EFFECTS OF SUPPLEMENTAL METHIONINE SOURCES IN FINISHING PIG DIETS ON CARCASS CHARACTERISTICS, CUTTING YIELDS, AND MEAT QUALITY

BY

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THESIS

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Abstract

While supplemental methionine (Met) is widely used within the swine industry, data are limited regarding the effect of Met sources on carcass cutability and meat quality. The objective was to determine the effects of L-Met (LM, 99%), DL-Met (DLM, 99%), or calcium salt of DL-Met hydroxyl analogue (MHA, 84%) in finishing pig diets on carcass characteristics and meat quality. At d 66 of age, pigs (N = 240) were allocated to 60 single-sex pens for a four phase feeding system that lasted 104 days. On d56 pens were randomly allotted to one of three experimental diets for 48 day with a phase change at d83. For the final 7 wk of the finisher phase, pigs (BW= 79.9 ± 0.80 kg) were fed diets containing LM, DLM, or MHA, with the supplemental Met source providing 25% of standardized ileal digestible (SID) Met + cysteine (Cys) requirement. One pig per pen was slaughtered at study conclusion, and left sides of carcasses were fabricated into subprimal cuts to determine carcass-cutting yields. Loin quality including proximate composition and shear force was measured. Data were analyzed using the MIXED procedure of SAS. Hot carcass weight was not different (P = 0.34) between treatments (LM 104.5 kg; DLM 103.0 kg; MHA 101.5kg), moreover loin eye area was not different (P = 0.98) between treatments (LM 52.65 cm²; DLM 52.49 cm²; MHA 52.81 cm²). Boneless carcass cutting yield was not different (P = 0.56) between treatments (LM 54.97 kg; DLM 54.82 kg; MHA 54.52 kg). Loin pH was not different (P = 0.24) between treatments (LM 5.45; DLM 5.48; MHA 5.45). However, drip loss tended to be reduced (P = 0.11) by the DLM treatment (5.58%) compared with LM (7.03%) and MHA (6.68%) treatments. Shear force was not different (P = 0.85) between treatments (LM 3.03 kg; DLM 3.06 kg; MHA 3.10 kg). However, cook loss tended to be reduced (P = 0.06) by the DLM treatment (16.20%) compared with LM (18.18%) and MHA (18.50%) treatments. These data suggest that only minimal differences in carcass cutability and meat quality can be attributed to Met source in finishing pig diets.

Key words: Carcass composition, meat quality, methionine, pork, sulfur amino acid, swine nutrition

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CHAPTER 1: REVIEW OF LITERATURE

Introduction

Amino acids (AA) are the building blocks of proteins and fall into one of two categories; essential and nonessential. Those that can be made by the body occur by de novo synthesis. However, AA that cannot be made through de novo synthesis must be obtained through the diet (East, 1929). Methionine (Met) is one of the essential AA and is typically the second limiting AA for pigs. Not only is Met an essential amino acid, but it is important in initiating protein translation, methyl donation, providing sulfur, antioxidant production, and immune function. Methionine is a naturally occurring amino acid in plant protein ingredients normally included in swine diets; however, the concentration of Met within any individual plant ingredient is insufficient to meet Met requirement. Due to the deficiency of Met in standard corn-soybean meal diets, Met is often supplemented. Supplementation enables producers to ensure AA requirements are met.

Furthermore, Met can be supplemented in multiple forms; L-Met, D-Met, DL-Met, MHA, etc. Supplementary Met is commonly supplied as DL-Met due to cost and ease of manufacture. However, Met is readily absorbed and used in the body as L-Met. Other forms of Met undergo transamination to convert into L-Met. Due to the additional steps required for absorption and utilization of alternative supplemental sources, many studies have evaluated the bioavailability of different forms (Drażbo et al., 2015; Lebret et al., 2018; Liu et al., 2007; Shoveller et al., 2010; Zelenka et al., 2013; Zimmermann et al., 2005). The addition of supplemental Met from various forms improve lean accretion and nitrogen retention (Li et al., 2017). Lean accretion is important in production animals by increasing saleable product and carcass yields.

While multiple studies have reported effects of different Met sources on health status and live performance, fewer have investigated the potential for differences in carcass yield and quality between Met sources. The effect of Met source on carcass yield and meat quality has predominantly been researched in broilers. Methionine is typically the first limiting amino acid for poultry and therefore has been studied extensively (Esteve-Garcia & Llauradó, 1997; Kiraz & Şengül, 2018; Liu et al., 2007; Wallis, 1999). While studies evaluating the effects of Met sources on growth performance of nursery pigs have been conducted, fewer studies have evaluated the potential for differences in carcass characteristics and meat quality traits of growing-finishing pigs.

Methionine in the diet / pig supplementation

Sources of methionine

Methionine naturally occurs in two chiral isoforms, D- and L-Met. While these two forms are mirror images of each other, D-Met must undergo a transformation to L-Met to be used metabolically (Fell 1959; Wu 2009). Additionally, Met as 2-hydroxy-4-methylthio butanoic acid (**MHA**), is produced as a liquid (**MHA-FA**, 88%) or solid form (**MHA-Ca**, 84%).

Soybean meal is a high protein ingredient, but a poor source of Met, therefore many diets are supplemented with Met to prevent deficiencies (Swine NRC, 2012). One common form of Met is DL-Met; this particular form is a 50:50 ratio of D- and L- enantiomers, 98-99% pure, and is produced as a crystalline. However, Met sources are not equal in bioefficacy. While the body is only able to readily use L-Met, D-Met can be converted and used with the same bioefficacy. The mixture of D- and L-Met has been extensively proven to have a 99% purity and its relative bioefficacy is very comparable to that of L-Met (Featherston et al., 1962; Grau & Almquist, 1943). In contrast, MHA is reported to have a relative bioefficacy of 65-80% compared to L-

Met. Each of the sources mentioned can be used to reach the requirement level, but with differences in the inclusion percentage. These differences are dictated by percentage of active ingredients source. When supplementing with MHA to reach the requirement level of Met, it takes a greater amount on a weight basis than DL-Met because its purity difference.

Methionine metabolism

Like other AA, Met is absorbed into the bloodstream through luminal AA transport systems. These AA are then transported out of the enterocyte through a sodium potassium exchange allowing for AA to enter the circulatory system (Soriano-García et al., 1998; Zebrowska et al., 1982). Methionine can be transported across the small intestinal lumen to the blood stream by sodium-dependent and –independent transporters. Interestingly, Met sources are absorbed differently where MHA are absorbed by sodium-dependent and sodium-independent monocarboxylate transporters while DL-Met is absorbed by Met specific sodium-dependent and sodium-independent transporters (Romanet et al., 2021). The affinity for L-Met is higher than that of D-Met in transporter cells (G. Wu, 2021). Once entering the circulatory system, D- or L-Met travels to multiple tissues.

Biological functions of methionine

The biological functions of Met are initiating protein translation, methyl donation, antioxidant production, and immune function (Yang et al., 2020). Each of these functions work together to promote overall growth and maintenance of the body. Additionally, Met works as a functional amino acid because it is a precursor for other AA and compounds.

Protein translation and methyl donation. Methionine can be utilized to synthesize proteins, nitrogen compounds, and be a source of energy. Furthermore, Met is a precursor to multiple important bioactive compounds like S-adenosyl-methionine, homocysteine, cysteine,

and succinyl-CoA (G. Wu et al., 2004). Upon entering the methyl cycle, methionine adenosyltransferase interacts with Met by transferring an adenosine group from ATP onto the sulfur molecule to create S-adenosyl-methionine (SAM). Primary functions involving SAM include methyl donation as well as providing a source of methylene and amino groups (Brosnan & ME, 2006). Additionally, as a primary methyl donor, SAM contributes to the methylation of DNA that allows for regulating gene activity and cellular differentiation via epigenetic mechanisms (Jerbi et al., 2017).

Methyl cycle. S-adenosyl-methionine undergoes methyl transferase producing S-adenosyl-homocysteine (SAH) and a methyl group, which then hydrolyzes into homocysteine. Two potential pathways are created for homocysteine; first, recycling back to Met by methionine synthase; or second, producing cysteine and downstream succinyl-CoA. The production of succinyl-CoA is then used in the TCA cycle to produce ATP and $FADH_2$. Homocysteine is recycled back to Met when vit B12 is present and receives a methyl group from folate or betaine from 5-methyl-tetrahydrofolate (5-methyl-THF). Within the folate cycle, THF is able to produce folate which is important for nucleic acid synthesis. Alternatively, when homocysteine is important for the production of cysteine that plays an important role in the production of hair, nails, and a precursor of several other compounds; PAPS (3'phosphoadenosine-5'phosphosulfate), cystine, and glutathione.

