 to 308 µm did not impact the ATTD of P.

Reducing the size of feed particles reduces the nutrient encapsulating effects of cell walls and increases the surface area, enhancing enzyme interaction and potentially improving nutrient absorption and overall performance in pigs. For instance, Huang et al. (2015) observed that weaned piglets fed finely ground corn showed greater ADG, ADFI, and gain to feed ratio (G:F) than those fed coarsely ground corn. Paulk et al. (2015) found that reducing sorghum particles from 724 to 319 µm improved G:F in pigs, and Mavromichalis et al. (2000) reported similar improvements in G:F when wheat particle size was reduced from 1,300 to 600 µm and from 600 to 400 µm. De Jong et al. (2016) also noted that decreasing wheat particle size from 728 to 326 µm led to a linear improvement in G:F. However, the relationship between particle size and growth performance is not universally consistent. Morel and Cottam (2007) found that reducing barley particle size did not impact ADG and G:F in growing pigs. Likewise, Lawrence et al. (2003) reported that reducing the particle size of extruded expelled soybean meal from 965 to 639 µm or solvent-extracted soybean meal from 1,226 to 444 µm did not affect ADG, ADFI, or G:F. These findings indicate that particle size reduction's effectiveness might depend on the type of grain and its proportion in the diet.

Gastric ulcers are a prevalent issue in pig farming, especially severe ulcers in the stomach's esophageal area, which can lead to decreased productivity and even sudden deaths due to hemorrhages (Friendship, 2004). The esophageal region is especially vulnerable when pigs are fed with very finely ground ingredients, as this increases acidity and pepsin activity because of less protective mucus in that area. A primary cause of ulcer development is the fine grinding of feed (Maxwell et al., 1970). For instance, Wondra et al. (1995) reported a higher ulcer incidence in pigs fed corn ground to 400 μm compared to those fed corn ground to 1,200 μm. Moreover, Jo et al. (2021) found a tendency for increased esophageal keratinization in pigs when the dietary particle size was reduced from 900 to 600 μm. Nevertheless, the impact of particle size on ulcer formation is not consistently observed. Ball et al. (2015) reported no significant impact of particle size on stomach ulceration in pigs. Also, there are additional factors such as pelleted feed, starvation, as well as intensive farming practices and housing conditions, that play a role in increasing ulcer risks in pigs (Gottardo et al., 2017; Rojas and Stein, 2017).

While fine feed particles can enhance energy and nutrient digestibility in pigs, coarser particles might be more beneficial for gut health and microbiota diversity. The size of feed particles affects not only the stomach's environment but also other parts of the gastrointestinal tract, particularly the large intestine. Coarser feed particles are known to influence the presence of enteric pathogens by promoting beneficial bacteria that produce SCFA, which can improve gut health and inhibit the growth of harmful bacteria like *Salmonella* spp. and *E. coli* (Kiarie and Mills, 2019). Diets consisting of larger particle sizes are linked with higher concentrations of VFA such as acetic, propionic, and butyric acids in the cecum and colon, substances known for their effectiveness in suppressing harmful bacteria (Hedemann et al., 2005). The reason might be that larger particles allow more starch to reach the large intestine, leading to increased SCFA production, which in turn helps in controlling the growth of detrimental bacteria. Mikkelsen et al. (2004) observed that pigs consuming coarser diets had higher levels of butyric acid in the cecum and colon than those on finer diets, along with significantly fewer coliform bacteria in the distal small intestine, cecum, and mid colon. Additionally, pigs on a coarse, non-pelleted diet exhibited an increased in vitro death rate of *Salmonella enterica* serovar Typhimurium DT12 in stomach content and lower coliform bacteria counts in the distal small intestine, cecum, and mid colon. Kim et al. (2009) found that increasing lupin particle size from 567 to 1,304 µm led to higher levels of acetic, propionic, and butyric acids in the gastrointestinal tract. Regina et al. (1999) noted higher acetate and L-lactate levels in the proximal stomach of pigs on coarsely ground meals compared to those on finely ground meals. Additionally, Rojas et al. (2016) observed that increasing feed particle size from 339 to 865 µm resulted in increased acetate, propionate, and butyrate concentrations in the cecal contents, from 1,846 to 2,537, 617 to 872, and 226 to 702 µg/mL respectively, and a decrease in pH in the cecal and colon contents from 6.64 to 6.04 and from 6.25 to 5.85, respectively.

2.8.2 Extrusion

Extrusion is a thermal and mechanical process commonly used in the feed industry that involves pressing feed materials through a barrel using a single or twin-screw extruder (Lancheros et al., 2020). This technique combines continuous cooking under conditions of pressure, moisture, and high temperature, and it employs shear force and can be applied to both individual feed ingredients and complete feeds. The process significantly improves starch gelatinization, which aids in its breakdown by α-amylase, and it also mechanically breaks down cell walls, enhancing the efficiency of digestive enzymes in accessing various nutrients (Camire et al., 1990). Therefore, extrusion is a key method for increasing the digestibility of nutrients, especially in cereal grains. Beyond nutritional benefits, extrusion helps in improving the storage life of feeds, prevents the segregation of ingredients, and inactivates antinutritional factors present in feed components (Traylor et al., 1999).

Stein and Bohlke (2007) observed that the AID and SID for CP and most AA, except for proline, improved with higher extrusion temperatures. They noted an increase in AID for starch from 89.8 to 95.9%, and for energy from 71.5 to 79.0%, as well as an increase in the ATTD of energy from 89 to 93.3% in pea-containing diets. Rodriguez et al. (2020) found that extruded grains showed a higher AID of starch compared to non-extruded grains, along with an increased SID of CP and most AA except for lysine and proline in corn. They also observed enhanced ATTD of energy and the DE and ME in extruded corn and sorghum. Zhang et al. (2022) reported an increase in the DE and ME values and the ATTD of GE and nutrients in diets with extruded corn DDGS and full-fat rice bran. Rojas et al. (2016) reported that extrusion at 115°C improved the AID of starch and most indispensable AA, as well as the ME in diets with medium and high fiber content. However, the effects of extrusion on cereal grains are not always consistent. Rodriguez et al. (2020) noted that wheat extrusion did not influence the ATTD of GE, DE, and ME, and extrusion did not impact the SID of CP and AA in wheat and sorghum. Muley et al. (2007) reported that corn extrusion at 82.8°C did not improve the digestibility of AA.

2.8.3 Pelleting

In modern practices of pig farming, diets are not usually in their original mash form. Instead, after mixing the ingredients, they are typically turned into pellets. Pelleting is a hydrothermal process that uses steam, heat, and pressure. The ingredients, once steamed, are pressed through dies to form the pellets (Lahaye et al., 2008). The objectives of pelleting include reducing ingredient segregation, increasing the bulk density, enhancing palatability, decreasing dust, minimizing feed waste, improving handling and transport, and altering the structures of starch and protein (Chae and Han, 1998). However, the high temperatures in the pelleting process can cause protein heat damage due to the Maillard reaction (González-Vega et al., 2011). The Maillard reaction is a spontaneous, enzyme-free process where reducing sugars' carbonyl groups react with the free amino groups in amino acids like lysine, forming Maillard reaction products with diverse sensory qualities.

Pelleting of cereal grains has been shown to improve their digestibility in the small intestine. This enhancement is primarily due to the gelatinization of starch that occurs during the pelleting process, leading to increased energy digestibility within the grains. Furthermore, this process contributes to improved digestibility of protein and AA, as it involves protein denaturation during the processing stage (Lancheros et al., 2020). Rojas et al. (2016) observed that pelleting at 85°C enhanced the AID of starch and most indispensable AA and increased the digestibility of GE. The ME in pelleted diets was found to be higher than in meal diets, particularly for low- and medium-fiber diets. Le Gall et al. (2009) reported that pelleting increased energy digestibility from 76.6 to 78.1%, NDF digestibility from 58.5 to 63.7%, and EE digestibility from 36 to 61%. This process also improved the DE and ME by 0.3 MJ/kg of DM in growing pigs. Chassé et al. (2022) observed that pelleting enhanced the AID of DM, CP, and energy by 17, 27, and 17%, respectively, in diets consisting of corn and soybean meal for growing pigs, along with a 63% increase in the AID of NSP. Pelleting is also known to improve pig performance, potentially due to decreased feed wastage, enhanced nutrient digestibility, and improved feed palatability (Skoch et al., 1983). Wondra et al. (1995) discovered that pelleted diets resulted in a 5% increase in ADG and a 7% increase in the G:F. Nemechek et al. (2016) reported that pigs on pelleted diets experienced higher ADG and hot carcass weight compared to those on meal diets.

CHAPTER THREE: HYPOTHESES AND OBJECTIVES

The studies in this dissertation tested the following hypotheses:

1. The energy contents of hybrid rye would be improved by fine grinding.

2. Reduced particle size and dietary multi-carbohydrase (MC) supplementation would increase the standardized ileal digestibility (SID) of amino acids (AA) in hybrid rye fed to growing pigs.

3. The phosphorus (P) digestibility of hybrid rye would be improved by the increasing level of phytase supplementation and reduced particle size.

4. Multi-carbohydrase supplementation would improve growth performance, nutrient digestibility, short-chain fatty acids (SCFA) production, and gut health of weaning pigs

The overall objective was to optimize the utilization of hybrid rye in swine diets through the application of feed processing techniques and enzyme supplementation.

The specific objectives were:

1. To determine the digestible energy (DE), metabolizable energy (ME), and net energy (NE) of hybrid rye and to investigate the effects of particle size and variety on energy content of hybrid rye.

2. To determine the effects of hybrid rye particle size and MC supplementation on the apparent ileal digestibility (AID) and SID of AA in hybrid rye.

3. To determine the individual and interactive effects of hybrid rye particle size and phytase levels (0, 500, and 2,500 FTU/kg) on the apparent total tract digestibility (ATTD) and the standardized total tract digestibility (STTD) of P in hybrid rye fed to growing pigs.

4. To investigate the effects of MC supplementation on growth performance, nutrient digestibility, SCFA production, and gut health of weaning pigs fed the wheat-based or hybrid rye-based diets.

CHAPTER FOUR: MANUSCRIPT I

Net energy content of hybrid rye fed to growing pigs and effects of particle size on energy values

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4.1 ABSTRACT

An experiment was conducted to determine the digestible energy (DE), metabolizable energy (ME), and net energy (NE) contents of hybrid rye fed to growing pigs using indirect calorimetry and to investigate the effects of particle size on energy values. Thirty growing barrows (18.06 ± 0.83 kg initial body weight) were individually housed in metabolism crates and randomly allotted to one of five dietary treatments to give six replicates per treatment. Two hybrid rye varieties (Bono and Gatano) were ground in a hammermill mounted with a 4.0-mm or 3.2-mm screen to obtain coarse and fine particle size, respectively. Dietary treatments included a corn-soybean meal basal diet and four additional diets containing 300 g/kg of the test ingredients in a 2×2 factorial based on hybrid rye variety (Bono or Gatano) and particle size (Coarse or Fine). Pigs were fed experimental diets for 15 days, including 10 days for adaptation and 5 days for total fecal and urine collection to determine the DE and ME of the experimental diets. Pigs were then moved into indirect calorimetry chambers to determine 24-h heat production and 12-h fasting heat production. All data were analyzed using the MIXED procedure of SAS with the individual pig as the experimental unit. The geometric mean diameter (D_{gw}) for coarsely versus finely ground Bono and Gatano were 1,111 versus 594 µm and 1,210 versus 717 µm, respectively. The apparent total tract digestibility (ATTD) of dry matter (DM) and gross energy (GE) were greater ($P < 0.05$) in diets containing finely than coarsely ground hybrid rye. The DE and ME of finely ground hybrid rye were greater ($P < 0.05$) than those of coarsely ground hybrid rye, and the NE of finely ground hybrid rye tended to be greater ($P = 0.084$) than that of coarsely ground hybrid rye. No differences were observed for the DE, ME, and NE contents between Bono and Gatano. The determined NE for coarsely and finely ground Bono were 11.06 and 11.77 MJ/kg DM, and the respective NE values for coarsely and finely ground Gatano were 11.09 and 11.57 MJ/kg DM. In conclusion, the

energy values of different hybrid rye varieties might be comparable. Particle size reduction could significantly improve the DE and ME values of hybrid rye fed to growing pigs, while the NE value was only numerically improved.

Keywords: hybrid rye, indirect calorimetry, energy digestibility, particle size, pigs

4.2 INTRODUCTION

Rye can be used as an alternative feedstuff in swine diets. However, the traditional rye is highly susceptible to ergot contamination, which may cause adverse health effects in animals (Coufal-Majewski et al., 2016). The recently developed hybrid rye contains mitigated ergot contamination and diminished concentrations of antinutritional factors, such as trypsin inhibitors and alkylresorcinols (Miedaner and Geiger, 2015; Schwarz et al., 2015). Also, the newly bred hybrid rye's metabolizable energy (ME) content is similar to that of barley and sorghum, making it an attractive feed ingredient for use in swine diets (McGhee and Stein, 2020).

Digestible energy (DE) and ME systems often overestimate the energy values of fibrous or high protein feed ingredients (Noblet et al., 1994). Since hybrid rye contains a relatively high level of fiber (McGhee and Stein, 2021), the net energy (NE) system may provide a more accurate estimate of the amount of energy that is available for pigs. Also, particle size is important for swine diets because the reduced particle size can increase the surface area of ingredients, improving nutrient and energy digestibility (Wondra et al., 1995; Rojas and Stein, 2015; Koo and Nyachoti, 2021). However, the NE value of hybrid rye has not been reported yet. Similarly, the effect of particle size on hybrid rye’s energy content has not been investigated.

Therefore, the objectives of this study were to determine the DE, ME, and NE of hybrid rye and to investigate the effects of particle size and variety on energy content of hybrid rye. It was hypothesized that the energy contents of hybrid rye would be improved by fine grinding.

4.3 MATERIALS AND METHODS

The animal use protocol was reviewed and approved by the University of Manitoba Animal Care Committee (Winnipeg, MB, Canada; AC11419). All pigs were raised according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

4.3.1 Experimental animals and diets

Thirty growing barrows (TN70 × TN Tempo; Topigs Norsvin, Winnipeg, MB, Canada) with an initial body weight (BW) of 18.06 ± 0.83 kg, were individually housed in adjustable metabolism crates (1.8 × 0.6 m) in a temperature-controlled room (24 ± 1°C). Each metabolism crate was equipped with a stainless-steel feeder and a nipple drinker that allowed pigs to have unlimited access to water throughout the experimental period.

Two hybrid rye varieties (Bono and Gatano; KWS Lochow GmbH, Bergen, Germany) were used in this study. The hybrid rye was ground using a hammermill fitted with 4.0-mm and 3.2-mm screens to obtain coarse and fine particle size, respectively. All experimental diets were supplemented with vitamins and minerals to meet or exceed the requirement of growing pigs (NRC, 2012). The corn-soybean meal (SBM) basal diet was formulated to ensure that the basal diet containing 300 g/kg of hybrid rye met requirements for standardized ileal digestible indispensable amino acids (AA), standardized total tract digestible phosphorus, vitamins, and minerals (NRC, 2012).

Table 4.1 Particle size analysis of hybrid rye[1].

Hybrid rye variety	Bono		Gatano	
Particle size	Coarse	Fine	Coarse	Fine
Distribution of particles, %				
Sieve opening, µm				
2,360	1.33	0.60	1.36	0.73
1,700	44.82	2.34	45.94	3.74
1,180	17.67	23.95	20.30	30.29
850	12.89	23.21	13.78	25.88
600	7.35	14.98	6.49	13.14
420	4.14	9.10	3.24	7.14
297	3.09	6.15	2.40	4.67
212	2.01	4.88	1.48	3.60
150	1.61	3.08	1.20	2.13
103	1.08	2.27	0.88	1.73
73	0.84	1.07	0.72	0.87
53	0.32	1.20	0.48	1.07
37	2.85	7.16	1.72	5.00
Coarse particles (≥ 1,700 µm)	46.14	2.94	47.30	4.47
Medium particles (297 to 1,700 µm)	45.14	77.39	46.22	81.12
Fine particles (< 297 µm)	8.71	19.67	6.49	14.41
Geometric mean particle size, µm	1,111	594	1,210	717
Standard deviation, µm	2.43	2.77	2.20	2.55
Surface area, cm^2/g	60.7	128.7	51.3	98.4

[1]Two hybrid rye varieties (Bono and Gatano) were ground using a hammermill fitted with 4.0-mm and 3.2-mm screens to obtain coarse and fine particle size, respectively.

Table 4.2 Analyzed nutrient composition of hybrid rye, as-fed basis.

	Hybrid rye[1]	
Variety	Bono	Gatano
Analyzed composition		
DM, %[2]	89.24	89.35
GE, MJ/kg[2]	16.43	16.39
CP, %[2]	8.32	7.13
EE, %[2]	1.18	1.16
Starch, %	54.90	52.59
Ash, %	1.47	1.49
NDF, %[2]	11.93	12.94
ADF, %[2]	3.18	4.08
Calcium, %	0.05	0.06
Phosphorus, %	0.30	0.27

[1]Two hybrid rye varieties (Bono and Gatano) were finely ground with grain miller for 50-200 µm of fitness for chemical analysis.

[2]ADF, acid detergent fiber; NDF, neutral detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; GE, gross energy.

4.3.2 Experimental design and sample collection

The experiment was conducted in two consecutive periods (15 pigs per period) using the same facility, experimental conditions, and procedures because only three indirect calorimetry chambers were available. Pigs were randomly allotted to one of five experimental diets in a completely randomized design with three replicates per treatment per period to give a total of six replicates per treatment. The five experimental diets included a corn-SBM basal diet and four additional diets formulated by a simple substitution of the basal diet with either coarse or fine hybrid rye (Bono or Gatano) at a ratio of 7:3 (Koo et al., 2021; Table 4.3).

Table 4.3 Composition and nutrient content of experimental diets, as-fed basis.

	Corn-SBM basal diet[1]				
Item	Basal	Bono Coarse	Bono Fine	Gatano Coarse	Gatano Fine
Ingredients, %					
Corn	67.00	46.90	46.90	46.90	46.90
Soybean meal	28.20	19.74	19.74	19.74	19.74
Hybrid rye	–	30.00	30.00	30.00	30.00
Monocalcium phosphate	1.20	0.84	0.84	0.84	0.84
Limestone	1.10	0.77	0.77	0.77	0.77
Vegetable oil	1.00	0.70	0.70	0.70	0.70
Salt	0.40	0.28	0.28	0.28	0.28
Lys HCL	0.10	0.07	0.07	0.07	0.07
Vitamin-mineral premix[2]	1.00	0.70	0.70	0.70	0.70
Analyzed composition					
DM, %[3]	89.64	89.21	88.79	88.92	88.68
GE, MJ/kg[3]	16.85	16.61	16.56	16.56	16.47
CP, %[3]	18.38	16.88	15.69	15.81	15.69
EE, %[3]	3.97	3.43	3.25	3.38	3.04
Starch, %	46.3	47.68	46.34	47.16	45.31
Ash, %	4.17	3.64	3.61	4.11	3.88
NDF, %[3]	7.77	9.10	9.57	9.93	9.34
ADF, %[3]	3.17	3.08	3.31	4.17	3.85
Calcium, %	0.63	0.47	0.54	0.45	0.52
Phosphorus, %	0.53	0.48	0.52	0.49	0.53

[1]SBM = soybean meal; The five experimental diets included a corn-SBM basal diet and four additional diets formulated by mixing 300 g/kg of coarse or fine hybrid rye (Bono or Gatano) with 700 g /kg of the basal diet, respectively.

[2]Provided the following per kg of the basal(hybrid rye) diets: vitamins A, 3,143(2200) IU; vitamin D_3, 315(220) IU; vitamin E, 23(16) IU; vitamin K, 0.7(0.5) mg; thiamine, 1.4(1.0) mg; riboflavin, 5(3.5) mg; niacin, 43(30) mg; pantothenic acid, 14.3(10.0) mg; vitamin B12, 0.03(0.02) mg; folic acid, 0.43(0.3) mg; Cu, 8.6(6.0) mg as copper sulfate; I, 0.2(0.14) mg as calcium iodate; Fe, 143(100) mg as ferrous sulfate; Mn, 5.8(4.0) mg as manganese oxide; Se, 0.43(0.3) mg as sodium selenite; Zn, 143(100) mg as zinc oxide; biotin 0.07(0.05) mg.

[3]ADF, acid detergent fiber; NDF, neutral detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; GE, gross energy.

Pigs were fed their assigned diets at the amount of 550 kcal ME/kg BW$^{0.60}$ per day based on their BW on day 1, 5, and 10, which was close to ad libitum intake (Noblet et al., 1994). During the experiment, pigs were fed at 0830 h and trained to consume their daily feed allowance within 1 h. In each period, pigs were fed experimental diets for 15 days, including a 10-day adaptation period followed by a 5-day period for total collection of feces and urine, according to the marker-to-marker procedure, which was performed to determine DE and ME, as previously described by Woyengo et al. (2010). Briefly, in the morning (0830 h) of day 11, each pig received 5 g of ferric oxide as an indigestible marker mixed with 100 g of assigned feed. The remaining feed was provided after all the marked feed was consumed. Fecal collection was initiated when the marker appeared in feces. In the morning (0830 h) of day 16, pigs received 100 g of marked feed, and fecal collection was terminated when the marker appeared in feces. Feces were collected once daily in the morning and were stored at −20°C immediately for further analysis. Urine collection was started at 0830 h on day 11 and stopped at 0830 h on day 16. Urine was collected once daily in the morning in a plastic container containing 20 mL of 3 *N* HCL to minimize N losses and weighed. A subsample (10% of the total weight) was obtained, filtered through glass wool, and stored at −20°C.