Methylation. The methyl cycle is also important in producing SAM for methylation, which is key for DNA replication, RNA replication, and protein methylation. DNA methylation occurs at the cytosine-phosphate-guanine site and converts cytosine into 5-methylcystine. Once methylated, mRNA is able to start the process of protein synthesis through translation. Methylation occurs at promotor regions of the DNA; this is how certain genes are expressed and

others are not. Alternatively, Met is also important in fat deposition through oxidative catabolism. Carnitine synthesis from S-adenosyl-methionine transports long chain fatty acids to the mitochondrial matrix allowing for beta-oxidation to break down the fatty acid molecules to produce acetyl-CoA (Fugui Yin, Yulong Yin, 2013).

Antioxidant production. As one of two AA that provide sulfur, Met is also one of the AA most susceptible to oxidation from reactive oxygen species (**ROS**) (Vogt, 1995). When the body undergoes stress, production of ROS can lead to cellular damage of proteins, lipids, and DNA (Yang et al., 2020). Damage to Met may increase the likelihood of decreased protein translation initiation (Levine et al., 1996). Methionine functions as an endogenous antioxidant where it produces Met sulfoxide reductases capable of reducing Met sulfoxides back to Met. Furthermore, Met also functions as a precursor for Cys, which is needed for glutathione (**GSH**) production, another cellular antioxidant (Levine et al., 1996, 1999).

Immune function. Furthermore, Met functions as a precursor for several molecules with critical immune function, polyamines, taurine, and GSH being a few. Polyamines have been referred to as "molecular grease" because high amounts of polyamines are found in cells that are rapidly dividing to support DNA transcription and RNA translation (East, 1929). Taurine works as a membrane stabilizer, a nitrogenous compound in immune cells, and helps to modulate pro inflammatory cytokine production (Grimble, 1996). Finally, GSH is needed to initiate T-lymphocytes, leukocytes, and produce cytokines needed for mounting an immune response (G. Wu et al., 2004).

Methionine deficiency

As an essential amino acid, Met cannot be produced via de novo synthesis. If the diet is deficient in Met, it will cause depressed muscle growth that will negatively impacts yields for

producers. A limiting amino acid is the amino acid with which the dietary supply provides the lowest proportion of the requirement ("Nutr. Requir. Swine," 2012). Given the number of biological processes requiring Met, failing to meet pigs' requirements will result in growth suppression as well as multiple other negative effects.

Methionine deficiency

Methionine is reported as the second limiting amino acid, behind Lys, in a corn-soybean diet due to the limited concentration in soybean meal. Similar to Lys, Met is important for muscle growth. Methionine requirements are dependent on age, sex, weight, gestational status, and health status. For finishing pigs ranging from 75-100 kg and 100-135 kg, the Met requirement on a standardized ileal digestible (SID) basis (%) is 0.21 and 0.18, respectively (Swine NRC, 2012).

Diets that are deficient in Met largely impact muscle and fat development. Yang (2021) reported that when Met was reduced by 40.5% relative to the recommendation of the 2012 Swine NRC, feed intake of growing pigs did not change, but both average daily gain and gain to feed conversion decreased. This trial also reported reduced muscle cell differentiation could be the cause of the reduced growth performance (Yang et al., 2021). In addition to reduced muscle cell differentiation, plasma urea nitrogen concentration was significantly increased in the Met deficient diet suggesting Met was the first limiting amino acid and when Met was fully used, other AA were removed as waste (Yang et al., 2021). In a study conducted in young growing pigs 42-52 days of age for ten days evaluated, the effects of Met deficient does have an effect on growth, metabolism, and lipolysis (Conde-Aguilera et al., 2016). Diets deficient in Met cause an increased de novo lipogenesis in adipose tissue of growing pigs; leading to an increase in total lipogenesis (Castellano et al., 2015). There was an increase in the amount of subcutaneous

adipose tissue in the LD muscle. Another study showed young pigs 42-52 days of age with a Met deficiency had decreased growth and glycolytic metabolism, resulting in decreased LM muscle weights (Conde-Aguilera et al., 2016). With these findings, it is well recognized that both growing and finishing pigs are susceptible to the effects of Met deficient diets. In neonatal pigs (3-10 d old), Bauchart-Thevet (2009) found that a sulfur amino acid deficiency suppressed the proliferation of epithelial cells causing further damage to growth potential.

Historically, the effects of Met deficiency have been evaluated in poultry since Met is the first limiting amino acid. Studies demonstrating the negative effects of Met deficiency in broilers can serve as a model for Met deficiency in pigs as both species are fast-growing meat species selected for growth efficiency and lean muscle development. While the research for Met deficiency in broilers is more extensive, impacts to muscle development are similar to the findings in pig studies.

Synthetic methionine supplementation

Current research findings demonstrate few differences in growth performance and meat quality of broilers supplemented different Met sources. However, early research conducted in both broilers and pigs indicated DL-Met may outperform MHA (Dilger & Baker, 2008; Lemme et al., 2002a; Zelenka et al., 2013). In Lemme's (2002a) study where DL-Met out performed MHA, the differences in intestinal absorption were thought to be a reason for differences in relative effectiveness of Met sources. The addition of a supplemental Met resulted in increased breast muscle for broilers (Esteve-Garcia & Llauradó, 1997; Liu et al., 2007; Pokoo-Aikins et al., 2022). Y. Liu (2007) reported a decrease in abdominal fat in addition to increased breast muscle. Important to note that studies that take bioefficacy into account did not show differences in growth performance in broilers feed either supplement.

In addition to the positive effects of Met supplementation in broilers, positive increases in growth and muscle development have also been observed in pigs. Roth (1987) observed greater longissimus dorsi size and reduced back fat at the 13-14th rib when growing pigs (60-90 kg) were supplemented DL-Met compared to diets supplemented L-Cys. Similar findings were reported by Opapeju (2012) where final body weight, average daily gain, and feed-to-gain ratio were greater in pigs supplemented either DL-Met at 0.04 and 0.08% or MHA at 0.062 and 0.123. At the higher additions of either supplement, a greater improvement in growth performance was observed when compared to the lower percent inclusion. B. Zimmermann (2005) established that the use of either DL-Met or MHA-FA will benefit growth when diets are deficient, but the use of MHA-FA has a bioefficacy of 65% relative to DL-Met.

When fed in excess, Met can increase the protein accretion in pigs. Studies conducted in broilers resulted in larger breast muscles (Esteve-Garcia & Llauradó, 1997; Liu et al., 2007) and a study conducted by Roth (1987) found similar results in pigs where the loin eye area and muscling had increased. While increasing the Met in the diet will increase muscling, and thereby increase carcass yield, excess amounts of Met in the diet can contribute to excess waste through higher blood urea nitrogen numbers (Whang & Easter, 2000). A blood urea nitrogen level can indicate protein turn over in the body. The liver will break down proteins and release nitrogen that can travel to the kidneys to be removed from the blood. A low BUN is indicative of the protein level being used toward lean accretion. However, feeding Met in extreme excess is not only expensive but also potentially can depress growth.

Conversely, the addition of more Met does not continue to improve growth past the requirement. Edmonds (2021) reported late-finishing growth of pigs can be susceptible to increased Met creating a decrease in growth performance. A decrease in ADG and ADFI were

observed for pigs supplemented with DL-Met at 1-2.3%. These findings were in efforts to understand how an increase in Met would impact pigs that were in the finisher for longer, in particular how methionine could be used to depress growth in times of market suspension. Too little Met in the diet will decrease growth, but too much Met in the diet may also decrease growth performance.

Lean accretion

Lean accretion

The meat industry relies on production animals' ability to develop muscle. The ability of animals to do so efficiently has important effects on carcass yield, profitability, and sustainability of meat production. Production animals utilize feeding-induced muscle protein synthesis by having greater anabolic metabolism over catabolic metabolism to promote protein synthesis thereby increasing amount of muscle. Prenatal muscle development occurs through hyperplasia, increasing the number of muscle cells. However, the number of muscle cells is largely considered fixed at birth (Aberle et al., 2012). After birth, muscle continues to develop through hypertrophy, increasing in size. Hypertrophy occurs by increasing both the diameter and length of myocytes (Aberle et al., 2012), to the point where muscle cells can increase by 10 to 15 times their original size. The maximum size of muscle fibers in pigs is achieved by approximately 150 days after birth (Aberle et al., 2012). Hypertrophy is regulated by a wide variety of factors including diet, sex, and genetics. Amino acids and glucose are used in the diet to increase protein synthesis and protein deposition (Staun, 1963). Sex and genetics also regulate the body's response to feeding-induced muscle protein synthesis through hormones like Insulin-like Growth Factor-1 (IGF-1) (Casas-Carrillo et al., 1997) and genetics that are more likely to have increased lean accretion like Piétrain and Large White (Gregory et al., 1977).

Regulation of hypertrophy

While diet greatly affects the growth of an animal, at the cellular level, muscle hypertrophy is dependent on cellular signaling and protein accretion pathways. At the cellular level, protein accretion occurs through activation of the mammalian target of rapamycin (**mTOR**) pathway. While mTOR is involved in skeletal muscle hypertrophy through several different involvements, the mTOR pathway primarily initiates protein synthesis through two different pathways. First, active mTOR phosphorylates S6 kinase that downstream encourages translation. Second, mTOR phosphorylates 4E-BPs removing it from eIF4E allowing for the initiation complex that increases the rate of protein synthesis (Jeyapalan et al., 2007). The activation of mTOR starts upstream when a growth factor binds to a ligand, on the surface of the cell membrane, where it activates PI3K and Akt. After mTOR has encouraged protein translation to occur the initiation, elongation, and termination of the translation of the mRNA strand (Avruch et al., 2006; Jeyapalan et al., 2007; Long et al., 2005). Methionine is used in transcription of proteins by binding to the AUG start codon on the polypeptide chain (Drabkin & RajBhandary, 1998). Inside the ribosome, the mRNA strand is translated into AA by corresponding tRNA molecules releasing the amino acid they carry and links it to the previous amino acid. This will produce a peptide chain by elongation. These AA can be used by satellite cells on sarcomeres to increase muscle size or utilized by other tissues in the body to repair damage and as a source of energy.

Additionally, animals grow according to the growth curve which is dictated by the priority of partitioning nutrients according to the metabolic rate of that animal (Campbell, 1988). Nutrients absorbed from the blood stream aid development of the following categories; brain and central nervous system, bone, muscle, and fat. Development of muscle will continue to increase

linearly with dietary protein increase, but will plateau once the maximum hypertrophy is reached (Whittemore et al., 1988). It is important to note that the plateau of muscle hypertrophy is caused by the amount and ability of nuclei and satellite cells on skeletal muscle. Muscle hypertrophy is also limited by the muscle's ability to diffuse oxygen across a large fiber (van Wessel et al., 2010). This limits muscle hypertrophy because there is a shift from hypertrophic growth to being readily available for muscle repair (Gattazzo et al., 2020; White et al., 2010).

Pork meat quality

Supplementing Met in the diet is a common practice in the pork industry and the effects of Met on growth performance of pigs has been extensively researched. However, Met source's impact on pork meat quality is limited. Within the US, pork quality is typically measured through ultimate pH, color, marbling, water holding capacity, and fat quality.

Ultimate pH and pH decline

One of the greatest drivers of meat quality in pork is pH (Bee et al., 2007). The pH of living muscle tissue is approximately 7.2, relatively neutral, while the pH of meat ranges from 5.2-6.5. Every protein has an isoelectric point or pH at which the amount of positively charged ions are equal to the amount of negatively charged ions. As the isoelectric point of myosin is approximately 5.2, meat with an ultimate pH near that point will likely experience negative meat quality traits (J.R. Bendall, 1988; Savell et al., 2005).

Ultimate pH is primarily determined by the amount of glycolysis that occurs postmortem. During the slaughter process a loss of blood circulation occurs during exsanguination, causing a loss of homeostasis and cells ability to receive oxygen. Due to the lack of lack of oxygen, muscles are forced to utilize anaerobic glycolytic metabolism. Glycolysis results in pyruvate that results in lactate that then is converted to lactic acid. The buildup of lactic acid drives muscle pH

down, normally resulting in a pH of ~ 5.6 (Aberle et al., 2012; Bee et al., 2007; Lee et al., 2010; Monin & Sellier, 1985).

However, pH is not identical across all muscles as differences in muscle fiber type may also affect ultimate pH. When a muscle is more glycolytic in nature (have less oxygen and functions more anaerobically) they inherently have higher glycogen concentrations causing more postmortem lactic acid build up and greater pH decline than oxidative muscles (Klont et al., 1998; Lee et al., 2010). It is important to note though, that oxidative muscles do have greater variation in pH and are more susceptible likely to experience shorter shelf-life (Klont et al., 1998). For loin quality evaluations, pH is typically measured in the loin at the 10^{th} rib location but pH is also important for ham quality. Since ham muscles are commonly used in further processed products WHC of ham muscles is very important. One primal less influenced by pH is the belly. Since fat comprises a great proportion of the belly primal than muscle, belly quality is normally considered a factor of fat quality, not pH (Arkfeld et al., 2016).

Variation in pH decline and postmortem metabolism contributes to the development of other meat quality factors like; color, firmness, and water holding capacity. When the rate of pH decline is rapid, there is greater denaturation of sarcoplasmic and myofibrilar proteins cause meat to appear paler in color and lower water holding capacity. The term is commonly associated with these characteristics is pale, soft, and exudative (PSE; Mancini & Hunt, 2005; Offer, 1991). While PSE is caused by a rapid decline in pH, ultimate pH is similar normal pork. The acute stress an animal may experience during pre-harvest handling and slaughter can cause the body to releases hormones (cortisol and adrenaline) that release glycogen and creating the fight or flight response. When the body then utilizes anaerobic glycolysis and the buildup of lactic acid is greater from the increased amount of glycogen in the blood from the actuate stress, this drives

pH down quickly resulting in PSE (Offer, 1991; SELLIER & MONIN, 1994). The presence of the halothane gene is a genetic condition associated with increased PSE potential. Pigs that carry the halothane gene are also more susceptible to stress; hyper-metabolism, muscle rigidity, and elevated body temperature antemortem (Soriano-García et al., 1998).

While PSE is caused by a rapid decline in pH, the extent of pH decline may also affect the quality. When ultimate pH decline is limited it causes dark firm and dry (**DFD**). This meat usually has less protein denaturation (MacDougall, 1982). This defect is commonly observed in beef and may be caused by long term stress which depletes of glycogen reserves. In contrast, when pH decline is extended to less than 5.4 it nears the isoelectric point of muscle proteins like myoglobin which is 5.2. This extent problem is referred to as acid meat. The Rendement Napole (**RN**) mutation has been linked to the acid meat condition. While the RN gene does not directly cause acid meat, it has been reported to cause increased muscle glycogen content (SELLIER & MONIN, 1994).

Dietary Met reportedly has had little impact on pH when fed on an equal bioequivalence (Li et al., 2017; Yuan et al., 2020). Studies using Met in excess of requirement report having higher pH than those fed at requirement (Lebret et al., 2018). Lebret (2018) reported that Met fed at five times that of a control diet, formulated to meet Met requirements from the Swine NRC 2012, did increase pH 5.55 to 5.66 in the longissimus lumborum muscle.

Color

The color of meat has been noted as the largest influencing factor of meat purchasing (Mancini & Hunt, 2005) and the color of meat is impacted by myoglobin concentration, myoglobin state, pH and water holding capacity; while many of these factors impact meat color,

the evaluation of meat color is used by consumers as an indicator of freshness. Colors changes in meat are dictated by the main protein in meat, myoglobin.

Myoglobin contains a heme ring that can make six bonds. While five of these bonds do not impact the color of meat, the sixth does (Richard Mancini, 2013). The compound attached to the sixth ligand of myoglobin will determine the color it appears. Traditionally, pork cuts are normally associated with a pinkish-red color, however, at times it may appear to have a purplish or brownish color. When the sixth ligand has nothing attached to it, it is not in the presence of oxygen and called deoxymyoglobin, it has a purple appearance. A red color is present when the sixth ligand is bound to O2 or CO is bound to it, this is called oxymyoglobin and carboxymyoglobin respectively. Finally, when meat has a brown color to it, it has H2O attached to the sixth ligand and is in a ferric state referred to as metmyoglobin (Richard Mancini, 2013). It is important to note that these states are reversible and get their color from the interaction of the sixth ligand interaction with the iron on the heme group (Mancini & Hunt, 2005).

It is widely accepted that consumers do not desire meat that appears brown in color. This has prompted the industry to examine what are potential reasons for meat to appear brown. This can be due to oxidation of a product and may be a result of the type of muscle fiber. Another cause may be environmental factors such as lighting. It has been reported that meat will discolor more quickly under ultraviolet lighting than other lights (SEIDEMAN et al., 1984). This is important because many meat departments use modified-UV lights to display packages. This is problematic and is one of the reasons modified atmosphere packaging (MAP) have become popular. MAP is uses specific gases in the air between the plastic wrap and the meat so it holds a bright cherry red color (Mancini & Hunt, 2005). However, this type of packaging can be tricky

to consumers as they relate color to freshness even though meat package in MAP could still be spoiled and appear a bright red color.