Before each period, the accuracy of indirect calorimetry chambers was validated by burning an alcohol lamp inside the system, according to the stoichiometric equation of ethanol combustion. Theoretically, the respiratory quotient (RQ) of ethanol combustion is 0.667, which is a ratio of CO_2 production to O_2 consumption (Benedict and Tompkins, 1916). The RQ value between 0.640 and 0.690 was considered acceptable, and no accuracy problems were observed for the two periods. On day 17, three pigs were transferred to the indirect calorimetry chambers (1.22 m × 0.61 m × 0.91 m; Columbus Instruments, Columbus, OH, USA) within 1 h of consuming their daily feed allowance in the metabolism crates to measure heat production (HP) and fasting HP (FHP), based on O_2 consumption, CO_2 production, and urinary nitrogen (N) excretion. The HP was continuously measured for 24 h (fed-state) and followed by 12 h (fasting-state). The following sets of three pigs were moved into the indirect calorimetry chambers every 2 days. Pigs had unlimited access to fresh water in the chambers, and excreted urine during the fed-state and fasting-state was collected separately, weighed, subsampled, and stored at −20°C for N analysis. The experimental temperature inside the chamber was maintained at 22°C, and personnel movement was limited in the chamber room to avoid distressing pigs during the measurements of HP and FHP.

4.3.3 Particle size analysis, sample preparation, and chemical analysis

Particle size distribution of hybrid rye was measured using a sieve shaker (RX-29, Superior Agri-System, MB, Canada) fitted with 13 multiple sieves (U.S. standard sieve Nos. 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, and 270). About 150 g ground hybrid rye samples were sieved for 10 min. The geometric mean diameter (D_{gw}), geometric standard deviation (S_{gw}), and surface area of ground hybrid rye samples were calculated, based on the ANSI/ASAE S319.2 (American Society of Agricultural and Biological Engineers [ASABE], 1995) standard method.

Fecal samples were oven-dried at 60°C for 5 days and then finely ground before chemical analysis. Urine samples from metabolism crates and chambers were thawed and pooled separately for each pig, sieved through cotton gauze, and filtered with glass wool prior to GE and N analysis. The dry matter (DM) of diets, ingredients, and feces was determined according to procedure 934.01 of the Association of Official Analytical Chemists (AOAC, 2006), and the gross energy (GE) of diets, ingredients, feces, and urine was measured using an adiabatic bomb calorimeter (model 6400; Parr Instrument Co., Moline, IL, USA), which had been calibrated using benzoic acid as a standard. Nitrogen of diets, ingredients, feces, and urine was determined according to procedure of 990.03 (AOAC, 2006) by a combustion analyzer (model CNS-2000; LECO Corp., St. Joseph, MI, USA), and crude protein (CP) was calculated as N × 6.25. Ash was determined according to procedure of 942.05 (AOAC, 2006), and ether extract (EE) was measured for diets and ingredients after hexane extraction according to procedure of 920.39A (AOAC, 2006) in an extraction apparatus. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) in diets and ingredients were analyzed using an Ankom 200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the method of Van Soest et al. (1991). The starch in diets and ingredients was measured using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd., Wicklow, Ireland). The concentrations of calcium and phosphorus were analyzed according to procedures 968.08 and 946.06 (AOAC, 2006), respectively, and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA, USA).

Urine samples were analyzed for GE as described by Kim and Nyachoti (2017). Briefly, approximately 0.5 g of cellulose was dried at 103°C for 24 h. Then, 2 mL of urine sample was sprayed over the cellulose, and the weight of the final mixture was recorded. The urine-cellulose mixture along with a sample of pure cellulose were dried in an oven at 50°C for 24 h and then weighed for the estimation of urine DM. The GE of the dried urine-cellulose and pure cellulose were determined using an adiabatic bomb calorimeter, and the GE of urine samples were calculated by the difference method.

4.3.4 Calculations and statistical analysis

The apparent total tract digestibility (ATTD) of DM, energy, and CP and N retention rate were calculated using the total collection method as described by the following equations:

$$\text{ATTD} = [(\text{CI} - \text{CO}_{\text{feces}})/\text{CI}] \times 100,$$

where CI is the component intake (g) and CO_{feces} is the component output in feces (g).

$$\text{N retention (\%)} = 100 \times [(NI - NO_{feces} - NO_{urine})/NI],$$

where NI is the N intake (g), and NO_{feces} and NO_{urine} are the N output in feces and urine (g), respectively.

The HP and FHP (Brouwer, 1965), retained energy (RE), and NE values were calculated using the following equations:

$$HP = [3.866 \times O_2 + 1.200 \times CO_2 - 1.431 \times \text{urinary N excretion}] \times 0.004184$$

where HP is in megajoules, O_2 is oxygen consumption in liters, CO_2 is carbon dioxide production in liters, and urinary N excretion is total urinary N excretion in grams. The FHP was also calculated using the same equation for HP.

$$RE = ME - HP,$$

where RE, ME, and HP are in megajoules per day. Retained energy as protein was calculated as N retention (g) × 6.25 × 5.68 (kcal/g) × 0.004184, whereas retained energy as lipid was calculated as the difference between RE and RE as protein (Labussiere et al., 2009).

$$DE = (GE_i - GE_f)/DMI$$

$$ME = (GE_i - GE_f - GE_u)/DMI$$

$$NE = (RE + FHP)/DMI,$$

where DE, ME, and NE are in megajoules per kilogram DM, respectively. The GE_i, GE_f, and GE_u, are the total GE intake, total GE output in feces, and total GE output in urine in megajoules, respectively. The RE and FHP are in megajoules per day, and dry matter intake (DMI) is in kilograms.

After the energy values of each experimental diet were determined, the DE, ME, and NE of test ingredients were calculated by subtracting the energy contribution of the corn-SBM basal diet from the energy contents of the diets containing 300 g/kg of hybrid rye (Adeola, 2001).

The predicted NE of test ingredients was calculated using the following two equations established by Noblet et al. (1994):

$$NE = [0.700 \times DE + 1.61 \times \%\ EE + 0.48 \times \%\ starch - 0.91 \times \%\ CP - 0.87 \times \%\ ADF] \times 0.004184$$

$$NE = [0.726 \times ME + 1.33 \times \%\ EE + 0.39 \times \%\ starch - 0.62 \times \%\ CP - 0.83 \times \%\ ADF] \times 0.004184$$

Where the two NE predicted equations were originally given in kcal/kg DM and were converted to MJ/kg DM using the conversion factor of 0.004184.

Data were identified by the UNIVARIATE procedures of SAS 9.4 for outliers and then analyzed using the MIXED procedure of SAS 9.4 (SAS, 2011). The individual pig was considered as the experimental unit, and diet and period were the fixed effects initially. However, the effect of period was excluded in the final model because it was not significant. The LSMEANS procedure was used to calculate mean values, and the PDIFF option of SAS was used to separate means. A probability of $P < 0.05$ was considered significant, whereas $0.05 < P < 0.10$ was considered a tendency.

4.4 RESULTS

The analyzed particle distribution of hybrid rye in the current study is presented in Table 4.1. The D_{gw} of coarsely and finely ground Bono were 1,111 and 594 µm, respectively, and the respective values for coarsely and finely Gatano were 1,210 and 717 µm. The surface areas of coarsely ground Bono and Gatano were 60.7 and 51.3 cm^2/g, respectively, and the respective values for finely ground Bono and Gatano were 128.7 and 98.4 cm^2/g. The chemical composition of hybrid rye is presented in Table 4.2. The GE values of Bono and Gatano were similar (16.43 vs 16.39 MJ/kg). The CP content was slightly greater in Bono (8.32 %) than in Gatano (7.13 %). The composition and analyzed nutrient contents of experimental diets are presented in Table 4.3. The analyzed nutrients and GE values of the experimental diets were in agreement with the formulated values.

As shown in Table 4.4, the ATTD of DM was greater ($P < 0.05$) in the basal diet than in the hybrid rye diets, except for the finely ground Bono diet. The ATTD of DM was greater ($P < 0.05$) in finely ground hybrid rye diets than in coarsely ground hybrid rye diets. Similarly, the ATTD of GE was greater ($P < 0.05$) in the basal diet than in the hybrid rye diets, and the ATTD of GE was greater ($P < 0.05$) in finely ground hybrid rye diets than in coarsely ground hybrid rye diets. The ATTD of CP was greater ($P < 0.05$) in the basal diet than in coarsely ground hybrid rye diets. Nitrogen intake was less ($P < 0.01$) in hybrid rye diets than in the basal diet. Urinary N excretion was less ($P < 0.05$) for pigs fed finely ground hybrid rye diets than for those fed the basal diet. No differences were observed for fecal excretion and N retention (%) among the experimental diets.

The energy contents of experimental diets are presented in Table 4.5. The DE, ME, and NE contents of the basal diet were greater ($P < 0.01$) than those of the hybrid rye diets. No differences were observed for the HP and FHP among the experimental diets. The RE was greater ($P < 0.01$) in the basal diet than in the hybrid rye diets. No differences were observed for RQ for fed and fasting states and energy efficiency, including the ME:DE and NE:ME ratios. The energy contents of test ingredients are presented in Table 4.6. The finely ground hybrid rye had greater ($P < 0.05$) DE and ME than the coarsely ground hybrid rye and tended to contain greater ($P = 0.084$) NE than the coarsely ground hybrid rye. No differences were observed for the DE, ME, and NE values between Bono and Gatano. There was no interaction between variety and particle size on the DE, ME, and NE of hybrid rye. The NE of Bono and Gatano determined by the indirect calorimetry method were 11.42 and 11.33 MJ/kg DM, respectively, whereas the NE calculated using published predicted equations were 10.74 and 10.62 MJ/kg DM, respectively (Table 4.7). The determined NE of hybrid rye was greater ($P < 0.05$) than the NE predicted using equation 2.

Table 4.4 Apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), and crude protein (CP) and nitrogen (N) balance of experimental diets fed to growing pigs.

	Corn-SBM basal diet[1]						
Item	Basal	Bono Coarse	Bono Fine	Gatano Coarse	Gatano Fine	SEM	*P*-value
ATTD, %							
DM[2]	90.3[a]	89.1[c]	90.0[ab]	88.9[c]	89.7[b]	0.21	<0.001
GE[2]	89.3[a]	87.7[c]	88.5[b]	87.5[c]	88.3[b]	0.23	<0.001
CP[2]	85.7[a]	83.2[b]	84.0[ab]	82.4[b]	84.2[ab]	1.06	0.012
N balance, g/d[2]							
N intake	23.6[a]	20.4[b]	19.3[b]	20.4[b]	18.8[b]	0.78	0.003
Fecal excretion	3.4	3.4	3.1	3.6	3.0	0.27	0.196
Urinary excretion	8.3[a]	7.4[ab]	6.5[b]	7.7[a]	6.3[b]	0.46	0.019
N retained	12.0	9.6	9.8	9.1	9.5	0.72	0.060
N retained, %	50.6	46.9	50.0	44.3	50.2	2.17	0.195

[a–c]Means without a common superscript within a row differ ($P < 0.05$).

[1]SBM = soybean meal; The five experimental diets include a corn-SBM basal diet and four additional diets formulated by mixing 300 g/kg of coarsely or finely hybrid rye (Bono or Gatano) with 700 g/kg of the basal diet, respectively.

[2]CP, crude protein; DM, dry matter; GE, gross energy; N, nitrogen.

Table 4.5 Energy values of experimental diets (MJ/kg, dry matter basis).

Item	Corn-SBM basal diet[1]					SEM	P-value
	Basal	Bono Coarse	Bono Fine	Gatano Coarse	Gatano Fine		
Energy value, MJ/kg							
DE[2]	16.79[a]	16.32[c]	16.49[b]	16.31[c]	16.41[bc]	0.04	<0.001
ME[2]	16.11[a]	15.49[c]	15.75[b]	15.52[c]	15.64[bc]	0.05	<0.001
HP[2,3]	5.95	6.32	6.42	6.50	6.03	0.23	0.101
HP, MJ/kg $BW^{0.6}$/d[2]	0.85	0.90	0.92	0.94	0.85	0.04	0.069
Total RE[2,4]	10.16[a]	9.17[bc]	9.34[bc]	9.02[c]	9.59[b]	0.26	<0.001
As protein	1.78	1.42	1.46	1.35	1.41	0.11	0.060
As lipid	8.38	7.74	7.88	7.67	8.18	0.29	0.119
FHP[2,5]	3.49	3.70	3.75	3.86	3.45	0.22	0.433
FHP, MJ/kg $BW^{0.6}$/d	0.49	0.53	0.54	0.56	0.49	0.03	0.263
Heat increment	2.46	2.62	2.67	2.64	2.59	0.11	0.692
NE[2,6]	13.64[a]	12.87[b]	13.08[b]	12.88[b]	13.04[b]	0.12	<0.001
Respiratory quotient							
Fed state	1.08	1.05	1.08	1.11	1.10	0.074	0.244
Fasting state	0.84	0.84	0.85	0.87	0.89	0.056	0.203
Energy efficiency							
ME/DE	0.96	0.95	0.96	0.95	0.95	0.004	0.356
NE/DE	0.81	0.79	0.79	0.79	0.79	0.007	0.090
NE/ME	0.85	0.83	0.84	0.83	0.83	0.007	0.342

[a–c]Means without a common superscript within a row differ ($P < 0.05$).
[1]SBM = soybean meal; The five experimental diets included a corn-SBM basal diet and four additional diets formulated by mixing 300 g/kg of coarsely or finely ground hybrid rye (Bono or Gatano) with 700 g/kg of the basal diet, respectively.
[2]BW, body weight; DE, digestible energy; DM, dry matter; FHP, fasting heat production; HP, heat production; ME, metabolizable energy; NE, net energy; RE, retained energy.
[3]Heat production = $[(3.866 \times O_2 + 1.200 \times CO_2 – 1.431 \times \text{urinary N})/\text{DM intake}] \times 0.004184$.
[4]Retained energy = (ME intake – HP)/DM intake.
[5]Fasting heat production = $[(3.866 \times O_2 + 1.200 \times CO_2 - 1.431 \times \text{urinary N})/\text{DM intake}] \times 0.004184$.
[6]Net energy = (RE + FHP)/DM intake

Table 4.6 Energy contents of hybrid rye ground in two particle sizes fed to growing pigs determined by the difference method using corn-soybean meal basal diet (MJ/kg, dry matter basis).

Item	Energy content of hybrid rye[1]				SEM[3]	P-value		
	Bono Coarse[2]	Bono Fine[2]	Gatano Coarse[2]	Gatano Fine[2]		Variety	PS[3]	Interaction
DE[3]	15.23	15.73	15.18	15.52	0.14	0.352	0.006	0.579
ME[3]	14.04	14.92	14.14	14.48	0.19	0.334	0.002	0.133
NE[3]	11.06	11.77	11.09	11.57	0.32	0.802	0.084	0.726

[1]Energy content of test ingredients were calculated using the difference method by subtracting the energy contribution of the basal diet from the energy content of the diets containing 300 g/kg of test ingredients.

[2]Geometric mean particle size for coarsely and finely ground Bono were 1,111 µm and 594 µm, respectively. Geometric mean particle size for coarsely and finely ground Gatano were 1,210 µm and 777 µm, respectively.

[3]DE, digestible energy; ME, metabolizable energy; NE, net energy; PS, particle size; SEM, standard error of the means.

Table 4.7 Comparison of determined and predicted net energy (NE) of hybrid rye ground in two particle sizes (MJ/kg, dry matter basis).

	NE of ingredient[2]				
Item	Determined	Predicted 1[1]	Predicted 2[1]	SEM[2]	P-value
Bono Coarse	11.06^{a}	10.71^{ab}	10.24^{b}	0.30	0.025
Bono Fine	11.77^{a}	11.12^{b}	10.90^{b}	0.23	0.008
Gatano Coarse	11.09^{a}	10.67^{ab}	10.31^{b}	0.25	0.024
Gatano Fine	11.57^{a}	10.96^{ab}	10.55^{b}	0.35	0.043

a–b Means without a common superscript within a row differ ($P < 0.05$).

[1] The two NE predicted equations from Noblet et al. (1994), where predicted 1) NE = [0.700 × DE + 1.61 × % ether extract + 0.48 × % starch − 0.91 × % CP − 0.87 × % ADF] × 0.004184; predicted 2) NE = [0.726 × ME + 1.33 × % EE + 0.39 × % starch − 0.62 × % CP − 0.83 × % ADF] × 0.004184. The two NE predicted equations were originally presented in kcal/kg DM and were converted to MJ/kg DM using the conversion ratio of 0.004184.

[2] NE, net energy; SEM, standard error of the means.

4.5 DISCUSSION

The production of hybrid rye is increasing in North America in recent years. Because of its high yield, winter hardiness, and drought tolerance, hybrid rye has become an attractive alternative to other small grains, such as barley and wheat to grow on poor soil (Geiger and Meidaner, 2009). Although some variations were observed for nutritional composition of hybrid rye in the current study due to generic differences, the GE (16.41 MJ/kg), starch (53.75%), ash (1.48%), calcium (0.06%), and phosphorus (0.29%) contents in hybrid rye agreed with published values (NRC 2012; McGhee and Stein, 2019; Archs Toledo et al., 2020). Although the CP (7.73%) in hybrid rye agreed with reported values (McGhee and Stein, 2019; Archs Toledo et al., 2020), this value was lower than that reported by NRC (2012). The concentrations of NDF (12.44%) and ADF (3.63%) in hybrid rye were similar to the values reported by NRC (2012). Due to its relatively high level of dietary fiber, the NE system is expected to be more appropriate than DE and ME system for assessing the available energy of hybrid rye for pigs (Strang et al., 2016). However, to our knowledge, there is no information about the NE value of hybrid rye yet. Therefore, the current study was performed to determine for the first time the NE content of hybrid rye fed to growing pigs. Also, this is the first study assessing hybrid rye regarding the energy content at different particle sizes and varieties by indirect calorimetry for growing pigs. Such results will provide background information for future studies assessing the energy value of hybrid rye. The outcomes of this study can assist in obtaining precise feed formulations for growing pigs considering particle size and varieties of hybrid rye.

In the feed manufacturing process, grinding is used to reduce the particle size of feed ingredients to improve nutrient and energy digestibility in pigs by increasing the surface area of feed ingredients to have better contact with digestive enzymes (Blasel et al., 2006; Rojas and Stein

2015). Also, reduced particle size helps facilitate further processing, such as mixing, transportation, and pelleting (Nahm, 2002). However, pigs fed fine particle size are more likely to develop gastric lesions and ulcerations, which may have negative impact on gut health (Kiarie and Mills, 2019). Therefore, the recommended optimal particle size for ground cereal grains ranges from 500 to 700 μm (Behnke et al., 1994). Nevertheless, fine grinding can increase energy consumption and decrease milling production rates, which may offset the benefits of improved nutrient and energy digestibility (Wondra et al., 1995). Since no studies have determined the effect of particle size on energy content of hybrid rye fed to pigs yet, in the current study, hybrid rye was finely and coarsely ground to obtain the particle size that were within the range of the optimal particle size and beyond the optimal particle size to determine the effects of particle size on the determined energy contents.

The decreased D_{gw} of ground hybrid rye corresponded to the increased surface area of ground hybrid rye. As the particle size of Bono decreased from 1,111 to 594 μm, the surface area increased by about 112%. Also, as the particle size of Gatano decreased from 1,210 to 717 μm, the surface area increased by about 92%. The main difference in particle size between finely and coarsely ground Bono was that the percentage of coarse particles (≥1,700 μm) in finely ground Bono (2.94%) was much less than that in coarsely ground Bono (46.14%). Similarly, the main difference in particle size between finely and coarsely Gatano was that the percentage of coarse particles (≥1,700 μm) in finely ground Gatano (4.47%) was much less than that in coarsely ground Gatano (47.30%).

In the current study, the ATTD of DM, GE, and CP were greater in the basal diet than in the hybrid rye diets, which is also in accordance with the finding that the DE, ME, and NE of the basal diet were greater than those of the hybrid rye diets. This can be explained by the fact that 300 g/kg of the basal diet was replaced by the test ingredients, which contain relatively high fiber content. Previous studies reported that dietary NDF is negatively correlated with DM and GE digestibility (Le Goff and Noblet, 2001; Kim and Nyachoti, 2017). The N intake of the basal diet was greater than that of the hybrid rye diets because the CP content in the basal diet was higher than that in the hybrid rye diets. Although feeding hybrid rye diets was assumed to lead to greater HP and FHP generation than the basal diet due to the higher level of dietary fiber, no differences were observed for HP and FHP of pigs fed hybrid rye diets and the basal diet in this study. A similar observation has been reported by others (Ayoade et al., 2012; Heo et al., 2014), which might be due to the fact that addition of dietary fiber can change the overall metabolism and the physical activities of pigs in the chambers (Schrama et al., 1998). The ME:DE ratio of the five experimental diets in this study was comparable to the typically reported values of around 0.96 for complete diets, as noted by Noblet and van Milegen (2004) as well as Kim and Nyachoti (2017). The average NE to ME ratio of 0.84 across the five experimental diets was in line with the value reported by Velayudhan et al. (2015) and Kim and Nyachoti (2017). However, this figure is marginally higher than the one proposed by Noblet et al. (1994), which suggested that the efficiency of utilizing ME for NE in complete swine diets was approximately 0.75. This variation might be due to differing genetic backgrounds of the animals used in the experiments, as suggested by Kil et al. (2013). Particle size reduction improved the ATTD of DM and GE in hybrid rye diets in this study, which can be explained by the fact that reduced particle size increases the surface area of feed ingredients, thus allowing for better contact with the pig's digestive enzymes (Wondra et al., 1995). There were greater DE and ME contents in finely ground hybrid rye than in coarsely ground hybrid rye, which is in agreement with previous reports that fine grinding can increase DE and ME for pigs (Fastinger and Mahan, 2003; Rojas and Stein, 2015). The determined DE and ME contents of hybrid rye in the current study were within the range of values reported by McGhee and Stein (2020). Because there is limited information about NE content, prediction equations are commonly used to estimate the energy contents of feed ingredients (NRC, 2012). In this study, the determined NE values for hybrid rye did not match with the predicted NE values, notably those obtained with equation 2. This discrepancy can be rationalized by the understanding that prediction equations fail to account for potential changes in the energy efficiency of nutrients, specifically starch and fiber, that may occur due to varying levels of grinding intensity, as reported by Koo and Nyachoti (2021). More research is needed to validate these findings and to delve into the differences between determined and predicted NE values when using prediction equations to estimate NE content in hybrid rye of various particle sizes.

4.6 CONCLUSION

The net energy contents of coarsely and finely ground Bono and Gatano were 11.06, 11.77, 11.09, and 11.57 MJ/kg (dry matter basis), respectively. Compared with coarse grinding, fine grinding can significantly improve the digestible energy and metabolizable energy but not the net energy content in hybrid rye fed to pigs. However, no differences in energy values were observed for the tested hybrid rye varieties.

TRANSITION STATEMENT

Manuscript I (Experiment 1) aimed to determine the DE, ME, and NE of hybrid rye, as well as evaluate the effects of particle size and variety on its energy content. The manuscript I identified that fine grinding of hybrid rye, compared to coarse grinding, leads to a significant improvement in DE and ME in pigs. Manuscripts II and III (Experiments 2 and 3) sought to explore the impact of particle size and enzyme supplementation on nutrient digestibility in hybrid rye for growing pigs. Specifically, Experiment 2 focused on investigating the effects of particle size and MC supplementation on AA digestibility in hybrid rye fed to growing pigs.

CHAPTER FIVE: MANUSCRIPT II

Standardized ileal digestibility of amino acids in hybrid rye ground to two particle sizes and fed with or without multi-carbohydrase supplementation to growing pigs

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5.1 ABSTRACT

A newly developed hybrid rye with enhanced ergot resistance has potential as an alternative energy source for pigs. An experiment was conducted to evaluate the effects of particle size (PS) and multi-carbohydrase (MC) supplementation on the apparent total tract digestibility (ATTD) of energy and nutrients and the standardized ileal digestibility (SID) of AA in hybrid rye fed to growing pigs. Bono and Gatano, two hybrid rye varieties, were ground to either a coarse (1,111 μm and 1,210 μm) or a fine (594 μm and 717 μm) PS using a hammer mill. Eighteen ileal-cannulated barrows (initial BW = 18.2 ± 1.0 kg) were randomly assigned to 1 of 9 dietary treatments in a replicated 9 × 3 incomplete Latin square design to give six observations per treatment. Among the nine experimental diets, eight featured two hybrid rye varieties (Bono or Gatano), either coarsely or finely ground, as the only source of AA with or without MC supplementation, while a N-free diet was used to estimate the endogenous loss of AA at the distal ileum. All diets contained titanium dioxide as an indigestible marker. Each period lasted 9 d, with the first 5 d being for adaptation followed by 2 d each for fecal and ileal digesta collection. Data were analyzed using the MIXED procedure of SAS, with the final model having variety, PS, MC supplementation, and their two-way or three-way interactions as the main effects. No significant interactions among the main effects were noted except for the two-way interactions ($P < 0.05$) between the variety and MC on the AID and SID of CP and most AA. The ATTD of DM, GE, and CP were greater ($P < 0.05$) in Bono diets than in Gatano diets. Finely ground hybrid rye diets had higher ($P < 0.05$) ATTD of NDF and EE compared to coarsely ground hybrid rye diets. Dietary MC supplementation increased ($P < 0.05$) the ATTD of DM, GE, CP, NDF, and EE in hybrid rye diets. Additionally, MC supplementation improved the SID of CP and most AA in hybrid rye. In conclusion, the nutrients digestibility of hybrid rye can be affected by its variety. Particle size

reduction improved the nutrient digestibility in hybrid rye, and MC supplementation improved the energy and AA digestibility in hybrid rye fed to growing pigs.

Keywords: hybrid rye, particle size, enzyme supplementation, digestibility, pigs

5.2 INTRODUCTION

A recently developed hybrid rye demonstrates reduced susceptibility to ergot contamination while maintaining high yield levels (Miedaner et al., 2021). Additionally, this new hybrid rye has metabolizable energy (ME) values comparable to those of barley and sorghum, thereby positioning it as a potential alternative energy source for swine nutrition (McGhee and Stein, 2020). Nevertheless, rye is characteristically high in non-starch polysaccharides (NSP), primarily arabinoxylans, cellulose, and β-glucans, which can increase the digesta viscosity and consequently affect the nutrient digestibility in pigs (Bach Knudsen, 1997; Salmenkallio-Marttila et al., 2005).

The utilization of multi-carbohydrase (MC), a composite of various carbohydrases, has been empirically substantiated to outperform the use of individual carbohydrase in the depolymerization of NSP in cereal grains, thereby enhancing nutrient digestibility and growth performance in pigs when fed a variety of feedstuffs (Omogbenigun et al., 2004; Emiola et al., 2009). Moreover, a decrease in particle size (PS) can improve the nutritional value of feed ingredients, as it enlarges the surface area available for enzymatic interaction during the digestive process (Chae and Han, 1998; Blasel et al., 2006).

Feed processing and enzyme supplementation have been identified as useful methods for enhancing digestive utilization of nutrients in NSP-rich feedstuffs (Zijlstra et al., 2010; De Vries et al., 2012). The combined application of these methods has emerged as a viable strategy to improve sustainability in pig and poultry production systems (Schedle, 2016). However, the possible effects of PS and MC supplementation on standardized ileal digestibility (SID) of amino acids (AA) in hybrid rye fed to growing pigs are yet to be elucidated. Therefore, the objective of this study was to determine the effects of hybrid rye PS and MC on the SID of AA in hybrid rye fed to growing pigs. It was hypothesis that reduced PS and dietary MC supplementation would increase the SID of AA in hybrid rye fed to growing pigs.

5.3 MATERIALS AND METHODS

The animal use protocol was reviewed and approved by the University of Manitoba Animal Care Committee (AC11419). All pigs were raised according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

5.3.1 Experimental animals and diets

Eighteen barrows (initial BW of 18.2 ± 1.0 kg; TN70 × TN Tempo; Topigs Norsvin, Winnipeg, MB, Canada) were housed individually in pens (1.47 × 1.14 m) with plastic-covered woven metal floors in a temperature-controlled room (22 ± 2°C). Each pen was equipped with a nipple drinker to allow pigs have unlimited access to water. Each pig was fitted with a T-cannula at the distal ileum as described by Nyachoti et al. (2002) and given a 14-d recovery period before being assigned to dietary treatments.

Two hybrid rye varieties (Bono and Gatano) were provided by KWS (KWS Lochow GmbH, Bergen, Germany) and ground using a hammer mill fitted with 4.0- and 3.2-mm screens to obtain coarse and fine PS, respectively. The MC preparation (supplemented at 60 g/MT of feed) was provided by Canadian Bio-System Inc. (Superzyme Conc; Calgary, Alberta, Canada;). The MC supplied 7,000 units of cellulase, 750 units of mannanase, 8,500 units of xylanase, 3,000 units of glucanase, 80,000 units of amylase, 4,000 units of invertase, and 6,500 units of protease per gram of enzyme. Experimental diets consisted of 92.9% finely or coarsely ground hybrid rye as the only source of AA that was fed with or without MC supplementation and an N-free diet to estimate the non-specific endogenous loss of AA at the distal ileum (Table 5.1). For each hybrid rye diet, a basal diet was mixed and then divided into two halves. One half served as the control, whereas the other half was supplemented with the MC preparation to give the treatment diet. All diets contained 0.3% titanium dioxide (TiO_2) as an indigestible marker.

Table 5.1 Ingredient composition of the experimental diets.

Diet	Bono[1]		Gatano[1]		
	Fine	Coarse	Fine	Coarse	N-free
Ingredient, %					
Hybrid rye	92.9	92.9	92.9	92.9	—
Cornstarch	—	—	—	—	68.1
Sucrose	—	—	—	—	20.0
Vegetable oil	3.0	3.0	3.0	3.0	2.5
Limestone	1.2	1.2	1.2	1.2	0.9
Monocalcium phosphate	1.2	1.2	1.2	1.2	1.8
Salt	0.4	0.4	0.4	0.4	0.4
Cellulose	—	—	—	—	5.0
Titanium dioxide	0.3	0.3	0.3	0.3	0.3
Vitamin-mineral premix[2]	1.0	1.0	1.0	1.0	1.0

[1]The hybrid rye diets utilized either finely or coarsely ground Bono or Gatano as the only source of amino acids (AA) and were formulated either with or without the inclusion of multi-carbohydrase (MC).

[2]Provided the following per kg of complete diet: vitamins A, 2,200 IU; vitamin D_3, 220 IU; vitamin E, 16.0 IU; vitamin K, 0.5 mg; thiamine, 1.0 mg; riboflavin, 3.5 mg; niacin, 30.0 mg; pantothenic acid, 10.0 mg; biotin 0.05 mg; folic acid, 0.3 mg; vitamin B_{12}, 0.02 mg; Cu, 6.0 mg as copper sulfate; I, 0.14 mg as calcium iodate; Fe, 100.0 mg as ferrous sulfate; Mn, 4.0 mg as manganese oxide; Se, 0.3 mg as sodium selenite; Zn, 100.0 mg as zinc oxide.

5.3.2 Experimental design and sample collection

The experiment was conducted as a replicated 9 × 3 incomplete Latin square design to give six observations per diet. Pigs were weighed at the beginning of each period and offered their daily ration as dry mash equivalent to 4% of BW divided into equal amounts at 0800 and 1600 h (Kiarie and Nyachoti, 2007). Water was available at all times throughout the experiment. Each period lasted for nine days, with the first 5 d serving as the adaptation period, followed by 2 d for fecal sample collection and the last 2 d for ileal digesta sample collection. Ileal digesta samples were collected continuously for 12 h (0800 to 2000 h) on d 8 and d 9, as described by Nyachoti et al. (2002). Digesta were collected in plastic bags attached to the barrel of the T-cannula by a hose clamp. Digesta collection bags contained 10 mL of 10% (vol/vol) formic acid to minimize bacterial activity. Fecal and digesta samples were immediately frozen at −20°C until further processing.

5.3.3 Particle size analysis, sample preparation, and chemical analysis

Hybrid rye PS distribution was measured by using a sieve shaker (RX-29, Superior Agri-System, MB, Canada) fitted with 13 multiple sieves (U.S. standard sieve Nos. 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, and 270). In general, 150 g ground hybrid rye samples were sieved for 10 min, and the geometric mean diameter (D_{gw}), geometric standard deviation (S_{gw}), and surface area were calculated according to the procedure of ANSI/ASAE S319.2 (American Society of Agricultural and Biological Engineers [ASABE], 1995).

Ileal digesta samples were thawed and pooled for each pig and period, homogenized in a blender (Waring Commercial, Torrington, CT, USA), sub-sampled, and freeze-dried. Fecal samples were dried in an oven at 50˚C for five days, pooled per pig within a period, and sub-sampled. Dried digesta, feces, and feed samples were ground to pass through a 1 mm screen before chemical analysis. Ingredients and diets samples were analyzed for dry matter (DM), nitrogen (N), AA, ether extract (EE), gross energy (GE), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, with ingredients additionally analyzed for calcium (Ca) and phosphorus (P) and diets additionally analyzed for TiO2. Fecal samples were analyzed for DM, GE, N, EE, ash, NDF, ADF, and TiO2, while ileal digesta samples were analyzed for DM, N, AA, and TiO2. Briefly, DM was determined according to procedure 934.01 of the Association of Official Analytical Chemists (AOAC, 2006). Nitrogen (N) was analyzed using a combustion analyzer (Model CNC-2000; LECO Corporation, St. Joseph, MI, USA), and crude protein (CP) content was calculated as N × 6.25. The AA profiles were determined according to procedure 982.30 E(a,b,c) of AOAC (2006). Tryptophan was determined following alkaline hydrolysis according to procedure 988.15 of AOAC (2006). The GE content was measured using an adiabatic bomb calorimeter (model 6400, Parr Instrument Co., Moline, IL, USA) using benzoic acid as the calibration standard. The EE content was determined after hexane extraction according to procedure 920.39 of AOAC (2006). The ash content was determined according to procedure 942.05 of AOAC (2006). The concentrations of Ca and P were analyzed according to procedure 968.08 and 946.06 of AOAC (2006), respectively, and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA, USA). The concentration of TiO_2 was determined according to procedures described by Lomer et al. (2000) and read on an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA, USA). The ADF and NDF contents were analyzed according to the method of Goering and Van Soest (1970) using Ankom 200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA).

5.3.4 Calculations and statistical analysis

Apparent ileal digestibility (AID) of AA were calculated using the following equation (Stein et al., 2007):

AID_{AA} (%) = 100 − [(AA_d/AA_f) × (Ti_f/Ti_d)] × 100,

where AA_d is the AA content in ileal digesta (mg/kg DM), AA_f is the AA content in feed (mg/kg DM), Ti_f is the TiO_2 concentration in feed (mg/kg DM), and Ti_d is the TiO_2 concentration in ileal digesta (mg/kg DM). The AID of CP and apparent total tract digestibility (ATTD) values were also calculated using the same equation.

The non-specific endogenous loss of AA (EAL) was measured at the distal ileum by feeding an N-free diet to growing pigs and calculated according to the following equation:

EAL = AA_d × (Ti_f/Ti_d),

where AA_d is the AA content in ileal digesta, Ti_f is the TiO_2 concentration in feed (mg/kg DM), and Ti_d is the TiO_2 concentration in ileal digesta (mg/kg DM). The basal ileal endogenous loss of CP was calculated using the same equation.

The SID values of AA were calculated using the following equation:

SID_{AA} (%) = AID_{AA} + (EAL/AA_f) × 100,

where AID_{AA} represents the AID of AA; EAL is the non-specific endogenous loss of AA (mg/kg DM), and AA_f is the AA content in feed (mg/kg DM). The SID of CP was calculated using the same equation.

Data were verified by the UNIVARIATE procedures of SAS 9.4 (SAS Inst. Inc., Cary, NC) for outliers and then analyzed using the MIXED procedure of SAS. The effects of pen and period were not significant in the study, so they were not included in the final model. The final model had factors that included variety, PS, MC supplementation, and two- and three-way interactions as the main effects. The LSMEANS procedure was used to calculate mean values, and the PDIFF option was used to separate means. Results were considered significant at $P < 0.05$ and considered a trend at $0.05 < P < 0.10$.

5.4 RESULTS

All pigs readily consumed their feed throughout the study. The D_{gw} of coarsely and finely ground Bono were 1,111 µm and 594 µm, and the respective values for coarsely and finely ground Gatano were 1,210 µm and 717 µm (Table 5.2). Bono had greater surface area when finely ground compared with coarsely ground (128.7 vs. 60.7 cm^2/g), and a similar pattern was observed in Gatano (98.4 vs. 51.3 cm^2/g). As stated in Table 5.3, the CP content was slightly greater in Bono (9.31%) than in Gatano (9.19%). In hybrid rye, Leu (0.58%) was the most abundant indispensable AA, whereas Trp (0.08%) was the least abundant indispensable AA. The analyzed nutrient composition of the experimental diets is displayed in Table 5.4.

There were no two- or three-way interaction effects for the ATTD of energy and nutrients in hybrid rye diets (Table 5.5). The ATTD of DM, GE, and CP were greater ($P < 0.05$) in Bono diets than in Gatano diets. The ATTD of NDF and EE were greater ($P < 0.05$) in finely ground hybrid rye diets than in coarsely ground hybrid rye diets, and the ATTD of GE tended to be greater ($P = 0.077$) in finely ground hybrid rye diets than in coarsely ground hybrid rye diets. Dietary MC supplementation improved ($P < 0.05$) the ATTD of DM, GE, CP, NDF, ADF, and ash in hybrid rye diets.

Two-way interactions between variety and MC ($P < 0.05$) were observed for the AID of CP and most AA including His, Ile, Leu, Lys, Met, Phe, Trp, Val, Cys, Glu, and Ser (Table 5.6 and 5.7). However, no other two-way interactions or three-way interactions were observed for the AID of CP and AA in hybrid rye. The AID values of CP and AA such as Met, Trp, and Cys, were greater ($P < 0.05$) in Bono than in Gatano, and Bono tended to contain greater ($P < 0.10$) AID for Ile, Thr, Gly, Ser, and Tyr than Gatano. There were no effects of PS on the AID of CP and AA in hybrid rye, with the exception that finely grinding tended to improve ($P = 0.076$) the AID of Tyr.

Dietary MC supplementation increased ($P < 0.05$) the AID of CP and all AA, although it showed a tendency to improve ($P = 0.096$) the AID of Tyr.

Likewise, there were two-way interactions ($P < 0.05$) between the variety and MC for the SID of CP and most AA (Table 5.8 and 5.9). No additional two-way interactions or three-way interactions were observed for the SID of CP and AA in hybrid rye. The SID for CP was greater ($P < 0.05$) in Bono than in Gatano. However, neither the variety nor PS had an effect on the SID values for AA in hybrid rye, with the only exception that Bono had a higher ($P < 0.05$) SID for Met than Gatano. The addition of MC improved ($P < 0.05$) the AID of CP and all AA, except that it tended to improve ($P = 0.060$) the SID of Tyr.

Table 5.2 Particle size analysis of hybrid rye[1].

Hybrid rye variety	Bono		Gatano	
Particle size	Fine	Coarse	Fine	Coarse
Distribution of particles, %				
Sieve opening, µm				
2,360	0.60	1.33	0.73	1.36
1,700	2.34	44.82	3.74	45.94
1,180	23.95	17.67	30.29	20.30
850	23.21	12.89	25.88	13.78
600	14.98	7.35	13.14	6.49
420	9.10	4.14	7.14	3.24
297	6.15	3.09	4.67	2.40
212	4.88	2.01	3.60	1.48
150	3.08	1.61	2.13	1.20
103	2.27	1.08	1.73	0.88
73	1.07	0.84	0.87	0.72
53	1.20	0.32	1.07	0.48
37	7.16	2.85	5.00	1.72
Coarse particles (≥ 1,700 µm)	2.94	46.14	4.47	47.30
Medium particles (297 to 1,700 µm)	77.39	45.14	81.12	46.22
Fine particles (< 297 µm)	19.67	8.71	14.41	6.49
Geometric mean particle size, µm	594	1,111	717	1,210
Standard deviation, µm	2.77	2.43	2.55	2.20
Surface area, cm^2/g	128.7	60.7	98.4	51.3

[1]Two hybrid rye varieties (Bono and Gatano) were ground using a hammermill fitted with 4.0-mm and 3.2-mm screens to obtain fine and coarse particle size, respectively.

Table 5.3 Analyzed nutrient composition of hybrid rye, as-fed basis.

	Hybrid rye[1]	
Variety	Bono	Gatano
Analyzed composition		
DM, %	89.73	89.57
GE, kcal/kg	3,859	3,845
CP, %	9.31	9.19
EE, %	1.18	1.42
Ash, %	1.36	1.41
NDF, %	11.24	13.35
ADF, %	2.90	3.12
Ca, %	0.07	0.07
P, %	0.25	0.25
Indispensable AA, %		
Arg	0.45	0.44
His	0.21	0.21
Ile	0.34	0.33
Leu	0.59	0.57
Lys	0.41	0.40
Met	0.17	0.16
Phe	0.41	0.41
Thr	0.31	0.30
Trp	0.08	0.07
Val	0.45	0.45
Dispensable AA, %		
Ala	0.42	0.41
Asp	0.69	0.69
Cys	0.22	0.19
Glu	2.01	2.00
Gly	0.42	0.42
Pro	0.77	0.81
Ser	0.36	0.35
Tyr	0.14	0.14

[1]Two hybrid rye varieties (Bono and Gatano) were finely ground with grain miller for 50-200 μm of fitness for chemical analysis.

Table 5.4 Analyzed nutrient composition of the experimental diets, as-fed basis.