Meat color can be measured by the naked eye (subjective) or with the use of an instrument (objective). The most common objective scale used in meat research is the CIE *L*a*b** system where *L** is lightness (light to dark), *a** is redness (red to green), and *b** is yellowness (yellow to blue) (Commission et al., 1960; Mancini & Hunt, 2005; Richard Mancini, 2013). Additionally, *L** is a scale from 0 to 100 where 100 is white and 0 is black, with pork usually between 31-61 *L** units. In addition to objective color measurements, subjective measurements allow for quality to be quickly measured and identify any deviations from normal color. Pork quality can be subjectively measured using National Pork Producers Council (**NPPC**) scales to determine color, marbling, and firmness. Color is determined by a scale 1-6 industry average being 3. Unsurprisingly, a colorimeter or spectrophotometer will be able to detect smaller differences that the naked eye may not see. The accepted difference in *L** values that can be perceived by the human eye is 2 *L** values, this aligns with half scores for the color standards sit by the NPPC (Brewer, Zhu, Bidner, et al., 2001). When differences are less than 3 *L** units in pork, the average consumer will not be able to detect the differences. Color may largely impact purchasing decision, it has been reported that visual color explains less than 1% of sensory traits; tenderness, juiciness, and flavor (Mancini & Hunt, 2005; Richardson et al., 2018).

Furthermore, meat color is impacted by pH, sex, diet, genetics, and stress (Hambrecht et al., 2005; Mancini & Hunt, 2005; Scheffler & Gerrard, 2007). As previously stated, meat will appear paler in color as the pH gets closer to its isoelectric point (Savell et al., 2005). This plus the low WHC is normally associated with PSE and can be triggered by the RN gene or stress at the time of slaughter. The diet of an animal can also effect meat color by affecting metabolism,

glycogen storage, chilling rate, and antioxidant accumulation (Richard Mancini, 2013). Using a low carbohydrate diet has been reported to produce darker loins. Additionally, dietary supplementation of Met 14 days before slaughter was reported to improve meat color by reducing lightness after seven days aging (Lebret et al., 2018). This study did however supplement Met in excess of five times the recommended amount to produce this result. It is important to note that most amino acids supplemented in grower-finisher diets have little effect on meat color (Lebret et al., 2018; Ma et al., 2020).

Marbling

Internal fat is first to deposit followed by intermuscular, subcutaneously, with intramuscular fat depositing. While internal fat is deposited first, there is not a halt to this fat development rather it slows allowing for excess energy to be used to deposited fat in other places like intramuscular fat (DW Pethick, 1996). Intramuscular fat is often referred to as marbling and is the recognizable streaks of fat in chops. It is recognizable for consumers as a positive eating experience as many consumers' desire beef steaks with greater marbling (Norman et al., 2003; SAVELL et al., 1986). This can preference is also observed in pork consumers but is influenced by the consumer type as some desire a leaner chop with less marbling present (Brewer, Zhu, & McKeith, 2001).

However, in pork there are conflicting results for effects of marbling on tenderness and juiciness. The NPPC marbling standards are determined by a 0-10 scale with the industry average being 2. One report by Moeller (2010) stated that loin chops with greater than 5-6% marbling were more likely to have greater tenderness and juiciness, but it is important to note that the majority of pork chops fall below the 5-6% marbling and chops in this study were cooked to a higher degree of doneness than the current recommended degree of doneness (63°C).

Other reports stated that when chops are cooked to the recommended degree of doneness (63°C), marbling has little impact on objective tenderness and juiciness or sensory attributes (Wilson et al., 2017).

A study in finishing pigs reported lipid content within the LD was not affected by dietary Met (K. G. Friesen, 1994). This study theorized that Met deficiency may result in nutrient repartitioning; with excess energy that would have been used for muscle deposition now directed toward fat deposition. This shift in energy repartitioning resulted in greater intramuscular fat content in pigs that were Met restricted after weaning (L. Wu et al., 2019).

Water holding capacity

As meat is approximately 75% water, water plays an important role in the consumers intent to purchase a product and the likelihood that they will purchase the product again (Cheng & Sun, 2008; Huff-Lonergan & Lonergan, 2005). This water being held within the muscle will contribute to sensory factors like juiciness and flavor. When there is a loss of water through either purge or cooking, the eating experience is described as less desirable. Water is held within meat at three different positions; bound, immobilized, and free. Bound water is held tightly by the muscle as this water is bound to proteins (Huff-Lonergan & Lonergan, 2005). By comparison, free water can be lost easily due to only weak forces like capillary action holding it in place. Immobilized water is held in place to the structure of a muscle cell but is not bound to it due to the steric effect that holds it in place (Huff-Lonergan & Lonergan, 2005). Therefore, when pH declines postmortem, changes in the net charge of bound water may affect muscle's ability to hold onto immobilized water. For this reason, water holding capacity (WHC) may have important effects on meat quality traits. The retention of water or WHC of meat is most greatly

impacted by pH and therefore has similar trends as pH (Cheng & Sun, 2008; Huff-Lonergan & Lonergan, 2005).

Moreover, WHC can be measured objectively and subjectively. The innate WHC can be measured objectively using drip loss and purge loss. Drip loss is water purged from meat and is measured by comparing the amount of water lost during a 24hr suspension. Cook loss is another important factor to consider when capturing the full picture for WHC. Subjective measurements of WHC can be measured through the use of trained or consumer panel evaluating juiciness (Hughes et al., 2014).

Furthermore, WHC can be improved by aging a product and allowing the sponge effect to occur. The sponge effect hypothesis theorizes that as meat wet ages, in most cases beef, the protein that are broken down create channels that water can be held in (Farouk et al., 2012). These channels could lead to lower WHC if cut prematurely but if there is more proteolysis occurring in the meat as it ages, there will be more channels created allowing for water to be retained more easily. This is one reason beef and lamb are aged before cut into primals or retail cuts (Farouk et al., 2012).

Studies focused on supplementing Met in pig diets reported improvements to WHC when compared to diets deficient in Met. Lebret (2018) found that increasing amounts of MHA-Met did improve WHC in pork. The improvement in WHC was determined by results for drip loss being higher in those pigs with greater amounts of Met. However, the improvement in WHC could be contributed to higher pH for diets that contained five times the recommended amount of Met for finishing pigs, rather than Met impacting only WHC.

Fat quality

Fat quality is an important aspect of pork quality since pork is widely consumed in the US as further-processed products. Quality of fat is largely measured by the fatty acid profile it exhibits (Miller et al., 1990; Wood et al., 2004). Unsaturated fats are softer and have a more oily appearance at room temperature, while saturated fats are firm or solid at room temperature. Therefore, the ratio of unsaturated to saturated fatty acid will affect firmness of products like bacon. Additionally, greater concentrations of unsaturated fatty acids are more likely to result in product oxidization and development of off-flavor and rancid aroma (Baer & Dilger, 2014; Wood et al., 2004). The impact of fat quality is visible to the naked eye when assessing bacon by utilizing the flop test. A flop test is conducted to measure how firm the belly is. A more desirable belly is firm, contains a higher ratio of saturated fats to unsaturated fats, and is less oily.

Fat that is deposited into the body is largely impacted by diet (Lebret, 2008; Lebret et al., 2018; L. Wu et al., 2019). The type dietary fat feed will largely impact the type of fat being deposited. Amino acids are largely important to protein deposition and have little effect on quality of fat deposition. Methionine has been reported to have little impact on the quality of fat but may impact the amount of fat depending on the growth and energy needs of the animal (L. Wu et al., 2019). Restriction of Met and other amino acids will also impact fat development. When restriction of Met occurs at the end of weaning, there is a shift in energy partitioning towards fat deposition and results in higher intramuscular fat content (Bastianelli & Sauvant, 1997). Age also impacts fat, because the older an animal is the slower their growth and deposition of protein therefore leading to increases in fat deposition. This is why bellies from older animals tend to be thicker than those of younger animals (Correa et al., 2008).

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CHAPTER 2: EFFECTS OF SUPPLEMENTAL METHIONINE SOURCES IN FINISHING PIG DIETS ON CARCASS CHARACTERISTICS, CUTTING YIELDS, AND MEAT QUALITY

Abstract: While supplemental methionine (Met) is widely used within the swine industry, data are limited regarding the effect of Met sources on carcass cutability and meat quality. The objective was to determine the effects of L-Met (LM, 99%), DL-Met (DLM, 99%), or calcium salt of DL-Met hydroxyl analogue (MHA, 84%) in finishing pig diets on carcass characteristics and meat quality. At d 66 of age, pigs (N = 240) were allocated to 60 single-sex pens for a four phase feeding system that lasted 104 days. On d56 pens were randomly allotted to one of three experimental diets for 48 day with a phase change at d83. For the final 7 wk of the finisher phase, pigs (BW= 79.9 ± 0.80 kg) were fed diets containing LM, DLM, or MHA, with the supplemental Met source providing 25% of standardized ileal digestible (SID) Met + cysteine (Cys) requirement. One pig per pen was slaughtered at study conclusion, and left sides of carcasses were fabricated into subprimal cuts to determine carcass-cutting yields. Loin quality including proximate composition and shear force was measured. Data were analyzed using the MIXED procedure of SAS. Hot carcass weight was not different (P = 0.34) between treatments (LM 104.5 kg; DLM 103.0 kg; MHA 101.5kg), moreover loin eye area was not different (P = 0.98) between treatments (LM 52.65 cm²; DLM 52.49 cm²; MHA 52.81 cm²). Boneless carcass cutting yield was not different (P = 0.56) between treatments (LM 54.97 kg; DLM 54.82 kg; MHA 54.52 kg). Loin pH was not different (P = 0.24) between treatments (LM 5.45; DLM 5.48; MHA 5.45). However, drip loss tended to be reduced (P = 0.11) by the DLM treatment (5.58%) compared with LM (7.03%) and MHA (6.68%) treatments. Shear force was not different (P = 0.85) between treatments (LM 3.03 kg; DLM 3.06 kg; MHA 3.10 kg). However, cook loss tended to be reduced (P = 0.06) by the DLM treatment (16.20%) compared with LM (18.18%)

and MHA (18.50%) treatments. These data suggest that only minimal differences in carcass cutability and meat quality can be attributed to Met source in finishing pig diets.

Key words: Carcass composition, meat quality, methionine, pork, sulfur amino acid, swine nutrition

Introduction

Methionine (Met) is an essential amino acid (AA) with important roles in synthesis of S-adenosylmethionine (SAM), methylation, protein synthesis, and in anti-oxidation biological processes (Levine et al., 1996). Methionine supplementation is common in commercial growing-finishing diets because high Met requirements of pigs coupled with low concentrations in industry-typical corn-soybean diets make Met the second limiting AA for pigs (Courtney-Martin & Pencharz, 2016; Fontecave et al., 2004).

Supplementary Met is commonly supplied as: DL-Met (99% pure), L-Met, or a methionine hydroxyl analog (MHA). MHA can be produced in liquid or solid form as a free acid (MHA-FA, 88%) or calcium salt (MHA-Ca, 84%). Historically, L-Met has not been used in commercial production due to the high cost of purification. However, novel microbial fermentation processes have recently led to some commercial availability of feed grade L-Met (99% pure). DL-Met is a 50:50 mixture of the D and L enantiomers, while MHA (2-hydroxy-4-methylthio butanoic acid) also includes enantiomers but is technically an organic acid due to the replacement of the amino group by a hydroxyl group (Yang et al., 2020). In the pig, both D-Met and MHA undergo oxidation and then transamination to become L-Met with the conversion of D-Met producing ammonia and MHA conversion producing water. MHA contains water (2%) and calcium (14%) and is commonly assumed to have 65% bioefficacy in comparison with L-Met (Shoveller et al. 2010; Zelenka et al. 2013). Despite these differences, it is largely accepted

that when formulated on a similar bioefficacy, few differences in pig growth performance are observed (Zimmerman et al 2005; Opapeju et al 2012).

Although previous research suggests no differences in growth performance between the liquid and crystalline forms of MHA compared with DL-Met (Opapeju et al., 2012, Zimmermann et al. 2005), a recent report has indicated improvements in loin chop marbling score and pH at 45 min postmortem in carcasses from pigs supplemented with liquid MHA compared with DL-Met (Yuan et al., 2017). However, few other reports of differences in meat quality or lean yield between Met sources exist. Therefore, the objective of this study was to determine the effects of three Met sources on pork carcass characteristics and meat quality. Overall, it was hypothesized that feeding different Met sources would have little impact on lean yield or quality when supplemented at concentrations based on differences in relative bioequivalence.

Materials and methods

All animal care and use procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois and followed standard practices described in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (2020).

Dietary Treatments

Pigs were fed for 104 d using a 4-phase feeding system. From d 0 (d 66 of age) to d 55, all pigs had *ad libitum* access to a corn-soybean meal grower diet. From d 0- d 55, pigs were fed a common grower diets (Supplemental Table 1) formulated to meet nutritional requirements of pigs 25–50 kg (Grower 1) and 50-75 kg (Grower 2), respectively (Swine NRC, 2012). From d 56 to d 104, pigs were fed assigned experimental diets. The basal experimental diets for phase 3 (d 56 to d 83) and phase 4 (d 84 to d 104) were designed to meet 75% of the estimated standardized

ileal digestible (**SID**) Met + Cys requirements of pigs weighing 75-100 kg and 100-135 kg (Table 1), respectively (Swine NRC, 2012). One of three Met sources were added to supplement the low-CP basal diet and fulfill estimated requirements (Table 1). Therefore, experimental Met treatments were added to the basal diet as follows: L-Met (**LM**), DL-Met (**DLM**), and MHA (**MHA**). For this study's formulation, the SID of L-Met and DL-Met was assumed to be 100% in accordance with Swine NRC guidelines (2012), whereas the SID of MHA Met was assumed to be 65% based on previous studies (Opapeju, 2012).

Experimental design

A total of 240 pigs (PIC 357 × Camborough sows) were raised in three separate blocks. The experimental design was a 2 × 3 factorial arrangement of sex and Met source with 10 pens per treatment combination. Prior to trial initiation, pigs were allotted to treatments by sex and body weight to minimize variation between pens for starting weight. Pigs were housed in single-sex pens with 4 pigs per pen (1.18 m^2/pig) where Met source was randomly assigned to pen. A total of 60 pens were used, each containing a nipple waterer, single-space dry-box feeder, and tri-bar slatted floors. Average initial body weight (BW) was 24.38 kg, 23.51 kg, and 29.14 kg for blocks 1 (n=36 pens), 2 (n=24 pens) and 3 (n=10 pens), respectively.

Diet analyses

Diets were analyzed for AA using standardized high-performance liquid chromatography procedures. The mixture is diluted with citrate buffer before chromatographic separation of hydrolysates was performed using cation exchange with pH adjusted to 2.2 using sodium hydroxide. Post-column derivatization was achieved using ninhydrin, with detection of approximately 14 AA at 570 nm light wavelength. Additionally, total AA constituting intact proteins were released by hydrolysis using hydrochloric acid-phenol reagent before repeating the

post-column derivatization. Concentration of AA that were bound as intact proteins were calculated by subtracting free/supplemental AA from AA from the total/acid hydrolysis assay.

Tryptophan was analyzed by alkaline hydrolysis. Feedstuffs were saponified under alkaline conditions with barium hydroxide in absence of air at 110°C for 20 h in an autoclave (110°C ± 2°C, pressure 0.4 bar, and steam sterilization). α-Methyl-tryptophan was added as an internal standard and the hydrolysate was adjusted to a pH of 3.0 and diluted with 30% methanol before reversed-phase chromatographic separation. Fluorescence detection was used to prevent interference by other AA and feed constitutes.

Growth performance

During the 104 d feeding trial, pigs were individually weighted at the start of each diet phase and at the end of the trial; phase 1 d 0 to 27, phase 2 d 27 to 56, phase 3 d 56 to 83, and phase 4 d 83 to 104. Feed consumption of pens were recorded on the same days as pig weights were collected. Data collected during growth period was used to calculate average daily feed intake (ADFI), average daily gain (ADG), and gain:feed ratio (G:F).

Blood urea nitrogen

Pigs were weighed on d 55 to determine the pig closest to the pen average, and this same pig was used for blood urea nitrogen (**BUN**), carcass quality, and yield evaluations. At d 55, 83, and 101, whole blood was collected via jugular venipuncture using 20-gauge, 2.54-cm vacutainer needles (EDTA BD Vacutainers; Thermo Fisher Scientific, Frederick, MD). Pigs were fasted for 4 to 6 h prior to blood collection. Samples were placed on ice immediately after collection and centrifuged at 1,300 × g at 4°C for 15 minutes. Plasma fraction was separated and aliquoted into cryovials before storing at -20°C pending analysis.

For determination of BUN, plasma samples were allow to thaw at ambient temperature (approximately 21°C), and subsequently diluted with deionized water at 1:20 (plasma:water) as per manufacturer's instructions. An internal standard curve (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156, and 0 mg/dL) was made using a urea nitrogen stock solution at 100 mg/dL. Blood urea nitrogen was measured according to manufacturer's directions (Urea Nitrogen Colorimetric Detection Kit; Thermo Fisher Scientific, Frederick, MD) in 96-well plates. Absorbance was measured at 450 nm using a Synergy HT microplate reader (BioTek, Winooski, VT). Concentrations of BUN were calculated using a standard curve and reported as the average of duplicate samples. Intra- and inter-assay precision of BUN kit protocol specified coefficient of variations were calculated by the manufacturer as 2.8 and 4.3, respectively.