	Bono[1]		Gatano[1]		
Item	Fine	Coarse	Fine	Coarse	N-free
DM, %	90.77	90.65	90.22	90.09	92.45
GE, kcal/kg	4,016	3,990	4,031	3,955	3,744
CP, %	8.32	8.79	7.85	7.94	0.63
EE, %	6.25	5.52	6.52	5.72	1.68
Ash, %	4.38	4.57	4.39	4.55	1.83
NDF, %	11.49	11.38	12.46	12.38	—
ADF, %	2.78	2.82	3.07	3.03	—
Indispensable AA, %					
Arg	0.44	0.42	0.40	0.41	0.01
His	0.19	0.19	0.18	0.17	0.00
Ile	0.31	0.30	0.28	0.27	0.01
Leu	0.54	0.52	0.50	0.49	0.03
Lys	0.38	0.37	0.34	0.35	0.02
Met	0.15	0.15	0.13	0.13	0.01
Phe	0.38	0.36	0.35	0.34	0.02
Thr	0.29	0.28	0.26	0.26	0.01
Trp	0.08	0.07	0.07	0.07	0.02
Val	0.43	0.41	0.39	0.38	0.01
Dispensable AA, %					
Ala	0.39	0.38	0.36	0.36	0.01
Asp	0.65	0.63	0.60	0.60	0.02
Cys	0.20	0.19	0.17	0.17	0.00
Glu	1.83	1.69	1.66	1.56	0.02
Gly	0.39	0.38	0.36	0.37	0.01
Pro	0.71	0.65	0.66	0.62	0.04
Ser	0.33	0.31	0.30	0.29	0.01
Tyr	0.18	0.16	0.15	0.17	0.01

[1]The hybrid rye diets utilized either finely or coarsely ground Bono or Gatano as the only source of amino acids (AA) and were formulated either with or without the inclusion of multi-carbohydrase (MC).

Table 5.5 Effects of particle size (PS) and multi-carbohydrase (MC) supplementation on apparent total tract digestibility (ATTD) of energy and nutrients in hybrid rye diets fed to growing pigs.

Treatments			ATTD, %						
Variety	PS	MC	DM	GE	CP	NDF	ADF	EE	Ash
Bono	Fine	With	85.89	85.45	68.01	69.77	47.82	74.10	55.22
Bono	Fine	Without	84.23	83.87	63.83	63.40	40.49	69.39	48.41
Bono	Coarse	With	84.71	84.42	65.21	66.12	47.22	70.38	52.95
Bono	Coarse	Without	84.30	83.74	66.45	64.39	34.08	68.21	52.62
Gatano	Fine	With	85.16	85.00	66.36	69.41	47.65	76.82	57.53
Gatano	Fine	Without	83.22	82.92	61.51	65.62	38.82	73.92	53.70
Gatano	Coarse	With	84.04	83.37	62.95	63.12	43.03	69.88	56.51
Gatano	Coarse	Without	82.10	81.71	55.56	60.88	36.47	68.72	50.48
		SEM	0.725	0.777	2.165	2.221	3.135	2.857	2.796
Main effects									
Variety		Bono	84.78^{x}	84.37^{x}	65.87^{x}	65.92	42.40	70.52	52.30
		Gatano	83.63^{y}	83.25^{y}	61.60^{y}	64.76	41.49	72.33	54.55
		SEM	0.390	0.415	1.189	1.194	1.801	1.452	1.435
PS		Fine	84.63	84.31	64.93	67.05^{x}	43.69	73.56^{x}	53.71
		Coarse	83.79	83.31	62.54	63.63^{y}	40.20	69.30^{y}	53.14
		SEM	0.397	0.416	1.243	1.143	1.760	1.394	1.467
MC		With	84.95^{x}	84.56^{x}	65.63^{x}	67.10^{x}	46.43^{x}	72.79	55.55
		Without	83.46^{y}	83.06^{y}	61.84^{y}	63.57^{y}	37.46^{y}	70.06	51.30
		SEM	0.377	0.401	1.207	1.139	1.531	1.432	1.378
P-value									
Variety			0.031	0.048	0.008	0.464	0.684	0.374	0.276
PS			0.110	0.077	0.128	0.036	0.123	0.042	0.781
MC			0.006	0.031	0.018	0.030	<0.001	0.184	0.045
Variety × PS			0.585	0.451	0.142	0.332	0.997	0.377	0.453
Variety × MC			0.382	0.506	0.137	0.743	0.570	0.729	0.741
PS × MC			0.545	0.554	0.642	0.191	0.693	0.599	0.603
Variety × PS × MC			0.546	0.829	0.201	0.627	0.368	0.922	0.295

$^{x, y}$ Means within a column without a common superscript are significantly different ($P < 0.05$).

Table 5.6 Effects of particle size (PS) and multi-carbohydrase (MC) supplementation on apparent ileal digestibility (AID) of crude protein (CP) and indispensable amino acids (AA) in hybrid rye fed to growing pigs.

Treatments			AID, %										
Variety	PS	MC	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Bono	Fine	With	71.04	76.86	77.33	77.18	77.43	70.42	82.97	79.79	69.47	74.38	75.78
Bono	Fine	Without	67.51	73.82	72.32	72.44	72.75	65.70	79.11	75.90	63.67	69.36	71.84
Bono	Coarse	With	69.37	72.35	71.96	73.38	73.31	64.60	78.45	76.31	64.49	75.20	72.17
Bono	Coarse	Without	68.90	73.96	73.47	73.44	73.44	64.53	79.98	76.07	65.06	73.27	72.37
Gatano	Fine	With	70.15	76.68	76.99	75.77	76.44	70.29	80.66	79.45	66.15	75.81	75.37
Gatano	Fine	Without	58.16	69.88	68.74	68.10	69.04	60.16	74.45	72.15	58.52	61.10	67.26
Gatano	Coarse	With	70.24	74.62	76.74	76.00	76.58	69.66	79.92	79.80	67.46	71.75	75.06
Gatano	Coarse	Without	60.72	70.25	69.59	67.67	69.70	56.36	74.08	72.94	59.23	68.50	67.85
		SEM	1.954	1.796	1.628	1.685	1.556	2.697	1.420	1.404	2.265	1.872	1.603
Main effects													
Variety		Bono	69.21^{x}	74.25	73.77	74.11	74.23	66.31	80.13^{x}	77.02	65.67	73.05^{x}	73.04
		Gatano	64.82^{y}	72.86	73.02	71.88	72.94	64.11	77.28^{y}	76.08	62.84	69.29^{y}	71.38
		SEM	1.230	0.969	1.000	1.022	0.938	1.589	0.840	0.872	1.252	1.221	0.968
PS		Fine	66.72	74.31	73.85	73.37	73.91	66.64	79.30	76.82	64.45	70.16	72.56
		Coarse	67.30	72.79	72.94	72.62	73.26	63.79	78.11	76.28	64.06	72.18	71.86
		SEM	1.295	0.962	0.996	1.040	0.942	1.571	0.875	0.873	1.280	1.259	0.976
MC		With	70.20^{x}	75.13^{x}	75.76^{x}	75.58^{x}	75.94^{x}	68.74^{x}	80.50^{x}	78.84^{x}	66.89^{x}	74.28^{x}	74.60^{x}
		Without	63.82^{y}	71.98^{y}	71.03^{y}	70.41^{y}	71.23^{y}	61.69^{y}	76.90^{y}	74.26^{y}	61.62^{y}	68.06^{y}	69.83^{y}
		SEM	1.125	0.923	0.875	0.896	0.810	1.422	0.808	0.736	1.162	1.105	0.847
P-value													
Variety			0.003	0.281	0.517	0.070	0.248	0.257	0.007	0.351	0.085	0.007	0.152
PS			0.674	0.240	0.437	0.536	0.556	0.143	0.244	0.588	0.809	0.137	0.539
MC			<0.001	0.018	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
Variety × PS			0.325	0.603	0.302	0.587	0.341	0.739	0.530	0.270	0.387	0.794	0.464
Variety × MC			0.003	0.063	0.014	0.023	0.033	0.019	0.020	0.016	0.106	0.044	0.015
PS × MC			0.600	0.171	0.106	0.391	0.235	0.847	0.159	0.311	0.374	0.009	0.273
Variety × PS × MC			0.917	0.663	0.248	0.258	0.338	0.312	0.219	0.425	0.285	0.123	0.481

$^{x, y}$ Means within a column without a common superscript are significantly different ($P < 0.05$).

Table 5.7 Effects of particle size (PS) and multi-carbohydrase (MC) supplementation on apparent ileal digestibility (AID) of dispensable amino acids (AA) in hybrid rye fed to growing pigs.

Treatments			AID, %						
Variety	PS	MC	Ala	Asp	Cys	Glu	Gly	Ser	Tyr
Bono	Fine	With	71.67	76.31	77.84	86.01	52.63	73.46	71.93
Bono	Fine	Without	67.06	71.23	73.97	82.83	48.83	69.87	67.72
Bono	Coarse	With	66.64	71.75	74.69	84.44	52.10	70.98	70.06
Bono	Coarse	Without	66.80	72.19	76.35	84.15	51.32	70.60	68.00
Gatano	Fine	With	69.15	74.89	78.35	85.76	52.36	72.67	71.83
Gatano	Fine	Without	61.73	67.99	70.58	79.72	39.79	64.09	66.53
Gatano	Coarse	With	70.38	74.71	77.17	86.52	50.35	74.06	63.83
Gatano	Coarse	Without	61.06	67.32	68.95	80.68	40.25	65.11	65.28
		SEM	2.221	1.700	1.293	0.999	4.491	1.654	2.099
Main effects									
Variety		Bono	68.04	72.87	75.71^{x}	84.36	51.22	71.23	69.43
		Gatano	65.58	71.23	73.76^{y}	83.17	45.69	68.98	66.87
		SEM	1.248	0.999	0.888	0.666	2.263	1.032	1.107
PS		Fine	67.40	72.61	75.19	83.58	48.40	70.02	69.50
		Coarse	66.22	71.49	74.29	83.95	48.51	70.19	66.79
		SEM	1.262	1.003	0.901	0.674	2.325	1.054	1.100
MC		With	69.46^{x}	74.42^{x}	77.01^{x}	85.68^{x}	51.86^{x}	72.79^{x}	69.41
		Without	64.16^{y}	69.69^{y}	72.46^{y}	81.85^{y}	45.05^{y}	67.42^{y}	66.88
		SEM	1.146	0.884	0.774	0.544	2.221	0.895	1.108
P-value									
Variety			0.126	0.180	0.039	0.100	0.090	0.063	0.093
PS			0.456	0.360	0.333	0.609	0.974	0.887	0.076
MC			0.002	<0.001	<0.001	<0.001	0.039	<0.001	0.096
Variety × PS			0.358	0.572	0.580	0.490	0.784	0.382	0.205
Variety × MC			0.058	0.052	<0.001	0.005	0.164	0.006	0.687
PS × MC			0.651	0.303	0.174	0.283	0.668	0.547	0.142
Variety × PS × MC			0.297	0.218	0.110	0.347	0.965	0.450	0.443

$^{x, y}$ Means within a column without a common superscript are significantly different ($P < 0.05$).

Table 5.8 Effects of particle size (PS) and multi-carbohydrase (MC) supplementation on standardized ileal digestibility (SID) of crude protein (CP) and indispensable amino acids (AA) in hybrid rye fed to growing pigs[1].

Treatments			SID, %										
Variety	PS	MC	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Bono	Fine	With	91.35	88.92	84.80	84.84	85.34	82.80	87.06	83.60	84.20	86.66	83.98
Bono	Fine	Without	88.43	86.75	80.22	80.33	81.13	78.42	83.47	83.10	78.92	81.62	80.23
Bono	Coarse	With	89.66	84.67	79.83	81.03	81.35	77.33	82.80	83.30	79.20	85.93	80.37
Bono	Coarse	Without	87.63	85.45	80.53	80.60	80.90	76.27	83.80	82.52	78.77	83.98	79.97
Gatano	Fine	With	91.56	89.53	85.28	84.23	85.08	83.65	85.33	86.80	82.50	87.98	84.15
Gatano	Fine	Without	80.16	82.48	77.06	76.56	77.52	73.16	79.14	79.54	74.32	75.36	76.06
Gatano	Coarse	With	91.50	88.15	84.60	84.17	84.90	83.02	84.58	86.75	83.18	85.97	83.60
Gatano	Coarse	Without	82.29	82.80	77.97	76.10	78.20	69.72	78.75	80.27	74.98	80.68	76.60
		SEM	1.953	1.798	1.627	1.687	1.551	2.695	1.419	1.404	2.264	1.873	1.604
Main effects													
Variety		Bono	89.27^{x}	86.45	81.35	81.70	82.18	78.70	84.28^{x}	83.88	80.27	84.55	81.14
		Gatano	86.38^{y}	85.74	81.20	80.27	81.43	77.39	81.95^{y}	83.34	78.75	82.50	80.10
		SEM	1.210	0.986	0.985	1.017	0.935	1.596	0.835	0.862	1.260	1.166	0.965
PS		Fine	87.88	86.92	81.84	81.49	82.27	79.51	83.75	84.01	79.98	82.91	81.11
		Coarse	87.77	85.27	80.71	80.48	81.34	76.58	82.48	83.21	79.03	84.14	80.13
		SEM	1.232	0.970	0.977	1.018	0.930	1.567	0.853	0.858	1.263	1.174	0.962
MC		With	91.02^{x}	87.82^{x}	83.63^{x}	83.57^{x}	84.17^{x}	81.70^{x}	84.94^{x}	85.86^{x}	82.27^{x}	86.64^{x}	83.02^{x}
		Without	84.63^{y}	84.37^{y}	78.92^{y}	78.40^{y}	79.44^{y}	74.39^{y}	81.29^{y}	81.36^{y}	76.75^{y}	80.41^{y}	78.22^{y}
		SEM	1.044	0.922	0.856	0.872	0.797	1.402	0.781	0.724	1.131	0.990	0.830
P-value													
Variety			0.044	0.582	0.902	0.236	0.496	0.494	0.026	0.590	0.347	0.130	0.368
PS			0.939	0.201	0.332	0.400	0.401	0.134	0.215	0.425	0.556	0.357	0.397
MC			<0.001	0.010	<0.001	0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
Variety × PS			0.582	0.383	0.307	0.532	0.289	0.645	0.492	0.258	0.317	0.755	0.400
Variety × MC			0.008	0.037	0.021	0.030	0.035	0.021	0.024	0.022	0.104	0.046	0.021
PS × MC			0.418	0.366	0.150	0.446	0.299	0.947	0.225	0.385	0.456	0.056	0.335
Variety × PS × MC			0.816	0.807	0.415	0.355	0.514	0.427	0.299	0.628	0.452	0.428	0.622

$^{x, y}$ Means within a column without a common superscript are significantly different ($P < 0.05$).

[1]Basal ileal endogenous losses for calculating standardized ileal digestibility (SID) of crude protein (CP) and indispensable amino acids (AA) were determined (mg/kg dry matter intake) as nitrogen, 3,019; arginine, 571; histidine, 156; isoleucine, 253; leucine, 461; lysine, 505; methionine, 67; phenylalanine, 277; threonine, 454; tryptophan, 95; valine, 370.

Table 5.9 Effects of particle size (PS) and multi-carbohydrase (MC) supplementation on standardized ileal digestibility (SID) of dispensable amino acids (AA) in hybrid rye fed to growing pigs[1].

Treatments			SID, %						
Variety	PS	MC	Ala	Asp	Cys	Glu	Gly	Ser	Tyr
Bono	Fine	With	83.72	85.72	84.68	90.10	86.80	85.88	83.90
Bono	Fine	Without	79.43	85.12	81.18	87.18	83.90	82.72	81.25
Bono	Coarse	With	78.68	81.45	81.53	88.50	87.16	83.03	81.35
Bono	Coarse	Without	78.22	81.18	82.52	87.87	83.72	81.93	79.93
Gatano	Fine	With	82.13	85.02	85.93	90.30	88.15	85.85	84.43
Gatano	Fine	Without	74.38	78.00	78.20	84.26	74.74	77.32	78.42
Gatano	Coarse	With	83.00	84.70	84.78	90.60	86.13	86.38	79.35
Gatano	Coarse	Without	73.68	77.43	77.00	85.07	76.00	78.30	77.88
		SEM	2.222	1.699	1.292	0.998	4.493	1.653	2.101
Main effects									
Variety		Bono	80.01	82.37	82.48	88.41	85.39	83.39	81.61
		Gatano	78.30	81.29	81.48	87.56	81.26	81.96	80.02
		SEM	1.257	0.995	0.867	0.655	2.279	1.012	1.073
PS		Fine	79.92	82.46	82.50	87.96	83.40	82.94	82.00
		Coarse	78.40	81.19	81.46	88.01	83.25	82.41	79.63
		SEM	1.255	0.990	0.863	0.659	2.310	1.017	1.052
MC		With	81.88^{x}	84.22^{x}	84.23^{x}	89.88^{x}	87.06^{x}	85.29^{x}	82.26
		Without	76.43^{y}	79.43^{y}	79.73^{y}	86.09^{y}	79.59^{y}	80.07^{y}	79.37
		SEM	1.133	0.867	0.732	0.527	2.177	0.864	1.042
P-value									
Variety			0.283	0.375	0.281	0.233	0.201	0.230	0.293
PS			0.340	0.297	0.262	0.647	0.964	0.654	0.119
MC			0.001	<0.001	<0.001	<0.001	0.024	<0.001	0.060
Variety × PS			0.314	0.494	0.884	0.478	0.942	0.279	0.770
Variety × MC			0.058	0.058	0.001	0.007	0.185	0.012	0.570
PS × MC			0.722	0.401	0.233	0.330	0.831	0.595	0.337
Variety × PS × MC			0.398	0.346	0.223	0.533	0.766	0.732	0.581

$^{x, y}$ Means within a column without a common superscript are significantly different ($P < 0.05$).

[1]Basal ileal endogenous losses for calculating standardized ileal digestibility (SID) of dispensable amino acids (AA) were determined (mg/kg dry matter intake) as alanine, 504; aspartic acid, 664; cysteine, 143; glutamic acid, 782; glycine, 1430; proline, 4769; serine, 425; tyrosine, 224.

5.5 DISCUSSION

Hybrid rye has gained attention as a promising feed ingredient for pigs, especially due to its reduced ergot alkaloid levels and higher yield (Miedaner and Geiger, 2015). The CP (9.25%) and AA contents in hybrid rye used in this study were lower than those reported by NRC (2012) and Rodehutscord et al. (2016) but within the range of values reported by McGhee and Stein (2018) and Strang et al. (2016), indicating that different rye cultivars may cause variations in protein and AA contents. Furthermore, this study identified compositional variance between the Bono and Gatano hybrid rye varieties, most notably in NDF content (11.24 vs. 13.35%), which could be attributed to genetic differences. The AID and SID of AA in hybrid rye, as determined in this study, were comparable to the values reported by NRC (2012). However, they were greater than the values reported by McGhee and Stein (2018). This variance in AID and SID of AA could be attributed to several factors, including the specific genotype of the rye used, the overall composition of the diet with particular emphasis on the levels of dietary fiber and antinutritional components, the type of the indigestible marker used, and the processing methods applied.

The grinding process holds a crucial role in feed manufacturing, serving to diminish PS while enhancing the digestibility of energy and nutrients in pigs (Rojas and Stein, 2015). However, too fine PS is associated with an increased risk of gastric ulcers in pigs (Kiarie and Mills, 2019). Therefore, the recommended PS for ground cereal grains is usually between 500 and 700 µm (Behnke, 1994). Nonetheless, the use of fine grinding can lead to higher electrical energy usage and thus elevate the overall manufacturing costs for feed (Koo and Nyachoti, 2021). Also, dietary MC supplementation has been shown to improve energy and nutrients digestibility in various feedstuffs fed to pigs (Omogbenigun et al., 2004; Emiola et al., 2009). However, to the best of our knowledge, no study has yet explored its effect on AA digestibility in hybrid rye. Moreover, there has been a lack of research concerning how PS affects AA digestibility in hybrid rye. Therefore, this study was conducted to investigate the effects of both PS and MC supplementation on AA digestibility in hybrid rye fed to growing pigs.

In the current study, a major difference in PS between fine and coarse Bono was the substantially lower proportion of coarse particles (⩾1,700 μm) in the finely ground Bono (2.94%), compared to 46.14% in its coarse counterpart. A comparable pattern was also observed for Gatano, with 4.47% coarse particles (⩾1,700 μm) in the finely ground Gatano and 47.30% in the coarsely ground Gatano. When compared to coarse grinding, fine grinding substantially enlarged the surface area of the hybrid rye. For Bono, the surface area increased by 112% when fine grinding was applied, as opposed to coarse grinding. Similarly, fine grinding led to a 92% increase in the surface area for Gatano when compared to its coarsely ground counterpart.