Slaughter and carcass characteristics

On d 104, one pig per pen was transported to the University of Illinois Meat Science Lab and held overnight in lairage without feed but with *ad libitum* access to water. On d 105, ending live weight was recorded immediately before slaughter. Pigs were immobilized using head-to-heart electrical stunning and terminated via exsanguination under the supervision of the Food and Safety Inspection Service of the United States Department of Agriculture. Hot carcass weight (**HCW**) and *longissimus thoracis and lumborum* (**LTL**) muscle pH was obtained 45 minutes after exsanguination. Measurements of 45 minute pH was taken on left sides, probe was inserted between the 10th and 11th rib in the LTL. Forty-five minute pH was measured using a Hanna Foodcare Portable pH Meter calibrated at 4°C with a Hanna glass electrode (Hanna 4198163 pH meter, -2.0-20.0 pH/±2000.0 mV; Hanna FC2323 meat specific electrode; 2-point calibration; pH 4 and pH 7).

Carcass sides were allowed to chill for at least 20 hours at 4°C. The left side of each carcass was then separated between the 10th and 11th rib to expose the LTL. The anterior face of the exposed LTL was traced on acetate paper. This was later traced using a Wacom digital tracing pad (Wacom, Vancouver, WA) and Adobe Photoshop CS6 (Adobe Inc., San Jose, CA, USA) was used to determine area. This process was repeated and the average of the two measurements were recorded as loin muscle area (**LMA**). Back fat thickness was measured at ¾ the distance of the LTL from the dorsal process of the vertebral column. Fat free lean (**FFL**) was calculated using the following equation described by Burson and Berg et alt. 2001: *Standardized fat-free lean = ((8.588 + (0.465 x HCW, lb) - (21.896 x fat depth, in) + (3.005 x LTL area, in2)) ÷ HCW) x 100*

Carcass Fabrication

At approximately 22 hours postmortem, carcasses were fabricated into primal and subprimal cuts to calculate cutting yields according to the methods described by Boler et al. (2011). The left side of each chilled carcass was weighed and fabricated into a pork leg (NAMP #401), skin-on whole loin, pork shoulder (NAMP #403), neck bones (NAMP #421), jowl (NAMP #419), skin-on natural fall belly (NAMP #408), and spareribs (NAMP #416) to meet the specifications described in the North American Meat Buyer's Guide (NAMI, 2014). Each primal was weighed before further fabrication. Skinned and trimmed hams were further fabricated to a five-piece ham using the Boler et al. (2011) method. The loins were separated into anterior and posterior portions between the 10^{th} and 11^{th} rib and were skinned and trimmed to the specifications of NAMP #410 bone-in loin. Both portions were weighed to determine the weight of the whole skinless bone-in loin. Each portion was then fabricated into a NAMP #414 Canadian back loin, NAMP # 415A tenderloin, and NAMP #413D sirloin. Whole shoulders were

skinned and trimmed to meet the specifications of a NAMP #404 skinned pork shoulder. Skinned shoulders were further fabricated into bone-in Boston butt (NAMP #406) and bone-in picnic shoulder (NAMP #405). Bones were removed to produce a boneless Boston butt (NAMP #406A) and a boneless picnic (NAMP #405A) with triceps brachii. Canadian back loins and natural fall bellies were reserved for later evaluation. Carcass cutting yields were determined using the following equations:

Bone-in lean cutting yield, % = [(trimmed ham (NAMP #402), kg + bone-in trimmed Boston butt (NAMP #406), kg + bone-in picnic (NAMP #405), kg + trimmed loin (NAMP #410), kg) / chilled left side weight, kg]×100

Bone-in carcass cutting yield, % = [(bone-in lean cutting yield components + natural fall belly (NAMP #408), kg + spareribs (NAMP #416)) / chilled left side weight, kg] × 100

Boneless carcass cutting yield, % = [(inside ham (NAMP #402F), kg + outside ham(NAMP #402E), kg + knuckle (NAMP #402H), kg) + inner shank, kg + lite butt, kg + Canadian back (NAMP #414), kg +tenderloin (NAMP #415A), kg + sirloin (NAMP #413D), kg) + boneless Boston butt (NAMP #406A), kg + boneless picnic (NAMP #405A), kg + natural fall belly (NAMP #408), kg) + spareribs (NAMP #416)) / chilled left side weight] × 100

Loin quality

At 1 d postmortem, loins were re-faced at the LTL surface posterior to the 10th rib. The portion removed while re-facing was used for the evaluation of drip loss through suspension method outlined by Boler et al. (2011).

Loin quality traits were evaluated on the ventral loin surface and re-surfaced, anterior chop face of boneless Canadian back loin at the approximate location of the 10th rib. Loins were allowed to bloom for at least 20 minutes on both the ventral surface and chop face before color

measurements were collected. Ultimate pH was measured using the same probe as 45 minute pH. Instrumental CIE *L** (lightness), *a** (redness), and *b** (yellowness) measurements (Commission Internationale de l'Eclairage (CIE), 1976) were measured using a Minolta CR-400 Chroma meter (Konica Minolta, Osaka, Japan) with a 2° observer, an 8 mm closed aperture, a D65 illuminant, and calibrated with a machine-specific white tile. Subjective NPPC color (NPPC, 1999), marbling (NPPC, 1999), and firmness (NPPC, 1991) were evaluated by a trained technician on the bloomed ventral loin surface and chop face of each loin. Loins were cut into 2.54 cm thick chops posterior to the 10th rib. The first two chops were vacuum packaged and stored for proximate analysis and Warner Bratzler Shear Force (**WBSF**). Proximate analysis chops were trimmed of external fat and frozen at -20ºC prior to analysis. Shear force chops were vacuum-packaged and aged for 14 days at 4°C before being stored at -20ºC until analysis.

Belly quality

Procedures outlined by Kyle et al. (2014) were used to evaluate fresh belly characteristics. Length was measured at the anterior to posterior midline, and width was measured at the dorsal to ventral midline of the belly. Belly thickness was reported as the average of from eight individual locations on the belly evaluated by inserting a probe through the lean side of each belly. Measurements 1 to 4 were collected at the midpoint between the latitudinal axis and the dorsal edge at 20%, 40%, 60%, and 80% of the length of the belly, respectively, starting at the anterior end. Measurements 5 to 8 were collected at the midpoint between the longitudinal axis and the ventral edge at 20%, 40%, 60%, and 80% of the length of the belly, respectively, starting at the anterior end. Belly flop was determined by placing bellies skin side down over a metal bar and measuring the distance between the inside edges of each end.

Loin proximate composition

Moisture and fat percentages of loin chops were determined using the chloroform:methanol extraction procedures described by Novakofski et al. (1989). In brief, chops were allowed to thaw for at least 60 minutes at approximately 23 °C and then homogenized with a food processor (Hamilton Beach, model 70720, Glen Allen, VA, USA). Duplicate 10g samples were dried in a convection oven set at 110 °C for a minimum of 24 hours and then weighed. Fat was then extracted using the soxhlet method where the sample was washed in a 4:1 chloroform:methanol mixture for a minimum of 8 hours. Samples were dried again in a convection oven set at 110 °C for a minimum of 24 h and weighed. Weights after drying and lipid extraction were used to calculate reported moisture and fat percentages.

Cook loss and Warner-Bratzler shear force

Chops used for cook loss and **WBSF** were thawed at 4°C for a minimum of 24 hours and weighed before cooking on Farberware Open Hearth grills (model 455N, Walter Kidde, Bronx, NY, USA). Temperature was continuously monitored during cooking using thermocouples (type K, range: -200ºC to 1250ºC, standard error: ±2.2ºC, Omega Engineering, Stamford, CT, USA) placed in the geometric center of chops. Thermocouples were connected to Omega HH378 Data Logger Thermometer (Omega Engineering, Norwalk, CT). Chops were cooked on one side until they reached 31°C, then flipped and cooked on the other side until reaching 63°C, at which point they were removed from grills. Chops were cooled to room temperature and were weighed a second time to calculate cook loss using the formula:

$$\textit{Cook loss, \%} = [(\textit{initial weight, kg} - \textit{cooked weight, kg}) \div \textit{initial weight, kg}] \times 100$$

Four cores with a width of 1.25cm were obtained parallel to muscle fibers and sheared using a Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable

Microsystems, Goldalming, UK) fitted with a WBSF blade as outlined by Richardson (2018). Cores were sheared perpendicular to the muscle fiber orientation with a speed of 3.33mm/s and load cell capacity of 100kg. Force to shear was recorded and the average of four cores was reported.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. In., Cary, NC) as a 2 × 3 factorial arrangement of treatments (sex × Met source) in a randomized complete block design. Pen (N = 60) served as the experimental unit. Fixed effects were Met source, sex, and their interaction. Block (N = 3) served as random variable. Least squares means were separated using the PDIFF statement. Effects were considered significant at $P \leq 0.05$. Blood urea nitrogen data were analyzed using the MIXED procedure with repeated measures modeling (Met source × sex × phase) of treatments in a completely randomized block design. Block served as a random variable in the MIXED procedure. Least squares means were separated using the PDIFF statement. Significance was considered at $P \leq 0.05$ and trends were considered at $P \leq 0.10$.

Results

Growth performance

Initial body weight (BW; (25.30 ± 3.5 kg) did not differ ($P \geq 0.37$) between Met sources (Table 3). However, initial BW of barrows and gilts did differ ($P < 0.01$) at trial initiation with barrows, 82.4kg, being heavier than gilts, 78.6kg. There were no differences ($P \geq 0.39$) between Met source for ADG, ADFI, or G:F during phase 1 (d 1-55) when all pigs were fed a common diet. There were no differences ($P = 0.43$) in BW of treatment groups at d 55, prior to beginning treatment diets. Diets with different sources of Met were fed in the final two phases of finishing (d 56-83 and d 84-104). There were no differences ($P \geq 0.25$) between Met sources for ADG,

ADFI, G:F in either phase. Final BW did not differ ($P \geq 0.45$) between Met sources. Therefore, Met source did not alter ($P \geq 0.20$) overall (d 1-104) growth performance. As expected, there were multiple differences between sexes for growth performance. Overall, barrows were heavier than gilts with greater ADG ($P < 0.001$) and overall ADFI ($P < 0.001$), while gilts had greater G:F ($P < 0.02$) than barrows. However, there were no interactions ($P \geq 0.08$) between Met source and sex for any growth performance measurements.

Blood urea nitrogen

There were no differences between Met source treatment ($P \geq 0.20$) for BUN concentrations at any measurement time (Figure 1). On d 55, BUN did not differ ($P = 0.67$) between sexes. However, by d 83, differences in BUN emerged between sexes. Barrow BUN levels were 2.01 mg/dL greater ($P \leq 0.001$) at phase 4 and 1.63 mg/dL greater ($P \geq 0.001$) at d 101 compared to gilts. Phase was different from each other ($P \leq 0.001$) with d 55 (11.55 mg/dL) and d 101(12.12 mg/dL) being lower than d 83 (16.21 mg/dL). No treatment × sex interaction for BUN was observed ($P \geq 0.39$).

Carcass characteristics

Ending live weight, HCW, and carcass yield did not differ ($P \geq 0.34$) between Met sources (Table 5). Despite a lack of statistical significance, HCW of L-Met-fed pigs was 2.7 kg greater than MHA-fed pigs. Similarly, carcasses from DL-Met fed pigs (103.0 kg) were also numerically heavier than MHA-fed pigs (101.8 kg), although not statistically different. Nonetheless, no differences in LMA, 10^{th} rib back fat depth, and FFL were observed ($P \geq 0.51$) between Met sources. Carcass differences between barrows and gilts were as expected, with barrows having greater ending live weight (**ELW**), HCW, and back fat thickness ($P < 0.01$) and

gilts having greater standardized FFL ($P < 0.001$). No interactions of Met source and sex were observed for any carcass characteristic ($P \geq 0.45$).

Carcass cutability

Source of Met did not alter (P > 0.12) whole or trimmed primal absolute or relative weights (Table 5). Trimmed ham weight expressed as a percentage of chilled side weight tended to be greater in DL-Met fed pigs (20.74%) than L-Met (20.15%) and MHA (20.44%) fed pigs. However, Met source did not alter absolute or relative weights for any ham components (Table 6). Shoulder primal cuts were not different in Met source from each other with exception of a trend in jowl weights (Table 7). Pigs fed L-Met had 0.10 kg heavier jowls than pigs fed DL-Met and MHA. On an absolute weight basis, tenderloin weights were greater for pigs fed L-Met than other Met sources (Table 8). However, differences in absolute weight did translate into a tendency ($P = 0.08$) in tenderloin as a percent of chilled side weight. As expected, given a lack of differences in primal cut weights, Met source had no effect on bone-in carcass cutting yield, bone-in loin cutting yield, or boneless carcass cutting yield (Table 10).

Generally, barrow carcasses produced heavier whole primal cuts on an absolute basis but no differences as a proportion of chilled side weight. For most trimmed primals, no differences in absolute weight were detected between sexes, but trimmed primals comprised a greater percentage of chilled side weight for gilts than barrows. No meaningful interactions between treatment and sex for any primal cut.

Loin and chop quality

There were no differences between Met source treatment (Table 10) for 45min pH ($P \geq 0.24$) or ultimate pH ($P = 0.24$). Ventral loin visual color, marbling, and subjective firmness were not different ($P \geq 0.41$) between Met sources. While ventral loin L*, *a**, and *b** did not differ

between Met sources ($P \geq 0.25$), there was a tendency for an interaction ($P < 0.07$) between Met source × sex for ventral loin surface redness. Barrows fed DL-Met and MHA and gilts fed L-Met tended to have a greater *a** than barrows fed L-Met and gilts fed DL-Met and MHA.

Chop visual color tended to be less in pigs fed L-Met (2.99) than DL-Met (3.29) and MHA (3.14). Despite differences in chop visual color, chop visual marbling ($P = 0.52$) and subjective color ($P = 0.38$) were not different between Met sources. There were no differences between Met sources for objective color measurements ($P \geq 0.18$). Chop moisture ($P = 0.84$) and extractable lipids ($P = 0.97$) did not differ between Met sources. Furthermore, Met sources were not different in WBSF. While quality had minimal differences, there were tendencies for pigs fed DL-Met to be reduced in drip loss ($P = 0.09$) and cook loss ($P = 0.07$) in comparison to L-Met and MHA.

Sexes did not differ in ventral loin or chop visual color ($P \geq 0.18$). Conversely, visual marbling ($P \leq 0.05$) and chop subjective firmness ($P = 0.08$) are different between sexes. Chop objective color did not differ between sex ($P \geq 0.28$) but objective color measurement of the ventral loin did differ between sexes, where for barrows had a greater *b** than gilts. There were differences in proximate analysis for sex. Chops from gilts (74.14%) had 0.55% greater ($P = 0.014$) moisture than barrows (73.59%), but barrows (2.75%) were had 0.67% greater ($P \leq 0.001$) extractable lipid compared to gilts (2.08%).

Fresh belly quality

Fresh belly length and width dimensions did not differ ($P \geq 0.56$) between treatments (Table 12). Likewise, there were no differences in belly thickness ($P = 0.95$) or flop distance ($P = 0.59$), both indicators of belly quality. There were no interactions between Met source and sex

for any belly quality trait ($P \geq 0.75$). As expected, barrow carcasses produced thicker bellies ($P \leq 0.0002$) with greater ($P \leq 0.01$) flop distances than ones from gilts.

Discussion and conclusion

Met needs to be supplemented to meet the needs of pigs and there are many forms of Met available. LMet is not commonly used because of its relatively high cost, thus DL Met and MHA are the two common forms found in swine diets. Both are converted to LMet by the pigs and have differences in bioefficacy. When diets are not formulated to correct for this, growth performance differences are detected (Zimmermann et al., 2005). This, in broilers, resulted in differences in carcass composition. When they are corrected, growth performance doesn't differ (Albrecht et al. 2019; Dilger & Baker, 2008; Lemme et al., 2002; Pokoo-Aikins et al. 2022; Zelenka et al., 2013).

In the present study, when differences in Met sources were used to supply 25% of SID Met + Cys requirement, growth performance did not differ. Similarly, carcass composition and cutability were not different across treatments. Dietary Met sources were not different from each other for HCW, loin muscle area, and 10^{th} rib back fat. Furthermore, differences between primal weights had slight numerical differences but no significant differences that would prove insight that one Met source is superior to another. Minimal differences in carcass composition and cutability in this trial provides information to pork producers that choice of Met source should not influence these qualities.

Previous reports in pigs and chicken suggest that Met source may alter meat pH, WHC, or color. Albrecht (2019) and Drażbo (2015) reported methionine source did impact breast reflectance values and thigh color, with MHA demonstrating improved color over birds fed DL-Met. In addition to chickens, pigs were reported to have increased pH and WHC when the

amount of Met was increased to five times the requirement during the finishing phase (70 kg to 105 kg) (Lebret et al., 2018). In the present study, trends for color were detected in ventral loin redness and chop visual color. Differences in ventral redness values were less than 1 a^* unit and are not considered to translate into visually detectable differences in redness to consumers. In support of this, visual ventral loin color, evaluated by a trained technician, was not different. Additionally, visual chop color tended to be different between Met sources by 0.3 but NPPC color scores are called in increments of 0.5, therefore this has little impact on quality.

Trends of improved water holding capacity from reduced drip loss and cook loss for DM-Met compared to L-Met and MHA treatments could impact consumers' preference in palatability. DL-Met retained water better by approximately 1.5% for drip loss and 2% for cook loss. While tendencies were identified, this may not translate into an improvement in chop juiciness for DL-Met fed pigs. No differences were found in tenderness, measured by WBSF, and contribute to major differences consumers identify for preferences in palpability.

The majority of pork products are further processed. Belly quality is important in the production of bacon and being indicative of quality of fat. Met source did not alter belly thickness or flop distance suggesting that no differences in bacon processing would result from different Met sources. This indicates that Met source does not impact fat differences.