Compared with Bono diets, Gatano diets showed lower ATTD of nutrients in terms of DM, GE, and CP, which could be due to the greater NDF content in Gatano. Previous studies have suggested that higher NDF levels can adversely affect the DM and GE digestibility and that greater concentrations of soluble dietary fiber can increase viscosity and reduce nutrient digestibility (Annison and Choct, 1991; Kim and Nyachoti, 2017). Additionally, reducing the PS in hybrid rye diets improved the ATTD of NDF and EE and tended to improve the ATTD of GE, which could be explained by the fact that reduced PS increases the surface area of feed ingredients, thus allowing for better contact with the pig's digestive enzymes (Wondra et al., 1995). Moreover, the inclusion of MC in hybrid rye diets improved the ATTD of DM, GE, CP, NDF, ADF, and ash, demonstrating that MC supplementation can break down NSP in hybrid rye, leading to better digestibility of an array of nutrients. The results of this study were in line with previous studies, such as those by Agyekum et al. (2016) and Huang et al. (2021), which demonstrated that multienzyme supplementation could significantly improve the ATTD of nutrients in diverse feed compositions. Similarly, enhancements in nutrient digestibility with enzyme supplementation were also noted by Kiarie et al. (2020) in roasted full-fat soybeans and expelled-extruded soybean meal diets and Niu et al. (2022) in diets high in canola meal.

No differences were found between Bono and Gatano regarding the SID of AA, which was consistent with the observations by McGhee and Stein (2018), who reported that the SID of AA in three varieties of rye was similar. However, Bono did exhibit a higher SID of CP compared to Gatano, possibly due to Gatano's higher fiber content. Additionally, reducing PS did not improve the SID of AA in hybrid rye, a finding that was in agreement with the study by Rojas and Stein (2015), where corn PS did not affect the SID of AA. However, studies with other ingredients such as distillers dried grains with solubles (DDGS) and lupins have shown that reducing PS can improve the SID of AA (Kim et al., 2009; Yáñez et al., 2011). These inconsistencies might be attributed to the impact of grinding intensity on the flow behavior of the digesta between hybrid rye and other ingredients. Dietary MC supplementation improved the SID of AA in hybrid rye, which was consistent with the previous study by Ayoade et al. (2012), in which MC supplementation increased the SID of AA, such as Leu, Met + Cys, Thr, Cys, Ser, and Tyr in extruded full-fat soybeans. However, Dadalt et al. (2016) reported that MC supplementation did not affect most AA digestibility in pea protein isolate for growing pigs. Similarly, a study by Kiarie et al. (2010) indicated that MC supplementation did not affect the digestibility of most AA in a wheat and barley-based diet fed to growing pigs. These different outcomes could be due to hybrid rye's higher NSP content compared to the other ingredients. Interestingly, this study also revealed interactions between variety and MC supplementation on the SID of CP and most AA. This might be because MC was more effective on Gatano than on Bono, potentially due to Gatano's higher fiber content.

5.6 CONCLUSION

The digestibility of nutrients in hybrid rye is influenced by the variety used. Reducing the hybrid rye particle size enhanced nutrient digestibility, whereas multi-carbohydrase supplementation improved the digestibility of energy and AA in hybrid rye diets for growing pigs.

TRANSITION STATEMENT

Manuscript II (Experiment 2) evaluated the impacts of particle size and MC supplementation on AA digestibility in hybrid rye fed to growing pigs. The manuscript II found that decreasing the particle size of hybrid rye improved nutrient digestibility, while adding multi-carbohydrase increased the digestibility of energy and AA in hybrid rye diets for growing pigs. Following this, Manuscript III (Experiment 3) was conducted to determine the effects of particle size and different levels of phytase supplementation on phosphorus digestibility in hybrid rye fed to growing pigs.

CHAPTER SIX: MANUSCRIPT III

Effects of particle size and phytase supplementation on apparent and standardized total tract digestibility of phosphorus in hybrid rye fed to growing pigs

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6.1 ABSTRACT

An experiment was conducted to investigate the effects of particle size (PS) and levels of phytase supplementation on the apparent (ATTD) and standardized (STTD) total tract digestibility of P in hybrid rye fed to growing pigs. Thirty-six growing barrows (23.6 ± 1.5 kg initial BW) were individually housed in metabolism crates and randomly allotted to one of six dietary treatments to give six replicates per treatment. The six dietary treatments were arranged in a 2 × 3 factorial with main effects of PS and phytase supplementation levels (0, 500, or 2,500 FTU/kg). Hybrid rye was ground using a hammermill mounted with 4.0-mm and 3.2-mm screens to obtain material with coarse and fine PS, respectively. Pigs were fed experimental diets for 11 d, including 5 d for adaptation and 6 d for total collection of feces. All data were analyzed using the MIXED procedure of SAS with PS, phytase, and their interaction as fixed effects. Orthogonal polynomial contrasts were used to test linear and quadratic effects of phytase level in both coarse and fine hybrid rye diets. The ATTD and STTD of P were greater ($P < 0.05$) in diets with fine than in those with coarse hybrid rye. Increasing levels of phytase supplementation linearly ($P < 0.01$) and quadratically ($P < 0.01$) improved the ATTD and STTD of P in hybrid rye diets. There were no interaction effects between PS and phytase supplementation on the ATTD and STTD of P in hybrid rye diets. In conclusion, PS reduction improved the digestibility of P in hybrid rye, and supplementing increasing levels of phytase improved P digestibility in linear and quadratic manner in growing pigs fed hybrid rye diets.

Keywords: digestibility, hybrid rye, microbial phytase, phosphorus, pigs

6.2 INTRODUCTION

Rye is a cereal grain that can be used as a feed ingredient for swine (Bengtsson et al., 1992). However, rye is traditionally not used in large amounts in livestock feeds because it is highly susceptible to ergot contamination, which produces ergot alkaloids that cause detrimental health issues in animals (Coufal-Majewski et al., 2016). Recently, a new commercial hybrid rye that is less susceptible to ergot contamination has been developed. The metabolizable energy (ME) content of the new hybrid rye is not different from barley and sorghum, indicating that it can be used as an alternative energy source in swine diets (McGhee and Stein, 2020).

Phosphorus is one of the most expensive nutrients added in swine diets. Minimizing P excretion must be a focus for feeding swine to reduce the environmental load of P (Misiura et al., 2020). However, the majority of total P in cereal grains is bound to phytic acid, which is not available to pigs (Adeola and Cowieson, 2011). Therefore, supplementing microbial phytase in diets is a common practice in the feed industry because the enzyme can break down phytic acid and increase the digestibility of P. Moreover, particle size (PS) reduction in cereal grains can increase nutrient digestibility because it increases the relative surface area of dietary particles, allowing better contact with digestive enzymes (Oryschak et al., 2002; Rojas and Stein, 2015; Lancheros et al., 2020).

Although rye contains a higher amount of intrinsic phytase than many cereal grains such as wheat, barley, and maize (Rodehutscord et al., 2016), the effects of microbial phytase levels on the P digestibility in hybrid rye has not been investigated. Also, the effects of PS on P digestibility of hybrid rye fed to growing pigs have not been reported. Therefore, the objective of this study was to determine the individual and interactive effects of hybrid rye PS and phytase levels (0, 500, and 2,500 FTU/kg) on the apparent total tract digestibility (ATTD) and the standardized total tract digestibility (STTD) of P in hybrid rye fed to growing pigs. It was hypothesized that the P digestibility of hybrid rye would be improved by the increasing level of phytase supplementation and reduced PS and that the efficacy of phytase would be greater in the finely ground hybrid rye than in the coarsely ground hybrid rye.

6.3 MATERIALS AND METHODS

The animal use protocol was reviewed and approved by the University of Manitoba Animal Care Committee (AC11419), and pigs were managed according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

6.3.1 Experimental animals and diets

Thirty-six growing barrows (TN70 × TN Tempo; Topigs Norsvin, Winnipeg, MB, Canada) with an initial body weight (BW) of 23.6 ± 1.5 kg were used in this study. Pigs were individually housed in adjustable metabolism crates (1.8 × 0.6 m) in a temperature-controlled room (24 ± 1°C) throughout the experiment. Each metabolism crate was equipped with a stainless-steel feeder, a nipper drinker, plastic-covered expanded metal floors, and a screen underneath the floors for fecal collection.

The hybrid rye used in this study was obtained from KWS (KWS Lochow GmbH, Bergen, Germany) and ground using a hammer mill fitted with 4.0- and 3.2-mm screens to obtain coarse and fine PS, respectively. Six diets consisting of either fine or coarse hybrid rye PS supplemented with phytase at 0, 500, and 2,500 FTU/kg of feed (Canadian Bio-System Inc., Calgary, AB, Canada) were formulated (Table 6.1). Hybrid rye was the only source of P in the diets, and vitamins and minerals, except for P, were provided to meet or exceed requirements for growing pigs (NRC, 2012).

Table 6.1 Composition and nutrient content of experimental diets, as-fed basis

Hybrid rye particle size[1]	Fine			Coarse		
Phytase, FTU/kg	0	500	2,500	0	500	2,500
Ingredients, %						
Hybrid rye	84.5	84.5	84.5	84.5	84.5	84.5
Sucrose	10.0	10.0	10.0	10.0	10.0	10.0
Limestone	1.1	1.1	1.1	1.1	1.1	1.1
Vegetable oil	3.0	3.0	3.0	3.0	3.0	3.0
Salt	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin-mineral premix[2]	1.0	1.0	1.0	1.0	1.0	1.0
Analyzed content						
DM, %	91.13	91.23	90.95	90.54	90.72	90.78
GE, kcal/kg	3,998	3,962	3,965	3,954	3,939	3,942
CP, %	8.25	8.06	8.00	8.63	8.13	8.31
EE, %	3.82	4.01	3.91	3.71	4.08	3.92
Ash, %	2.17	2.20	2.17	2.00	2.19	2.27
NDF, %	13.65	14.34	15.12	14.18	12.05	13.87
ADF, %	2.67	2.57	2.62	2.65	2.86	2.69
Ca, %	0.42	0.42	0.42	0.41	0.42	0.42
P, %	0.27	0.26	0.26	0.26	0.25	0.25

[1]Geometric mean particle size of fine and coarse hybrid rye were 569 and 1,104 μm, respectively.
[2]The vitamin-mineral premix provided the following per kg of complete diet: vitamins A, 2,200 IU; vitamin D3, 220 IU; vitamin E, 16.0 IU; vitamin K, 0.5 mg; thiamine, 1.0 mg; riboflavin, 3.5 mg; niacin, 30.0 mg; pantothenic acid, 10.0 mg; vitamin B12, 0.02 mg; folic acid, 0.3 mg; Cu, 6.0 mg as copper sulfate; I, 0.14 mg as calcium iodate; Fe, 100.0 mg as ferrous sulfate; Mn, 4.0 mg as manganese oxide; Se, 0.3 mg as sodium selenite; Zn, 100.0 mg as zinc oxide; biotin 0.05 mg.

6.3.2 Experimental design and sample collection

The experiment was conducted in two consecutive periods each lasting 11 d under the same facility and similar experimental conditions and procedures. In each period, 18 pigs were randomly assigned to one of six treatments in a 2 × 3 factorial arrangement to give three replicates per treatment per period and six replicates in total for each treatment. The two factors were PS (fine or coarse) and phytase supplementation level (0, 500, and 2,500 FTU/kg of feed).

Pigs were fed their assigned diets once daily at 0830 h based on BW on d 1 and d 5 at the rate of 550 kcal ME/kg $BW^{0.60}$, which was close to ad libitum intake (Noblet et al., 1994). Pigs had free access to water via a low-pressure nipple drinker throughout the experiment. In each period, pigs were fed experimental diets for a 5-d adaptation period followed by a 6-d period for total collection of feces according to the marker-to-marker procedure (Adeola, 2001). Briefly, in the morning (0830 h) of d 6, each pig received 5 g of ferric oxide as an indigestible marker mixed with 100 g of the total daily feed allowance. The remaining daily feed allowance was provided after all the marked feed was consumed. Fecal collection was initiated when the marker appeared in feces. In the morning (0830 h) of d 11, pigs received 100 g of marked feed as described above, and fecal collection was terminated when the marker appeared in feces. Feces were collected once daily in the morning and stored at −20° C for further analysis.

6.3.3 Particle size analysis, sample preparation, and chemical analysis

Particle size distribution was measured using a sieve shaker (RX-29, Superior Agri-System, Winnipeg, MB, Canada) fitted with 13 multiple sieves (U.S. standard sieve Nos. 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, and 270), and a sample of approximately 150 g for each fine and coarse hybrid rye was sieved for 10 min. The geometric mean diameter (D_{gw}), geometric standard deviation (S_{gw}), and surface area of each sample were calculated according to the ANSI/ASAE

S319.2 standard method (American Society of Agricultural and Biological Engineers [ASABE], 1995).

Fecal samples were dried in a forced-air oven at 50° C for five days, pooled per pig within a period, and sub-sampled. Ingredient, diet, and fecal samples were finely ground with a grain miller (HC-700, Boshi Electonic Instrument, Guangzhou, China) before chemical analysis. Ingredient and diet samples were analyzed for dry matter (DM), ash, crude protein (CP), ether extract (EE), gross energy (GE), neutral detergent fiber (NDF), acid detergent fiber (ADF), Ca, and P whereas fecal samples were analyzed for DM, Ca, and P only. Briefly, DM was determined according to procedure 934.01 of Association of Official Analytical Chemists (AOAC, 2006) whereas CP content was determined by measuring nitrogen (N) concentration using a combustion analyzer (Model CNC-2000; LECO Corporation, St. Joseph, MI, USA) and multiplying the determined N content by 6.25. The GE content was measured using an adiabatic bomb calorimeter (model 6400, Parr Instrument Co., Moline, IL, USA) using benzoic acid as the calibration standard whereas EE content was determined according to AOAC (2006; procedure 920.39). The NDF and ADF contents were analyzed according to (Goering and Van Soest, 1970) using Ankom 200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA), and ash content was determined according AOAC (2006; to procedure 942.05). The concentrations of Ca and P were analyzed according to procedures 968.08 and 946.06 of AOAC (2006), respectively, and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA, USA). Ingredient samples were analyzed for phytate content according to the method suggested by Haug and Lantzsch (1983). The concentration of phytate-bound P in ingredients was calculated as 28.2% of phytate (Tran and Sauvant, 2004), and the concentration of non-phytate P was calculated as total P (%) minus phytate-bound P (%). Ingredient samples were analyzed for phytase activity as described by Slominski et al. (2007).

6.3.4 Calculations and statistical analysis

The ATTD of P was calculated by the following equation:

ATTD of P (%) = $[(P_i - P_f) / P_i] \times 100$,

Where P_i is the total P intake (g) from d 6 to d 10 and P_f is the total fecal P output (g) relative to the same period. The same equation was used to calculate the ATTD of Ca.

The STTD of P was calculated using the following equation:

STTD of P (%) = $[(P_i - P_f + EPL) / P_i] \times 100$,

Where EPL is the basal endogenous loss of P and assumed to be 190 mg/kg DM intake for all pigs (NRC, 2012).

Two outliers were determined using the UNIVARIATE procedure of SAS since they were greater than 1.5 times the interquartile range and removed from further statistical analysis (Devore and Peck, 1993). Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) in a 2 × 3 factorial model including PS (fine or coarse), phytase supplementation levels (0, 500, and 2,500 FTU/kg), and their interaction as main effects. Nonsignificant interactions were removed from the model and the main effects were analyzed individually. The LSMEANS procedure was used to calculate mean values, and the PDIFF option was used to separate means. Orthogonal polynomial contrasts were performed to test linear and quadratic effects of phytase level on both finely and coarsely ground hybrid rye diets where the phytase effect was significant. Orthogonal polynomial coefficients for unequally spaced phytase levels were generated by the interactive matrix language procedure (PROC IML) of SAS. A probability of $P < 0.05$ was considered significant, whereas $0.05 < P < 0.10$ was considered a tendency.

6.4 RESULTS

All pigs remained healthy and readily consumed their daily diets throughout the experimental periods. As shown in Table 6.2, the D_{gw} of finely and coarsely ground hybrid rye were 569 µm and 1,104 µm, respectively. The S_{gw} (2.81 vs. 2.33 µm) and surface area (136 vs. 59 cm^2/g) of finely ground hybrid rye were higher than that of coarsely ground hybrid rye. The nutrient composition of the coarse and fine hybrid rye is presented in Table 6.3. The average DM, GE, CP, Ca, and P content in hybrid rye were 89.52%, 3,851 kcal/kg, 9.29%, 0.04%, and 0.30%, respectively (as-fed basis). The average concentration of phytate-bound P in hybrid rye accounted for 61% of total P content. The average intrinsic phytase activity in hybrid rye was 3,063 FTU/kg.

Table 6.2 Particle size analysis of hybrid rye[1]

Particle size	Fine		Coarse	
Distribution of particles, %	Actual	Cumulative	Actual	Cumulative
Sieve opening, µm				
2,360	0.47	0.47	1.00	1.00
1,700	2.08	2.56	44.22	45.22
1,180	21.25	23.81	16.95	62.18
850	24.88	48.69	13.87	76.05
600	15.87	64.56	7.56	83.61
420	8.88	73.44	4.08	87.68
297	5.78	79.22	3.12	90.80
212	4.64	83.86	2.04	92.84
150	2.82	86.68	1.64	94.48
103	2.08	88.77	1.48	95.96
73	1.48	90.25	2.84	98.80
53	4.03	94.28	0.80	99.60
37	5.72	100.00	0.40	100.00
Coarse particles (≥ 1,700 µm)	2.56	2.56	45.22	45.22
Medium particles (297 to 1,700 µm)	76.66	79.22	45.58	90.8
Fine particles (< 297 µm)	20.78	100.00	9.20	100.00
Geometric mean particle size, µm	569		1,104	
Standard deviation, µm	2.81		2.33	
Surface area, cm^2/g	136.0		59.0	

[1]Hybrid rye was ground using a hammermill fitted with 4.0-mm and 3.2-mm screens to obtain coarse and fine particle sizes, respectively.

Table 6.3 Analyzed nutrient composition in the ingredients, as-fed basis

	Hybrid rye	
Particle size	Fine	Coarse
Analyzed composition		
DM, %	89.64	89.40
GE, kcal/kg	3,854	3,847
CP, %	9.44	9.13
EE, %	1.33	1.30
Ash, %	1.53	1.55
NDF, %	15.34	16.86
ADF, %	2.58	2.41
Ca, %	0.04	0.04
P, %	0.30	0.29
Phytate, %	0.64	0.64
Phytate-bound P, %[1]	0.18	0.18
Phytate-bound P, % of total P	60.0	62.1
Non-phytate P, %[2]	0.12	0.11
Non-phytate P, % of total P	40.0	37.9
Phytase, FTU[3]	3,362	2,763

[1]Phytate-bound P was calculated as 28.2% of phytate.
[2]Non-phytate P was calculated as total P minus phytate-bound P.
[3]Intrinc phytase was expressed as phytase units (FTU) per kg.

The ATTD of Ca and P and STTD of P in hybrid rye diets are presented in Table 6.4. No differences ($P > 0.10$) in daily feed, P, and Ca intake were observed among the treatments. Daily total weight of feces was reduced ($P < 0.05$) as the PS decreased, and there were no effects of phytase and interaction effects of phytase and PS on daily total weight of feces. Pigs fed hybrid rye diets with phytase supplementation had lower ($P < 0.05$) concentrations of Ca in feces and daily Ca fecal output compared with those without phytase supplementation, but no linear or quadratic relationship was observed with increasing levels of dietary phytase. Increasing phytase supplementation linearly ($P < 0.001$) and quadratically ($P < 0.001$) decreased concentrations of P in feces and daily P fecal output. The ATTD and STTD of P were higher ($P < 0.05$) in fine hybrid rye diets than in coarse hybrid rye diets. There were no effects of PS on the ATTD of Ca and interactions of PS and phytase supplementation on the ATTD of Ca and P and the STTD of P. The ATTD of Ca tended to increase ($P < 0.10$) in a linear pattern in hybrid rye diets with increasing levels of dietary phytase. The ATTD and STTD of P increased in both linear ($P < 0.001$) and quadratic ($P < 0.01$) patterns in hybrid rye diets with increasing levels of dietary phytase.

Table 6.4 Apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility (STTD) of P in fine or coarse hybrid rye diets without or with phytase supplementation

Treatments		Feed intake, g/day			Fecal output					Digestibility, %		
PS[1]	Phytase (FTU/kg)	Feed intake	Ca intake	P intake	Total feces, g/day	Ca in feces, %	P in feces, %	Ca output, g/day	P output, g/day	ATTD of Ca	ATTD of P	STTD of P
Fine	0	979	4.11	2.64	85	1.45	1.41	1.21	1.20	70.5	55.0	62.0
Fine	500	962	4.04	2.50	89	1.16	1.04	1.03	0.92	74.4	63.3	70.6
Fine	2,500	994	4.18	2.59	89	1.16	0.96	1.00	0.86	76.1	67.0	74.3
Coarse	0	993	4.07	2.58	98	1.35	1.35	1.31	1.32	67.9	48.9	56.2
Coarse	500	1,003	4.21	2.51	98	1.20	1.03	1.14	1.01	72.7	59.6	67.2
Coarse	2,500	996	4.18	2.49	95	1.16	0.92	1.08	0.86	74.1	65.4	73.0
	SEM	21.79	0.09	0.06	7.30	0.28	0.05	0.19	0.06	4.97	1.89	1.89
Main effects												
PS	Fine	978	4.11	2.58	88	1.26	1.14	1.08	0.99	73.7	61.7	69.0
	Coarse	997	4.16	2.52	97	1.23	1.09	1.17	1.05	71.7	58.4	65.9
	SEM	14.97	0.06	0.04	6.23	0.27	0.05	0.18	0.06	4.68	1.80	1.83
Phytase	0	985	4.09	2.61	90	1.41^{a}	1.38^{a}	1.25^{a}	1.25^{a}	69.3^{b}	52.2^{c}	59.4^{c}
	500	980	4.12	2.50	93	1.18^{b}	1.03^{b}	1.08^{b}	0.96^{b}	73.6^{a}	61.6^{b}	69.1^{b}
	2,500	995	4.18	2.54	92	1.16^{b}	0.94^{c}	1.04^{b}	0.86^{b}	75.1^{a}	66.2^{a}	73.7^{a}
	SEM	17.59	0.07	0.04	6.86	0.28	0.04	0.18	0.05	4.73	1.51	1.49
P-Value												
PS		0.239	0.479	0.218	0.023	0.704	0.314	0.150	0.108	0.164	0.020	0.030
Phytase		0.788	0.535	0.118	0.838	0.003	<0.001	0.024	<0.001	0.008	<0.001	<0.001
Phytase linear[2]						0.227	<0.001	0.140	<0.001	0.099	<0.001	<0.001
Phytase quadratic[3]						0.161	<0.001	0.203	<0.001	0.204	0.001	<0.001
PS × Phytase		0.587	0.402	0.575	0.745	0.637	0.762	0.982	0.548	0.965	0.488	0.486

[1]PS, particle size.

[2-3]Polynomial orthogonal contrasts were only analyzed when the phytase effect was significant at $P < 0.05$.

[a-c] Means within a column without a common superscript are significantly different ($P < 0.05$).

6.5 DISCUSSION

Compared with traditional rye, the newly bred hybrid rye (KWS Lochow GmbH, Bergen, Germany) has a mitigated ergot contamination and also higher yield, making it an attractive feed ingredient to be used in swine diets (Miedaner and Geiger, 2015; Schlegel, 2016) . Although hybrid rye contains a considerable amount of intrinsic phytase, the P digestibility of hybrid rye might be further improved if exogenous phytase is supplemented (Archs Toledo et al., 2020). A previous study reported that the STTD of P in hybrid rye increased 14.2% when 1,000 FTU/kg of microbial phytase was added to swine diets (McGhee and Stein, 2019). However, there is limited information about the impacts of phytase levels on P digestibility of hybrid rye. In this study, the concentrations of P (0.3%) and phytate-bound P (0.18%) in hybrid rye were within the range of values reported previously (NRC, 2012; McGhee and Stein, 2019). The concentration of Ca (0.04%) was in agreement with the published value (McGhee and Stein, 2019) but lower than the value (0.08%) reported by NRC (2012). The difference of the concentration of Ca between the current study and NRC (2012) indicated that hybrid rye contained less concentration of Ca than the common rye. The intrinsic phytase activity of hybrid rye (3,063 FTU/kg) was similar to the published value (McGhee and Stein, 2019). The ATTD of P (52.2%) and STTD of P (59.4%) in hybrid rye without phytase supplementation determined in the current study were in agreement with the values reported by Archs Toledo et al. (2020) but slightly greater than the values reported by NRC (2012) and McGhee and Stein (2019).

Grinding is one of the most important steps in the feed manufacturing process in which PS of feed ingredients is reduced (Vukmirović et al., 2017). Decreasing PS improves nutrient digestibility in pigs by increasing the surface area, which allows the nutrients to have better contact with digestive enzymes (Blasel et al., 2006). However, too fine PS may negatively affect pig gut health and cause gastric lesions and ulcerations (Kiarie and Mills, 2019). Thus, it is generally recommended that the optimal PS for cereal grains ranges from 500 to 700 µm (Behnke, 1994). Nevertheless, fine grinding can increase electrical energy expenses and cause additional cost in feed manufacturing in comparison with coarse grinding (Koo and Nyachoti, 2021). Since no studies have determined the effects of PS on P digestibility of hybrid rye fed to pigs yet, and also there is limited information about the impacts of phytase levels on P digestibility of hybrid rye, the present study was performed to determine the effects of PS and phytase supplementation levels, individually or in combination, on the STTD of P in hybrid rye fed to growing pigs.

In the current study, the fine PS of hybrid rye (569 µm) was within the range of the optimal particle size, and the coarse PS of hybrid rye (1,104 µm) was beyond the optimal particle size. The main difference between fine and coarse hybrid rye PS was that the percentage of coarse particles (≥ 1,700 µm) in fine hybrid rye (2.56%) was much lower than that in coarse hybrid rye (45.22%). Also, as the PS decreased from 1,104 to 569 µm, the surface area increased by 77 cm^2/g. Compared with pigs fed the coarse hybrid rye diet, those fed the fine hybrid rye diets had lower total daily fecal output, which might be explained by the effect of PS reduction on increasing dietary DM digestibility in pigs (Kerr et al., 2020). Also, the current study showed that fine PS increased the ATTD and the STTD of P in hybrid rye, demonstrating that reduction in particle size by increasing surface area of hybrid rye is effective in improving P digestibility in pigs. This finding was in agreement with the study reported by Oryschak et al. (2002), in which reducing PS of the diets from 700 to 400 µm increased P digestibility and retention by 42% in growing pigs. However, Liu et. al (2012) reported that PS reduction of distillers dried grains with solubles (DDGS) did not influence the ATTD of P, and Rojas and Stein (2015) reported that reducing the PS of corn did not affect ATTD or STTD of P. The discrepancies between these studies and the current study might be due to the fact that the phytic acid content of DDGS is significantly lower than that of hybrid rye and that hybrid rye contains higher intrinsic phytase than other ingredients.

The main storage form of P in grains and oilseeds is phytate, which cannot be utilized by pigs and is thus excreted to environment as a waste because pigs lack endogenous phytase (Weremko et al., 1997; Woyengo and Nyachoti, 2013). Therefore, phytase is routinely added to pig diets to degrade phytate-bound P, and therefore improve P digestibility. The findings in this study showed that phytase supplementation increased the ATTD of P and the STTD of P in hybrid rye, which was in agreement with previous studies (Selle and Ravindran, 2008; McGhee and Stein, 2019). Moreover, increasing phytase levels from 500 to 2,500 FTU/kg further improved the STTD of P in both fine and coarse hybrid rye diets. This result demonstrated that hybrid rye might contain more substrate than what can be fully hydrolyzed by 500 FTU/kg microbial phytase, which was in accordance with the previous study (Archs Toledo et al., 2020). However, to determine the optimal phytase level that can maximize the P digestibility of hybrid rye, more graded levels of phytase should be used in the future research. Dietary Ca can be bound with phytate to form insoluble Ca-phytate complexes, which is indigestible in the intestinal tract of pigs (Selle et al., 2009). Therefore, as observed in the current study, the ATTD of Ca increased when phytase was added to the diets. Such increase in ATTD of Ca by microbial phytase supplementation was also reported previously by Gonzalez-Vega et al. (2015) and Lee et al. (2021). However, PS reduction of hybrid rye did not increase the ATTD of Ca in diets, which can be explained by the fact that the ATTD of Ca in diets mostly represented the digestibility of Ca in limestone as the contribution of Ca from hybrid rye was little.

6.6 CONCLUSION

The average standardized total tract digestibility of phosphorous in coarse and fine hybrid rye were 65.9% and 69.0%, respectively. Particle size reduction increased the apparent total tract digestibility and standardized total tract digestibility of phosphorus in hybrid rye. Supplementation of microbial phytase at 500 FTU/kg and 2,500 FTU/kg increased the standardized total tract digestibility of phosphorus in hybrid rye by 16.3% and 24.1%, respectively. Although hybrid rye has considerable quantities of intrinsic phytase, a high dose of dietary phytase supplementation is still recommended in hybrid rye diets to maximize the standardized total tract digestibility of phosphorous.

TRANSITION STATEMENT

Manuscripts II and III demonstrated that enzyme supplementation improved the energy and nutrients digestibility in hybrid rye diets for growing pigs. Manuscript IV (Experiment 4) was conducted to determine the effects of MC supplementation on the growth performance and gut health of weaning pigs fed hybrid rye or wheat-based diets.

CHAPTER SEVEN: MANUSCRIPT IV

Effects of multi-carbohydrase supplementation on growth performance, nutrient digestibility, short-chain fatty acids production, and gut microbiota composition in weaned piglets fed wheat or hybrid rye-based diets

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7.1 ABSTRACT

This study aimed to determine the effects of a multi-carbohydrase (MC) supplementation on growth performance, nutrient digestibility, short-chain fatty acids (SCFA) production, and gut microbiota composition in weaned piglets fed either wheat or hybrid rye-based diets. Ninety-six pigs (1:1 male to female ratio) with an initial body weight (BW) of 6.74 ± 0.43 kg were used in a 2-phase feeding study with phase I (1-14 d) and phase II (15-28 d). Pigs were assigned to one of four dietary treatments in a randomized complete block design with a 2 × 2 factorial arrangement (cereal source and MC supplementation) to give eight replicate pens per treatment and three pigs in each pen. The four dietary treatments included a wheat- or hybrid rye-based diet fed with or without MC supplementation. Results indicated that pigs fed the wheat-based diet had higher ($P <$ 0.05) average daily gain (ADG) and average daily feed intake (ADFI) than those fed the hybrid rye-based diet, but no differences ($P > 0.10$) in gain to feed ratio (G:F) were observed between pigs fed the wheat- and hybrid rye-based diets. The ATTD of gross energy (GE) and crude protein (CP) were greater ($P < 0.05$) in pigs fed the wheat-based diet than in those fed the hybrid rye-based diet, whereas the ATTD of neutral detergent fiber (NDF) and phosphorus (P) were greater ($P <$ 0.05) in pigs fed the hybrid rye-based diet than in those fed the wheat-based diet in phase 1. The ATTD of CP was greater ($P < 0.01$) in pigs fed the wheat-based diet than in those fed the hybrid rye-based diet in phase 2, and dietary MC supplementation improved ($P < 0.05$) the ATTD of NDF in phase 2. Interaction effects ($P < 0.05$) were observed for the concentration of acetic acid, propionic acid, and total SCFA in the cecum. For pigs fed the hybrid rye-based diet, dietary MC supplementation increased ($P < 0.05$) the concentrations of acetic acid, propionic acid, and total SCFA. The concentrations of valeric acid and branched chain fatty acids (BCFA) were higher (P < 0.05) in the hybrid rye-based diet than in the wheat-based diet. Dietary MC supplementation increased ($P < 0.05$) the relative abundance of Bifidobacterium and Lactobacillus. In conclusion, replacing wheat with hybrid rye in the nursery pig diet may reduce ADG as a result of decreased ADFI, but the feed efficiency is comparable between the hybrid rye-based diet and the wheat-based diet. Also, feeding hybrid rye diets and MC supplementation could enhance gut health outcomes in weaned piglets.

Keywords: hybrid rye, enzyme supplementation, growth performance, digestibility, gut microbiota, piglets

7.2 INTRODUCTION

In western Canada, wheat is one of the most common cereal grains used in swine diets, but small grains such as rye may replace its use at a competitive price (Smit et al., 2019). Traditionally, rye is not largely used for pigs because it is highly susceptible to ergot contamination, which can produce ergot alkaloids and cause detrimental health effects (Coufal-Majewski., 2016). However, a newly bred hybrid rye that is less susceptible to ergot infection has recently been developed, which makes it an attractive feed ingredient used in the swine industry (Geiger et al., 2009). Previous research indicated that substituting wheat with rye in the diets of weaned piglets did not decrease performance (Chuppava et al. 2020; Ellner et al., 2020). However, there is limited research comparing the growth performance of weaned piglets fed on hybrid rye to those fed on wheat throughout the nursery phase.

Wheat and hybrid rye contain considerable amounts of non-starch polysaccharides (NSP), which may increase digesta viscosity and adversely affect nutrient digestibility and growth performance of pigs (Salmenkallio-Marttila et al., 2005; Mirzaie et al., 2012; Arczewska-Wlosek et al., 2019). In contrast to wheat, hybrid rye has significantly higher levels of water-soluble arabinoxylans (McGhee and Stein, 2018). Multi-carbohydrase (MC), a combination of carbohydrases, can depolymerize NSP for more effective hindgut fermentation and release the encapsulated nutrients to enhance nutrient and energy digestibility, leading to improved growth performance and gut health outcomes (Jha et al., 2015). As indicated by McGhee et al. (2021) and Ellner et al. (2022), hybrid rye could replace most of the corn in a corn-soybean meal-based diet and be used as an alternative to wheat for nursery pigs without affecting growth performance. However, the effects of MC supplementation on growth performance, nutrient digestibility, and gut health of weaned piglets fed diets primarily composed of wheat or hybrid rye are yet to be elucidated. Therefore, this study aimed to investigate the effects of MC supplementation on these parameters in weaning pigs fed wheat- or hybrid rye-based diets. It was hypothesized that MC supplementation might improve growth performance, nutrient digestibility, short-chain fatty acids production, and gut health of weaned pigs and that substituting wheat with hybrid rye in their diet would not compromise growth performance.

7.3 MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the University of Manitoba Animal Care Committee (AC11419). All pigs were cared for according to the guidelines of the Canadian Council on Animal Care (2009).

7.3.1 Animals, housing, diets, and experimental design

A total of 96 weaned piglets (TN70 × TN Tempo; Topigs Norsvin, Winnipeg, MB, Canada) weaned at 21 d of age (1:1 male:female ratio) with an initial body weight (BW) of 6.74 ±0.43 kg were obtained from Glenlea Research Station, University of Manitoba for use in this study. Pigs were assigned to one of four dietary treatments in a randomized complete block design with a 2 × 2 factorial arrangement (diets and MC) to give 8 replicates per diet and three pigs per pen. Pens were equipped with plastic-covered expanded metal floors (1.2 × 1.5 m) and stainless-steel feeders and nipple drinkers to provide free access to feed and water throughout the 4-week experimental period. Room temperature was set at 30 ± 1°C for the first week and gradually decreased by 1°C every week. Hybrid rye was provided by KWS (KWS Lochow GmbH, Bergen, Germany), and the MC commercial product (Superzyme; Supplemented at 500 g/ton) was supplied by Canadian Bio-System Inc. (Calgary, Alberta, Canada). The MC supplied 1,000 units of cellulase, 1,700 units of xylanase, 250 units of glucanase, 12,000 units of amylase, 700 units of invertase, and 6,000 units of protease per gram of enzyme. The four experimental diets included the wheat- and hybrid rye-based diets fed with or without MC supplementation (Table 7.1 and 7.2). Diets were formulated to meet or exceed the estimated requirements for net energy, standardized ileal digestible (SID) amino acids, vitamins, and minerals for weaned pigs according to NRC (2012). However, diets were not formulated to be isocaloric or isonitrogenous. All diets contained 0.3% titanium dioxide (TiO_2) as an indigestible marker. Pigs were fed the phase-1 diets from d 1 to d 14 and phase-2 diets from d 15 to d 28.

Table 7.1. Ingredient composition and analyzed nutrient content of phase I diets (as-fed basis)

Diet	Wheat[1]		Hybrid rye[1]	
	Without MC	With MC	Without MC	With MC
Ingredient, %				
Wheat	47.78	47.78	—	—
Soybean meal	26.00	26.00	26.00	26.00
Fish meal	5.00	5.00	5.00	5.00
Dry whey	14.00	14.00	14.00	14.00
Hybrid rye	—	—	47.44	47.44
Monocalcium phosphate	0.06	0.06	0.22	0.22
Limestone	1.03	1.03	0.99	0.99
Vegetable oil	3.95	3.95	3.95	3.95
Salt	0.55	0.55	0.55	0.55
Vitamin-mineral premix[2]	1.00	1.00	1.00	1.00
L-Lysine-HCL	0.25	0.25	0.30	0.30
DL-Methionine	0.05	0.05	0.16	0.16
L-Threonine	0.03	0.03	0.09	0.09
Titanium dioxide	0.30	0.30	0.30	0.30
Analyzed composition				
DM, %	89.22	89.45	90.42	90.37
GE, kcal/kg	4,122	4,157	4,172	4,146
CP, %	24.7	25.1	22.7	22.9
EE, %	4.77	4.59	4.60	5.01
NDF, %	8.02	7.88	11.20	10.67
ADF, %	5.25	5.64	5.98	5.11
Ca, %	0.68	0.56	0.71	0.67
P, %	0.58	0.58	0.57	0.57

[1]Wheat- and hybrid rye-based diets were formulated without or with MC supplementation.
[2]Provided the following per kg of complete diet: vitamins A, 2,200 IU; vitamin D_3, 220 IU; vitamin E, 16.0 IU; vitamin K, 0.5 mg; thiamine, 1.0 mg; riboflavin, 3.5 mg; niacin, 30.0 mg; pantothenic acid, 10.0 mg; vitamin B_{12}, 0.02 mg; folic acid, 0.3 mg; Cu, 6.0 mg as copper sulfate; I, 0.14 mg as calcium iodate; Fe, 100.0 mg as ferrous sulfate; Mn, 4.0 mg as manganese oxide; Se, 0.3 mg as sodium selenite; Zn, 100.0 mg as zinc oxide; biotin 0.05 mg.

Table 7.2. Ingredient composition and analyzed nutrient content of phase II diets (as-fed basis)

Diet	Wheat[1]		Hybrid rye[1]	
	Without MC	With MC	Without MC	With MC
Ingredient, %				
Wheat	56.57	56.57	—	—
Soybean meal	35.00	35.00	35.00	35.00
Hybrid rye	—	—	56.25	56.25
Monocalcium phosphate	0.54	0.54	0.75	0.75
Limestone	1.27	1.27	1.20	1.20
Vegetable oil	4.40	4.40	4.40	4.40
Salt	0.70	0.70	0.70	0.70
Vitamin-mineral premix[2]	1.00	1.00	1.00	1.00
L-Lysine-HCL	0.17	0.17	0.22	0.22
DL-Methionine	0.05	0.05	0.12	0.12
L-Threonine	—	—	0.06	0.06
Titanium dioxide	0.30	0.30	0.30	0.30
Analyzed composition				
DM, %	87.09	87.59	88.74	88.90
GE, kcal/kg	4,095	4,088	4,105	4,114
CP, %	25.10	25.40	23.20	23.00
EE, %	4.78	4.98	4.46	4.46
NDF, %	10.35	10.70	11.70	12.10
ADF, %	4.37	4.39	4.82	4.66
Ca, %	0.61	0.61	0.66	0.61
P, %	0.57	0.57	0.58	0.57

[1]Wheat- and hybrid rye-based diets were formulated without or with MC supplementation.
[2]Provided the following per kg of complete diet: vitamins A, 1,750 IU; vitamin D_3, 200 IU; vitamin E, 11.0 IU; vitamin K, 0.5 mg; thiamine, 1.0 mg; riboflavin, 3.0 mg; niacin, 30.0 mg; pantothenic acid, 9.0 mg; vitamin B_{12}, 0.02 mg; folic acid, 0.3 mg; Cu, 5.0 mg as copper sulfate; I, 0.14 mg as calcium iodate; Fe, 100.0 mg as ferrous sulfate; Mn, 3.0 mg as manganese oxide; Se, 0.25 mg as sodium selenite; Zn, 80.0 mg as zinc oxide; biotin 0.05 mg

7.3.2 Experimental procedure and sample collection

Pigs were weighed and feed disappearance recorded weekly to determine average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). Fecal samples were collected from each pen by grab sampling on the last 2 d of each phase (day 13 and 14 for phase1; day 27 and 28 for phase 2) to determine nutrient digestibility. Subjective fecal scores were recorded daily by the same person for each pen through observations for each individual pig and signs of stool consistency according to the following criteria: 1 = normal; 2 = soft; 3 = moderate diarrheic feces; 4 = liquid and severe diarrhea (Yi et al., 2005). On d 29, one pig per pen (total 32 pigs) was randomly selected and anesthetized by intramuscular injection of ketamine and xylazine (20 and 2 mg/kg, respectively; Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada) and euthanized by a captive bolt. The abdominal cavity was open, and digesta samples from the ileum (30 cm before the ileocecal junction), cecum, and colon were collected to determine the pH using a pH probe (AB15 plus, Fisher Scientific, ON, Canada). Cecum and colon digesta samples were obtained for short chain fatty acids (SCFA) and microbial analyses. Also, organs (liver, spleen, and kidney) and the gastrointestinal tract (stomach, small intestine, and large intestine) were removed, emptied of digesta, blotted dry with paper towels, and weighed.

7.3.3 Sample preparation and chemical analysis

Fecal samples were dried in an oven at 50˚C for five days, pooled per pig within a phase, and sub-sampled. Dried feces and feed samples were ground to pass through a 1 mm screen before chemical analysis. Feed samples were analyzed for dry matter (DM), gross energy (GE), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), phosphorus (P), and TiO_2, whereas feces were analyzed for DM, GE, CP, NDF, Ca, P, and TiO_2. Briefly, DM was determined according to procedure 934.01 of the Association of Official

Analytical Chemists (AOAC, 2006). The GE was measured using an adiabatic bomb calorimeter (model 6400, Parr Instrument Co., Moline, IL, USA) using benzoic acid as the calibration standard. Nitrogen (N) was analyzed using a combustion analyzer (Model CNC-2000; LECO Corporation, St. Joseph, MI, USA), and CP content was calculated as N × 6.25. The EE content was determined after hexane extraction according to procedure 920.39 of AOAC (2006). The concentrations of Ca and P were analyzed according to procedures 968.08 and 946.06 of AOAC (2006), respectively, and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA, USA). The concentration of TiO_2 was determined according to procedures described by Lomer et al. (2000) and read on an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA, USA). The ADF and NDF contents were analyzed according to the method of Goering and Van Soest (1970) using Ankom 200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA). The concentrations of SCFA in the cecum and colon digesta were analyzed by gas chromatography as described by Liao et al. (2020).

7.3.4 Microbial DNA extraction and quantitative real-time PCR

Microbial genomic DNA was extracted from cecum digesta samples to determine the relative abundance of selected bacteria using a QIAamp® DNA Stool Mini Kit (QIAGEN, Canada) according to the manufacturer's instruction and quantified using a NanoDrop (Thermo Fisher Scientific, Waltham, MA, United States). Quantitative real-time PCR was performed for DNA templates using a Bio-Rad CFX Real-time system (BioRad, Hercules, CA, USA), and the sequences of primers were obtained from published studies (Table 7.3). Normalizing the expression of selected bacteria groups with that of total eubacteria was performed, and the relative abundance values were calculated using the $2^{-\Delta\Delta CT}$ method (Rao et al., 2013).

Table 7.3. Primers used for quantifying pig gut bacteria groups by real-time PCR.

Bacteria group	Primer Sequence	Reference
Bifidobacterium spp.	F: TCGCGTCYGGTGTGAAAG	Rinttilä et al.
	R: CCACATCCAGCRTCCAC	(2004)
Clostridium cluster IV	F: GCACAAGCAGTGGAGT	Matsuki et al.
	R: CTTCCTCCGTTTTGTCAAC	(2002)
Enterococcus spp.	F: CCCTTATTGTTAGTTGCCATCATT	Rinttilä et al.
	R: ACTCGTTGTACTTCCCATTGT	(2004)
Lactobacillus spp.	F: AGCAGTAGGGAATCTTCCA	Walter et al.
	R: CACCGCTACACATGGAG	(2001)
Escherichia coli	F: CAATTTTCGTGTCCCCTTCG	Wealleans et al.
	R: GTTAATGATAGTGTGTCGAAAC	(2017)
Total Eubacteria	F: CGGYCCAGACTCCTACGGG	Lee et al.
	R: TTACCGAGGCTGCTGGCAC	(1996)

7.3.5 Calculations and statistical analysis

The apparent total tract digestibility (ATTD) was calculated using the following equation:

$$ATTD\ (\%) = 100 - [(N_{feces}/N_{diet}) \times (Ti_{diet}/Ti_{feces})] \times 100,$$

where N_{feces} is the nutrient concentration (%) in feces, N_{diet} is the nutrient concentration (%) in diet, Ti_{diet} is the titanium concentration (%) in diet, and Ti_{feces} is the titanium concentration (%) in feces.

Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with the pen of three pigs as the experiment unit. Cereal source (wheat vs. hybrid rye), MC supplementation, and their interaction were considered the main effects, with the sex and block considered as random effects. The LSMEANS procedure was used to calculate mean values, and the PDIFF option was used to separate means. A probability of $P < 0.05$ was considered significant, whereas $0.05 < P < 0.10$ was considered a tendency.

7.4 RESULTS

7.4.1 Chemical composition of hybrid rye and wheat

All pigs readily consumed the assigned feed throughout the study. The GE in hybrid rye and wheat were 3,857 kcal/kg and 3,951 kcal/kg, respectively (Table 7.4). The CP was lower in hybrid rye than in wheat (9.38% vs. 14.90%), whereas the NDF and ADF were higher in hybrid rye than in wheat (14.34% vs. 8.84% and 4.30% vs. 2.70%, respectively).

Table 7.4. Analyzed ingredients nutrient composition, as-fed basis

	Wheat	Hybrid rye	Soybean meal
Item			
DM, %	86.90	88.52	89.25
GE, kcal/kg	3,951	3,897	4,213
CP, %	14.90	9.38	46.50
EE, %	1.03	1.18	1.35
NDF, %	8.84	14.34	13.27
ADF, %	2.70	4.30	8.09
Ca, %	0.03	0.06	0.50
P, %	0.36	0.26	0.69

7.4.2 Growth performance

As shown in Table 7.5, there were no interaction effects ($P > 0.10$) of cereal source and MC supplementation on growth performance. Pigs fed the wheat-based diet had greater ($P < 0.05$) BW at the end of each week throughout the trial than those fed the hybrid rye-based diet. The ADG and ADFI of pigs fed the wheat-based diet were greater ($P < 0.05$) than those of pigs fed the hybrid rye-based diet in both phase-1 and phase-2, whereas no differences ($P > 0.10$) in G:F were observed between pigs fed the wheat-based diet and hybrid rye-based diet in both phases. Dietary MC supplementation did not affect ($P > 0.10$) growth performance.

Table 7.5. Effects of cereal source and multi-carbohydrase (MC) supplementation on growth performance in weaned piglets

Item	Wheat		Hybrid rye		SEM	*P*-value[2]		
	–MC[1]	+MC[1]	–MC	+MC		Cereal	MC	Cereal × MC
BW, kg								
d 0	6.75	6.75	6.75	6.72	0.164	0.940	0.940	0.934
d 7	8.42	8.44	7.85	7.80	0.252	0.023	0.959	0.897
d 14	11.52	11.36	10.43	10.34	0.358	0.006	0.719	0.920
d 21	15.14	15.18	13.71	13.51	0.457	0.002	0.859	0.798
d 28	20.60	20.31	17.69	17.75	0.579	<0.001	0.849	0.766
ADG, g/d								
d 1 to 7	274	282	189	180	19.6	<0.001	0.985	0.677
d 8 to 14	442	416	359	362	20.4	0.002	0.573	0.482
d 15 to 21	517	546	468	453	28.3	0.018	0.806	0.440
d 22 to 28	780	698	570	607	38.3	<0.001	0.567	0.130
Overall	503	486	396	400	31.4	0.003	0.829	0.728
ADFI, g/d								
d 1 to 7	315	321	225	221	24.2	<0.001	0.957	0.848
d 8 to 14	609	586	495	481	28.4	<0.001	0.517	0.893
d 15 to 21	820	830	674	684	31.7	<0.001	0.740	0.997
d 22 to 28	946	918	743	785	39.6	<0.001	0.862	0.380
Overall	684	664	534	543	42.6	0.002	0.894	0.737
G:F, g/g								
d 1 to 7	0.87	0.89	0.84	0.83	0.044	0.310	0.877	0.644
d 8 to 14	0.73	0.71	0.73	0.76	0.018	0.203	0.734	0.345
d 15 to 21	0.63	0.66	0.69	0.66	0.025	0.175	0.961	0.242
d 22 to 28	0.83	0.76	0.77	0.77	0.028	0.344	0.282	0.245
Overall	0.76	0.76	0.76	0.75	0.020	0.862	0.813	1.000

[1]–MC = without MC supplementation; +MC = with MC supplementation.
[2]Cereal, the main effect of cereal source. MC, the main effect of MC supplementation.

7.4.3 Apparent total tract digestibility of nutrients and energy and pH in the ileum, cecum, and colon contents

The effects of cereal source and MC supplementation on ileum, cecum, and colon pH and ATTD of nutrients and energy are shown in Table 7.6. There were no interaction effects ($P > 0.10$) of cereal source and MC supplementation on ileum, cecum, and colon pH. Also, no differences ($P > 0.10$) in the ileum, cecum, and colon pH were observed between pigs fed the wheat-based diet and the hybrid rye-based diet. Dietary MC supplementation did not affect ($P > 0.10$) ileum, cecum, and colon pH. No interactive effects ($P > 0.10$) were observed for cereal source and MC supplementation on the ATTD of nutrients in either of the two phases. The ATTD of GE and CP were greater ($P < 0.05$) in pigs fed the wheat-based diet than in those fed the hybrid rye-based diet (85.0% vs. 83.6% and 82.4% vs. 77.5%, respectively), whereas the ATTD of NDF and P were greater ($P < 0.05$) in pigs fed the hybrid rye-based diet than in those fed the wheat-based diet (75.2% vs. 63.0% and 57.5% vs. 54.2%, respectively) in phase 1. The ATTD of CP was greater ($P < 0.01$) in pigs fed the wheat-based diet than in those fed the hybrid rye-based diet (84.9% vs. 83.0%) in phase 2. The MC supplementation did not affect ($P > 0.10$) the ATTD of nutrients in phase 1, but it improved ($P < 0.05$) the ATTD of NDF (70.9% vs. 64.0%) in phase 2.

Table 7.6. Effects of cereal source and multi-carbohydrase (MC) supplementation on ileal, cecal, and colon pH and apparent total tract digestibility (ATTD) of nutrients in weaned piglets

Item	Wheat		Hybrid rye		SEM	*P*-value[2]		
	–MC[1]	+MC[1]	–MC	+MC		Cereal	MC	Cereal × MC
pH								
Ileum	6.05	6.23	6.06	6.31	0.197	0.812	0.283	0.840
Cecum	5.33	5.36	5.54	5.30	0.089	0.390	0.259	0.141
Colon	5.63	5.88	5.65	5.73	0.079	0.423	0.052	0.288
Phase I								
DM, %	83.87	85.31	83.61	83.31	0.620	0.080	0.367	0.171
GE, %	84.42	85.64	83.92	83.20	0.597	0.020	0.685	0.116
CP, %	81.65	82.95	77.35	77.47	1.036	<0.001	0.495	0.573
NDF, %	61.93	63.97	75.06	75.24	1.923	<0.001	0.569	0.633
Ca, %	46.75	49.35	39.60	44.24	3.795	0.120	0.350	0.791
P, %	55.65	52.60	58.08	56.88	1.330	0.018	0.123	0.491
Phase II								
DM, %	84.48	84.54	83.02	85.00	1.118	0.660	0.371	0.396
GE, %	86.42	86.12	83.84	85.57	1.000	0.129	0.482	0.320
CP, %	86.00	86.80	82.12	83.94	1.198	0.009	0.285	0.671
NDF, %	64.29	68.94	61.69	70.51	3.255	0.875	0.049	0.527
Ca, %	50.55	51.60	53.92	61.09	4.090	0.128	0.324	0.461
P, %	49.25	47.26	54.50	53.33	3.156	0.086	0.621	0.898

[1]–MC = without MC supplementation; +MC = with MC supplementation.
[2]Cereal, the main effect of cereal source. MC, the main effect of MC supplementation.

7.4.4 Organ weights and concentrations of microbial metabolites

The effects of cereal source and MC supplementation on the relative organ weights and SCFA concentrations in the cecum and colon are shown in Table 7.7. There were no interaction effects ($P > 0.10$) of cereal source and MC supplementation on the relative organ weights and SCFA concentrations in the cecum and colon. The relative spleen weights of pigs fed the wheat-based diet were greater ($P < 0.05$) than those of pigs fed the hybrid rye-based diet (2.86 g/kg/BW vs. 2.24 g/kg/BW), whereas the relative large intestine weights of pigs fed the hybrid rye-based diet were greater ($P < 0.05$) than those of pigs fed the wheat-based diet (24.77 g/kg/BW vs. 22.22 g/kg/BW). Dietary MC supplementation had no effects ($P > 0.10$) on the relative organ weights. Interactive effects ($P < 0.05$) were observed for the concentrations of acetic acid, propionic acid, and total SCFA in the cecum. The concentrations of acetic acid, propionic acid, and total SCFA in the cecum were higher ($P < 0.05$) in the hybrid rye-based diet with MC supplementation than that without MC supplementation. The concentrations of valeric acid and branched chain fatty acids (BCFA) in the cecum were higher ($P < 0.05$) in the hybrid rye-based diet than in the wheat-based diet. No interaction effects ($P > 0.10$) were observed for the SCFA concentrations in the colon. The concentration of valeric acid in the colon was higher ($P < 0.05$) in the hybrid rye-based diets than in the wheat-based diets.

Table 7.7. Effects of cereal source and multi-carbohydrase (MC) supplementation on the relative organ weights (g/kg/BW) and short chain fatty acids (SCFA) in the cecum and colon of weaning piglets

Item	Wheat		Hybrid rye		SEM	P-value[2]		
	–MC[1]	+MC[1]	–MC	+MC		Cereal	MC	Cereal × MC
Organ weight								
Liver	38.31	35.32	36.40	37.76	1.30	0.838	0.535	0.105
Spleen	2.86	2.86	1.94	2.53	0.23	0.010	0.203	0.205
Kidney	7.29	6.94	6.76	7.18	0.26	0.589	0.894	0.156
Stomach	8.75	8.24	7.70	9.01	0.45	0.756	0.383	0.055
Small intestine	37.86	36.55	39.41	39.44	2.32	0.348	0.784	0.776
Large intestine	23.09	21.34	25.58	23.95	1.16	0.036	0.155	0.987
Cecum, mmol/kg								
Acetic acid	73.31[a]	71.76[a]	57.41[b]	76.74[a]	4.114	0.196	0.040	0.017
Propionic acid	46.10[ab]	39.60[bc]	35.54[c]	51.31[a]	3.476	0.870	0.193	0.004
Butyric acid	23.34	17.83	22.28	22.33	2.378	0.476	0.261	0.252
Valeric acid	3.98	2.63	6.71	5.33	0.939	0.007	0.157	0.986
BCFA[3]	0.34	0.51	0.96	0.57	0.149	0.030	0.475	0.069
Total SCFA	147.01[a]	132.30[ab]	122.89[b]	156.26[a]	7.597	0.991	0.230	0.004
Colon, mmol/kg								
Acetic acid	77.34	70.48	69.10	75.11	3.666	0.627	0.909	0.090
Propionic acid	40.14	33.44	39.98	45.38	3.185	0.075	0.840	0.068
Butyric acid	26.21	23.91	29.90	23.40	3.087	0.611	0.165	0.502
Valeric acid	5.05	5.14	9.65	6.65	1.152	0.013	0.217	0.191
BCFA[3]	2.08	2.66	2.51	2.86	0.394	0.425	0.244	0.765
Total SCFA	150.79	135.60	151.11	153.39	8.529	0.297	0.455	0.315

[a,b,c] Means within a column without a common superscript are significantly different ($P < 0.05$).
[1]–MC = without MC supplementation; +MC = with MC supplementation.
[2]Cereal, the main effect of cereal source. MC, the main effect of MC supplementation.
[3]Branched chain fatty acids included isovaleric acid, isobutyric acid, and 2-methylbutyric acid.

7.4.5 Fecal score and gut microbiota

As shown in Fig.7.1, pigs fed the hybrid rye-based diet showed lower ($P < 0.05$) fecal scores than those fed the wheat-based diet for the first two weeks after weaning. Also, dietary MC supplementation decreased ($P < 0.05$) fecal scores for the first week after weaning. There were no interaction effects ($P > 0.10$) of cereal source and MC supplementation on the relative abundance of the microbes in cecum digesta, including Bifidobacterium, Lactobacillus, Enterococcus, Clostridium cluster IV, and Escherichia coli (Fig. 7.2). The supplementation of MC increased ($P < 0.05$) the relative abundance of Bifidobacterium and Lactobacillus.

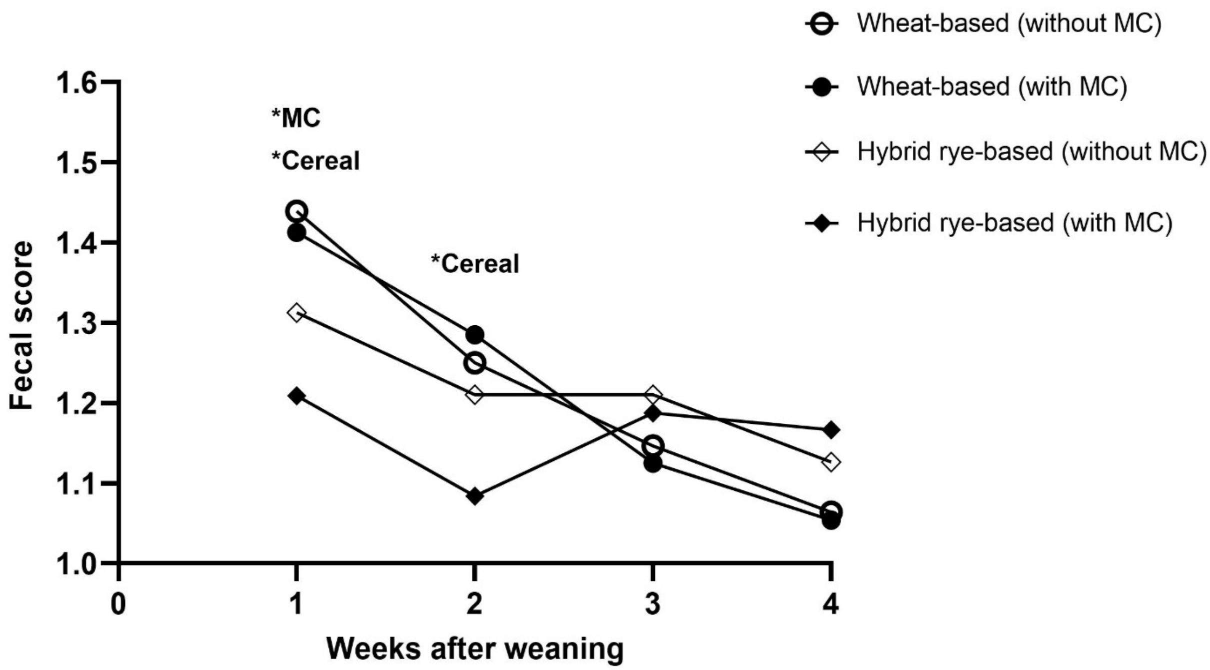

Figure 7.1 Effects of cereal source and multi-carbohydrase (MC) supplementation on the fecal score in weaned pigs. Wheat-based and hybrid rye-based diets were formulated without or with MC supplementation. Fecal score was recorded for each pen following observations of an individual pig and signs of stool consistency according to the following criteria: 1 = normal; 2 = soft; 3 = moderate diarrheic feces; 4 = liquid and severe diarrhea. No interaction effects of cereal source and MC supplementation were observed throughout the study. *$P < 0.05$.

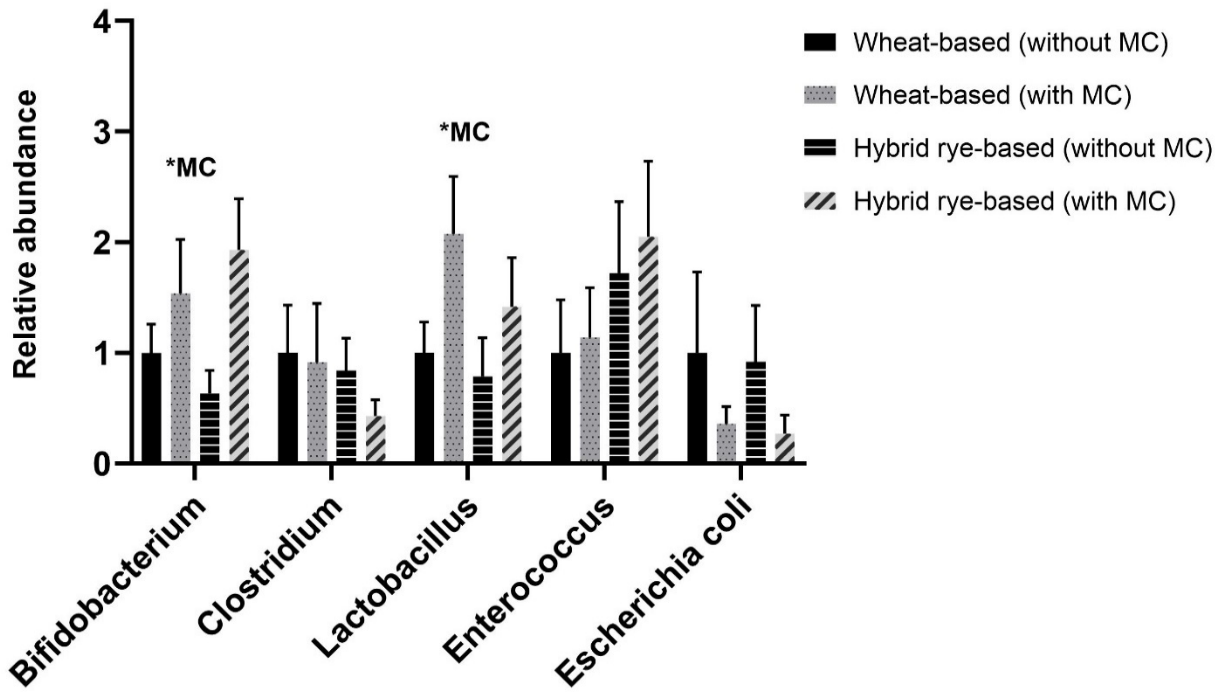

Figure 7.2. Effects of cereal source and multi-carbohydrase (MC) supplementation on the relative abundance of selected microbes in cecal digesta. Wheat-based and hybrid rye-based diets were formulated without or with MC supplementation. Each value represents the mean ± SEM. *$P <$ 0.05.

7.5 DISCUSSION

In the current study, weaned pigs fed the hybrid rye-based diet had lower ADG and ADFI compared to those fed the wheat-based diet, which is in agreement with the previous report that increasing hybrid rye levels in a wheat-based diet reduced ADG and ADFI of growing-finishing pigs (Smit et al., 2019). The authors suggested that this might be caused by the greater NSP content in hybrid rye compared to wheat, which can make digesta more viscous, and thus pigs are more likely to feel full. However, Ellner et al. (2021) reported that hybrid rye could be used as an alternative to wheat for weaned piglets without affecting growth performance. Chuppava et al. (2020) reported that feeding a diet containing 69% hybrid rye did not affect the growth performance of weaned pigs. The discrepancies between these studies and the current study might be explained by the fact that the CP content of the hybrid rye-based diet in the current study was lower than that of the wheat-based diet, whereas the experimental diets in other studies were isonitrogenous. Although ADG and ADFI were lower in pigs fed the hybrid rye-based diet than those fed the wheat-based diet, their feed efficiency was comparable because ADG and ADFI were reduced in parallel. Similar results were also reported by Schwarz et al. (2014), in which no differences in feed efficiency were observed between the hybrid rye-based and barley-based diets. Also, the present study showed that dietary MC supplementation did not affect growth performance, which is in agreement with the study reported by Olukosi et al. (2007). However, several studies reported that the addition of carbohydrase improved feed efficiency in pigs (Fan et al., 2009; Koo et al., 2017). The reason for these inconsistent results has not been established yet.

The ATTD of GE was higher in the wheat-based diet than in the hybrid rye-based diet in phase 1, which can be explained by the fact that wheat contains more starch and less NDF than hybrid rye. Previous studies reported that dietary NDF is negatively correlated with GE digestibility and that greater concentrations of soluble dietary fiber can increase viscosity and reduce nutrient digestibility (Annison and Choct, 1991; Kim and Nyachoti, 2017). Similarly, the ATTD of CP was greater in the wheat-based diet than in the hybrid rye-based diet, which might be due to the greater concentration of total dietary fiber in the hybrid rye-based diet. However, the ATTD of NDF in hybrid rye-based diet was greater than in the wheat-based diet, indicating that pigs fed hybrid rye had more microbial fermentation. This finding is in agreement with the study by McGhee and Stein (2018), who reported that hybrid rye had more microbial fermentation than barley, wheat, corn, and sorghum. The greater ATTD of P in the hybrid rye-based diet than in the wheat-based diet might be due to the higher intrinsic phytase content of hybrid rye (Song et al., 2022). Dietary MC supplementation increased the ATTD of NDF in phase 2, demonstrating that MC supplementation can hydrolyze NSP in cereal grains to release more nutrients and thus improve nutrient digestibility. This finding is in agreement with the study by Huang et al. (2021), in which dietary supplementation of MC and phytase complex improved the ATTD of nutrients in a corn-wheat-SBM based diet. Pigs fed the hybrid rye-based diet had a greater relative weight of large intestine compared to those fed the wheat-based diet, which might be due to the higher fiber content in the hybrid rye-based diet. Similarly, Nyachoti et al. (2000) reported that feeding high fiber diets to pigs increased the relative size of the liver, colon, and cecum.

Interactive effects were observed for the concentration of acetic acid, propionic acid, and total SCFA in the cecum, indicating that the impacts of MC supplementation on SCFA production differed between the hybrid rye-based diet and wheat-based diet. This might be due to the fact that the amounts of soluble NSP and total NSP in hybrid rye were higher than in wheat. For the hybrid rye-based diet, MC supplementation increased the concentrations of acetic acid, propionic acid, and total SCFA in the cecum, suggesting that exogenous enzyme promoted NSP degradation and

SCFA production in the cecum. This finding is in agreement with the study reported by Zhang et al. (2017), in which cell wall-degrading enzymes such as cellulase and xylanase increased the concentrations of acetate, propionate, and total SCFA in the digesta. Also, compared with the wheat-based diet, the hybrid rye-based diet produced greater concentrations of valeric acid and BCFA in the cecum and valeric acid in the colon. This could be due to the high fiber content in hybrid rye, which may bind to dietary protein and lower its digestibility in the small intestine. Consequently, more protein remains undigested until it reaches the large intestine, where it can undergo microbial fermentation, potentially leading to an increase in BCFA production.

Lactobacillus and Bifidobacterium can produce lactic acids as the primary metabolic product of carbohydrate fermentation. Lactobacillus and Bifidobacterium are considered as beneficial microorganisms because they can maintain microbial balance in the host intestinal tract and prevent the proliferation of unfavorable pathogens (Cho et al., 2009). The addition of exogenous carbohydrase in diets can produce short-chain oligosaccharides from cell wall NSP and have potential prebiotic effects (Kiarie et al., 2013). Therefore, the finding in this study showing that MC supplementation increased the relative abundance of Bifidobacterium and Lactobacillus, is in agreement with the results of the studies reported by Long et al. (2020) and Zhang et al. (2014). Also, the reduced fecal scores were observed for pigs fed the hybrid rye-based diet during the first two weeks after weaning and for those fed with MC supplementation during the first week after weaning, indicating that hybrid rye and MC supplementation might mitigate the occurrence of diarrhea in weaned pigs. These results are consistent with Kiarie et al. (2008), who demonstrated that the use of NSP hydrolysis products can reduce the fecal scores in piglet, and with Ellner et al. (2021), who found that piglets on a hybrid rye diet had the lowest fecal scores. Similarly, Chuppava et al. (2020) discovered that incorporating a high inclusion of hybrid rye (69%) in pig diet can help in reducing the excretion of *Salmonella* in the feces of young pigs.

7.6 CONCLUSION

The study findings reveal that feed efficiency remained consistent between the wheat- and hybrid rye-based diets in weaned pigs. This is noteworthy considering that the replacement of wheat with hybrid rye resulted in a decrease in both average daily gain and average daily feed intake. Additionally, incorporating hybrid rye in the diets of weaned piglets, as well as the application of exogenous multi-carbohydrase supplementation in both wheat- and hybrid rye-based diets, can improve the gut health outcomes of weaned pigs.

CHAPTER EIGHT: GENERAL DISCUSSION

Rye (*Secale cereale* L.) is a cereal grain primarily cultivated in North America and Europe, known for its resilience in harsh conditions such as cold climates, droughts, and infertile soils. Traditionally, its use in pig feed has been limited due to high levels of antinutritional factors and a vulnerability to ergot contamination, which can harm animal health (Bengtsson et al., 1992; Bushuk, 2001; Coufal-Majewski, 2016). However, recent developments have led to the creation of a new hybrid rye variety. This improved variant exhibits lower antinutritional factors and a reduced likelihood of ergot contamination. Similar to other cereal grains, hybrid rye is rich in starch and possesses a higher protein content than corn, as well as having a substantial number of fermentable fibers beneficial for the intestinal health of pigs. The metabolizable energy (ME) content of this newly developed hybrid rye is comparable to that of barley and sorghum, making it an attractive ingredient for pig feed (McGhee and Stein, 2020). Nonetheless, the presence of high levels of non-starch polysaccharides (NSP), particularly water-soluble arabinoxylans, in hybrid rye may impede the absorption of nutrients and energy and affect pig growth performance, posing environmental issues due to the excretion of unutilized nutrients (Cervantes-Pahm et al., 2013; Cervantes-Pahm et al., 2014; Bach Knudsen et al., 2016; Bach Knudsen et al., 2017).

Grinding is essential in animal farming, as it significantly influences the digestibility of energy and nutrients in pigs by changing the size of cereal grain particles. Finer particles provide a larger surface area for digestive enzymes in the pig's gut, enhancing nutrient and energy absorption (Huang et al., 2015). However, too fine feed may raise the risk of gastric ulcers in pigs and increase electrical energy consumption in feed production, leading to higher production costs (Vukmirović et al., 2017; Kiarie., 2019). Additionally, the use of multi-enzyme supplements, which include various enzymes like carbohydrases, protease, phytase, and lipase, is more effective than single-enzyme supplements. These supplements efficiently break down cell walls and release NSP hydrolysis products, thus enhancing the ingredient's nutritional value and contributing to better animal health, economic efficiency, and reduced environmental impact (Meng et al., 2005; Kiarie et al., 2007; Adeola and Cowieson, 2011; Slominski, 2011; Woyengo et al., 2011). Integrating these enzyme supplements into the feed, especially for NSP-rich cereal grains like hybrid rye, may significantly improve their digestibility and nutritional value for non-ruminant animals. The integration of feed processing and enzyme addition has been identified as an effective strategy to improve the nutritional values of NSP-rich feedstuffs, with the combined application of these technologies offering notable benefits, ultimately leading to more efficient and sustainable pig and poultry farming (Schedle, 2016). Consequently, the overall objectives of this study were to optimize the utilization of hybrid rye in swine diets through applying feed processing techniques and incorporating enzyme supplements. The results from the studies conducted to determine the above objectives are discussed here.

In Manuscript I, the study aimed to evaluate the net energy (NE) content of hybrid rye and to examine the impact of particle size and variety on its energy content. The hypothesis was that fine grinding would enhance the energy content of hybrid rye. According to the findings, the NE for coarsely and finely ground grain of Bono variety was 11.06 MJ/kg DM and 11.77 MJ/kg DM, respectively, while for Gatano variety coarse and fine grinding, it was 11.09 MJ/kg DM and 11.57 MJ/kg DM. No significant differences were observed for the digestible energy (DE), ME, and NE values between the two hybrid rye varieties. Both DE and ME values were higher in finely ground hybrid rye compared to the coarsely ground rye, and NE tended to increase in finely ground grain of hybrid rye as well. These results suggest that reducing particle size can improve the energy content of hybrid rye when fed to growing pigs.

In this study, it was observed that decreasing the particle size of hybrid rye significantly increased its surface area. For example, when Bono's particle size was reduced from 1,111 to 594 μm, there was an approximate 112% increase in surface area. Likewise, reducing Gatano's particle size from 1,210 to 717 μm resulted in a 92% increase in surface area. This reduction in particle size enhanced the apparent total tract digestibility (ATTD) of dry matter (DM) and gross energy (GE) in the hybrid rye diets. This improvement can be attributed to the fact that a smaller particle size increases the feed's surface area, facilitating more effective interaction with the pig's digestive enzymes (Wondra et al., 1995). The study found higher DE and ME in finely ground hybrid rye compared to its coarsely ground counterpart, supporting previous studies (Fastinger and Mahan, 2003; Rojas and Stein, 2015), which indicated that fine grinding could enhance DE and ME contents in pig diets. The DE and ME values obtained in this study for hybrid rye were consistent with the range reported by McGhee and Stein (2020). Nonetheless, the NE values obtained in this research differed from those predicted, suggesting that there may be a need to develop new prediction equations specifically designed to estimate the NE content of hybrid rye when considering different particle sizes.

In manuscript II, the research focused on investigating the impact of particle size reduction and multi-carbohydrase (MC) supplementation on the ATTD of energy and nutrients and the standardized ileal digestibility (SID) of amino acids (AA) in hybrid rye diets for growing pigs. The hypothesis proposed that smaller particle sizes and the addition of MC would enhance both the ATTD of energy and nutrients and the SID of AA. The findings revealed that diets with finely ground hybrid rye had higher ATTD of neutral detergent fiber (NDF) and ether extract (EE) and showed a tendency for greater ATTD of GE compared to those with coarsely ground hybrid rye, likely due to the increased surface area from smaller particles enhancing enzyme interaction

(Wondra et al., 1995). Moreover, diets supplemented with MC showed improved ATTD of DM, GE, crude protein (CP), NDF, EE, and greater SID of CP and most AA in hybrid rye. This suggests that MC supplementation is effective in breaking down NSP in hybrid rye, thereby enhancing the digestibility of a range of nutrients. Overall, the study concluded that reducing hybrid rye particle size enhances its nutrient digestibility, and supplementing with MC improves the digestibility of energy and AA in hybrid rye diets for growing pigs.

This study found that the SID of AA in hybrid rye was comparable to the values reported by NRC (2012) yet exceeded those reported by McGhee and Stein (2018). The variation in SID of AA results could be influenced by various factors such as the distinct genotype of the rye, the specific composition of the diet focusing on dietary fiber and antinutritional factors, the type of indigestible marker utilized, and the processing methods applied. The study revealed that reducing hybrid rye's particle size did not improve the SID of AA, a finding consistent with similar research on corn by Rojas and Stein (2015) but contrasting with other studies (Kim et al., 2009; Yáñez et al., 2011), possibly due to the effects of grinding intensity on the movement of digesta, altering passage rate of the digesta through the gastrointestinal tract when comparing hybrid rye with other ingredients. In terms of ATTD of DM, GE, and CP, diets with Gatano hybrid rye showed lower levels compared to those with Bono, potentially due to Gatano's higher NDF content. Previous studies (Annison and Choct, 1991; Kim and Nyachoti, 2017) have suggested that elevated NDF levels can negatively affect the digestibility of DM and GE, and that increased soluble dietary fiber might elevate viscosity, hindering nutrient absorption. This is consistent with the observation that Bono exhibited a higher SID of CP compared to Gatano, likely owing to the latter's elevated fiber content. Interestingly, the study also revealed that the interaction between hybrid rye variety and

MC supplementation affected the SID of CP and most AA, indicating that MC supplementation was more effective with Gatano, possibly because of its higher fiber content.

The research in manuscript III was focused on examining the combined and individual impacts of particle size in hybrid rye and varying levels of phytase (0, 500, and 2,500 FTU/kg) on the ATTD and the standardized total tract digestibility (STTD) of phosphorus (P) in diets fed to growing pigs. The study hypothesized that smaller hybrid rye particles and increased phytase supplementation would lead to greater P digestibility. The study's results supported this hypothesis, revealing that the ATTD of P in diets with finely ground hybrid rye was 61.7%, which was higher compared to 58.4% in diets with coarsely ground hybrid rye. Similarly, the STTD of P was 69.0% in diets with fine rye, surpassing the 65.9% in coarse hybrid rye diets. Moreover, increasing phytase levels showed a linear and quadratic enhancement in the ATTD of P, rising from 52.2% to 66.2%, and in the STTD of P from 59.4% to 69.1%. These findings suggest that reducing particle size effectively improves P digestibility in hybrid rye, and increasing phytase levels further enhances this effect in a linear and quadratic fashion in growing pigs fed hybrid rye diets.

The current study's findings on the ATTD of P (52.2%) and STTD of P (59.4%) in hybrid rye without microbial phytase supplementation align with the results reported by Archs Toledo et al. (2020) and are slightly higher than those reported by NRC (2012) and McGhee and Stein (2019). The research demonstrated that a finer particle size in hybrid rye effectively enhances both the ATTD and the STTD of P, suggesting that the increased surface area resulting from reduced particle size plays a significant role in improving P digestibility in pigs. Although hybrid rye naturally contains high levels of intrinsic phytase (Slominski et al., 2007), this study revealed that additional microbial phytase supplementation further elevates the ATTD of P and the STTD of P in hybrid rye diets. Additionally, the study documented that raising the level of microbial phytase from 500 to 2,500 FTU/kg notably improved the STTD of P in both finely and coarsely ground hybrid rye diets, suggesting that the 500 FTU/kg of microbial phytase might not be sufficient to fully hydrolyze all available substrates in hybrid rye, as also noted by Archs Toledo et al. (2020). The study also observed an increase in the ATTD of calcium (Ca) with the addition of microbial phytase to the diet since dietary calcium can form indigestible Ca-phytate complexes in pigs' intestinal tracts (Selle et al., 2009). However, the reduction in particle size of hybrid rye did not lead to an increase in the ATTD of Ca, likely due to the minimal contribution of Ca from hybrid rye and the fact that the Ca digestibility in the diets is largely influenced by the digestibility of Ca from limestone.

The manuscript IV focused on evaluating the effects of MC supplementation on the growth, nutrient digestibility, short-chain fatty acids (SCFA) production, and gut microbial composition in weaned piglets fed diets based on either wheat or hybrid rye. The hypothesis was that MC supplementation could enhance the growth, nutrient digestibility, and intestinal health of weaning pigs, and that replacing wheat with hybrid rye in their diets would not hinder their growth. The study indicated that pigs consuming the wheat-based diet outperformed those on the hybrid rye-based diet in terms of average daily gain (ADG) and average daily feed intake (ADFI) across both phases, while the gain to feed ratio (G:F) remained the same across both diet types. This supports the study by Smit et al. (2019) that higher inclusion levels of hybrid rye in a wheat-based diet decrease ADG and ADFI in growing-finishing pigs. The diminished ADG and ADFI with the hybrid rye diet could be due to its increased NSP content, potentially leading to viscous digesta and increased satiety in pigs. However, studies by Chuppava et al. (2020) and Ellner et al. (2021) suggested that hybrid rye can replace wheat in pig diets without affecting growth, a contrast possibly explained by the current study's lower CP content in the hybrid rye diet compared to the wheat diet, unlike the isonitrogenous diets in the other studies. Despite lower ADG and ADFI in pigs fed the hybrid rye diet, G:F was similar to that of wheat-fed pigs due to parallel reductions in ADG and ADFI. The lack of impact of MC supplementation on growth performance may be attributed to its limited effect on improving energy and nutrient digestibility, as evidenced by only the marginal increase in ATTD of NDF in the phase 2.

The study found that the ATTD of GE and CP was significantly higher in pigs on the wheat-based diet compared to those on the hybrid rye-based diet (85.0% vs. 83.6% for GE and 82.4% vs. 77.5% for CP, respectively). This difference may be due to the higher fiber content in the hybrid rye diet, as NDF is known to negatively impact the digestibility of both GE and CP (Kim and Nyachoti, 2017). Additionally, the ATTD of NDF was higher in the hybrid rye diet, suggesting more effective microbial fermentation in pigs fed this diet, which was in agreement with the findings by McGhee and Stein (2018). Pigs fed the hybrid rye diet also showed a higher ATTD of P, possibly due to the higher intrinsic phytase levels in hybrid rye. Moreover, the study noted that MC supplementation in the phase 2 enhanced the ATTD of NDF, demonstrating its role in breaking down the NSP in cereal grains.

It was observed that the concentrations of acetic acid, propionic acid, and the total SCFA in the cecum were notably higher in pigs fed the hybrid rye-based diet supplemented with MC, compared to those on the same diet without MC supplementation. This enhancement in SCFA levels suggests that MC supplementation plays a key role in promoting the breakdown of NSP found in hybrid rye within the entire gastrointestinal tract. As a result, this process facilitates the utilization of these fibers by gut microbiota, leading to increased production of SCFA. Moreover, the addition of MC also increased the relative abundance of beneficial gut bacteria, specifically Bifidobacterium and Lactobacillus. These bacteria are known for their positive effects on gut health, including the promotion of nutrient absorption and enhancement of the immune response. The increased presence of these bacteria, coupled with higher SCFA levels, indicates a more efficient fermentation process in the hindgut. This improved fermentation not only contributes to the breakdown of fibers into SCFA, which are crucial for maintaining gut health, but also plays a significant role in the overall development and health of the animal's digestive system, ensuring better nutrient utilization and potentially improving the animal's growth and health outcomes.

CHAPTER NINE: CONCLUSIONS AND FUTURE STUDIES

9.1CONCLUSIONS

The following conclusions can be drawn from the current research:

1. The determined NE contents of Bono hybrid rye was 11.42 MJ/kg DM, and the determined NE contents of Gatano hybrid rye was 11.33 MJ/kg DM.
2. The energy values of different hybrid rye varieties might be comparable.
3. Particle size reduction could significantly improve the DE and ME values of hybrid rye fed to growing pigs, while the NE value was only numerically improved.
4. The nutrients digestibility of hybrid rye can be affected by its variety.
5. Particle size reduction could improve the nutrient digestibility in hybrid rye
6. The MC supplementation could improve the energy and AA digestibility in hybrid rye fed to growing pigs.
7. Particle size reduction could improve the digestibility of P in hybrid rye
8. Supplementing increasing levels of phytase improved P digestibility in linear and quadratic manner in growing pigs fed hybrid rye diets.
9. Replacing wheat with hybrid rye in the nursery diet may reduce ADG as a result of decreased ADFI, but the gain to feed ratio is comparable between the hybrid rye-based diet and the wheat-based die
10. Feeding hybrid rye diets and MC supplementation could enhance the gut health of weaning piglets.

9.2 FUTURE STUDIES

The current study has shown that the nutritional value of hybrid rye for pigs can be enhanced through feed processing and enzyme supplementation, yet further research is needed to:

1. Determine the effects of mill type such as hammer mill and roller mill on the energy and nutrients digestibility in hybrid rye fed to growing pigs.
2. Exploring the effects of other feed processing techniques such as extrusion and pelleting on the nutritional value of hybrid rye for pigs.
3. Using the direct method to determine the NE content of hybrid rye fed to growing pigs and compare the difference between the direct method and difference method on the energy contents of hybrid rye.
4. Develop more phytase supplementation levels to determine the optimal phytase levels added in hybrid rye dies for pigs.
5. Determine the effects of mill type on the growth performance and gut health of weaning piglets when fed hybrid rye diets.

CHAPTER TEN: LITERATURE CITED

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