In conclusion, methionine sources had little effect on carcass characteristics and meat quality of finishing pigs. While methionine sources may be processed differently within the body, utilization of different sources did not impact growth performance, carcass characteristics, or meat quality in pigs. The lack of differences indicates that there would little benefit or disadvantage of using one Met source over another for carcass characteristics. These findings

provide greater understanding of the effects of methionine sources on carcass cutability and meat quality.

Tables and Figure

Table 1. Calculated ingredient and nutrient composition of experimental diets (%, as fed basis)

	Phase 3 (d56-83)			Phase 4 (d84-104)		
Ingredient, %	**L-Met**	**DL-Met**	**MHA**	**DL-Met**	**L-Met**	**MHA**
Corn, yellow dent	33.71	33.71	33.71	19.43	19.43	19.43
Sorghum	35.00	35.00	35.00	60.00	60.00	60.00
Soybean meal	2.50	2.50	2.50	.	.	.
Field peas	25.00	25.00	25.00	17.00	17.00	17.00
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.84	0.84	0.84	0.83	0.83	0.83
Dicalcium phosphate	0.55	0.55	0.55	0.35	0.35	0.35
L-Lys	0.26	0.26	0.26	0.32	0.32	0.32
L-Thr	0.12	0.12	0.12	0.09	0.09	0.09
L-Trp	0.05	0.05	0.05	0.03	0.03	0.03
L- Val	0.02	0.02	0.02	.	.	.
DL-Met	.	0.11	.	0.09	.	.
L-Met	0.11		.	.	0.09	-
MHA Met	.	.	0.17	.	.	0.14
Cornstarch	0.29	0.29	0.23	0.31	0.31	0.26
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[1]	0.15	0.15	0.15	0.15	0.15	0.15
Calculated composition						
ME, kcal/kg	3,397	3,397	3,394	3,445	3,445	3,443
Ca, %	0.52	0.52	0.52	0.46	0.46	0.46
P[2], %	0.24	0.24	0.24	0.21	0.21	0.21
Crude protein, %	12.79	12.79	12.79	10.99	10.99	10.99
SID AA[3], %						
Arg	0.72	0.72	0.72	0.53	0.53	0.53
His	0.27	0.27	0.27	0.23	0.23	0.23
Ile	0.41	0.41	0.41	0.34	0.34	0.34
Leu	1.03	1.03	1.03	0.98	0.98	0.98
Lys	0.73	0.73	0.73	0.61	0.61	0.61
Met	0.27	0.27	0.27	0.24	0.24	0.24
Met + Cys	0.42	0.42	0.42	0.36	0.36	0.36
Phe	0.51	0.51	0.51	0.44	0.44	0.44
Thr	0.48	0.48	0.48	0.40	0.40	0.40
Trp	0.15	0.15	0.15	0.12	0.12	0.12
Val	0.50	0.50	0.50	0.41	0.41	0.41

[1]The vitamin-mineral premix will provide the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[2]Standardized total tract digestible P.

[3]Amino acids are indicated as standardized ileal digestible AA.

Table 2. Analyzed diet and nutrient composition for Phases 3 and 4

	Phase 3 (d56-83)			Phase 4 (d84-104)		
Analyzed values	**L-Met**	**DL-Met**	**MHA**	**DL-Met**	**L-Met**	**MHA**
DM (NIR)	87.78	87.60	87.73	87.30	87.28	87.38
CP	16.54	16.20	17.03	12.68	12.55	12.61
Met (total)	0.32	0.33	0.23	0.26	0.28	0.19
Met (supplemental)	0.09	0.11	<0.01	0.08	0.10	<0.01
MHA	<0.01	<0.01	0.16	<0.01	<0.01	0.13
Cys	0.25	0.24	0.25	0.20	0.20	0.21
Met + Cys	0.57	0.57	0.48	0.45	0.48	0.39
Arg	1.09	1.06	1.10	0.72	0.75	0.75
Ile	0.69	0.66	0.68	0.50	0.49	0.52
His	0.41	0.39	0.41	0.30	0.29	0.30
Leu	1.42	1.35	1.41	1.17	1.16	1.24
Lys	0.91	0.89	0.93	0.67	0.70	0.71
Phe	0.81	0.78	0.82	0.60	0.60	0.63
Trp	0.20	0.19	0.20	0.15	0.14	0.15
Thr	0.67	0.65	0.70	0.51	0.50	0.51
Val	0.78	0.75	0.78	0.59	0.58	0.60
Gly	0.68	0.66	0.69	0.49	0.49	0.50
Ser	0.78	0.76	0.80	0.58	0.58	0.60
Pro	0.90	0.86	0.89	0.75	0.73	0.77
Ala	0.88	0.84	0.88	0.76	0.76	0.80
Asp	1.65	1.60	1.67	1.12	1.14	1.16
Glu	2.91	2.81	2.95	2.22	2.20	2.32

Table 3. Effects of methionine source and sex on growth characteristics[1]

	Diet			Sex			*P*-values		
Item	LMet	DLMet	MHA	Barrows	Gilts	SEM	Diet	Sex	Diet × Sex
Pens, n	20	20	20	30	30				
Phase 1 (d0-27) [2]									
BW d0, kg	25.6	25.7	25.7	25.3	25.3	1.84	1.00	0.37	0.99
ADG, kg/d	0.90	0.91	0.89	0.93	0.86	0.02	0.64	0.00	0.90
ADFI, kg/d	1.85	1.85	1.81	1.88	1.79	0.05	0.72	0.08	0.62
G:F	0.489	0.491	0.492	0.50	0.48	0.01	0.96	0.08	0.20
BW d27, kg	50.0	50.2	49.6	50.5	49.4	2.04	0.90	0.33	0.97
Phase 2 (d27-55)									
ADG, kg/d	1.09	1.09	1.06	1.14	1.03	0.02	0.39	<0.01	0.27
ADFI, kg/d	2.67	2.69	2.65	2.88	2.46	0.07	0.75	<0.01	0.65
G:F	0.412	0.408	0.404	0.396	0.419	0.01	0.64	<0.01	0.30
BW d55, kg	80.6	81.6	79.4	82.4	78.6	2.32	0.43	0.01	0.91
Phase 3 (d56-83)									
ADG, kg/d	1.20	1.20	1.19	1.25	1.14	1.14	0.93	<0.01	0.26
ADFI, kg/d	3.30	3.31	3.24	3.52	3.05	0.07	0.55	<0.01	0.87
G:F	0.364	0.365	0.368	0.357	0.374	0.01	0.92	0.02	0.33
BW d83	114.0	115.2	112.6	117.4	110.5	2.12	0.38	<0.01	0.93
Phase 4 (d84-104)									
ADG, kg/d	1.02	1.02	1.01	1.02	1.01	0.04	0.95	0.73	0.48
ADFI, kg/d	3.49	3.54	3.45	3.66	3.33	0.11	0.25	<0.01	0.54
G:F	0.294	0.289	0.296	0.280	0.306	0.01	0.75	<0.01	0.23
BW d104	135.5	136.6	134.2	139.1	131.8	2.40	0.45	<0.01	0.89
Overall (d0-104)									
ADG, kg/d	1.06	1.05	1.04	1.05	1.01	0.02	0.55	<0.01	0.79
ADFI, kg/d	2.80	2.84	2.76	2.95	2.64	0.07	0.20	<0.01	0.69
G:F	0.379	0.372	0.379	0.371	0.382	0.01	0.35	0.01	0.32

[1]Experimental dietary treatments were fed from d 56-104. Corn-sorghum-SBM diets where 25% of the Met+Cys requirement was met using 1 of 3 Met sources; LMet, DLMet, and MHA. Abbreviations: BW = Body Weight, ADG = Average Daily Gain, ADFI = Average Daily Feed Intake, G:F = Gain : Feed, SEM = Standard error of the means

[2] Pigs were approximately 25 kg when placed on trial.

Table 4. Effects of methionine source and sex on carcass characteristics on pigs slaughtered at the University of Illinois Meat Science Laboratory.

	Diet			Sex			*P*-values		
Item	LMet	DLMet	MHA	Barrows	Gilts	SEM	Diet	Sex	Diet × Sex
Pens, n	20	20	20	30	30				
Ending live weight, kg	133.5	132.0	131.0	135.5	129.0	3.82	0.47	<0.01	0.79
HCW[1], kg	104.5	103.0	102.0	106.0	100.0	2.30	0.34	<0.01	0.63
Carcass yield, %	78.35	77.89	77.93	77.93	77.82	0.65	0.39	0.12	0.45
Loin muscle area, cm^2	52.65	52.49	52.81	53.01	53.01	1.62	0.98	0.60	0.82
10th rib back fat depth, cm	2.01	1.98	2.13	2.37	1.71	0.10	0.51	<0.01	0.81
Standardized fat-free lean[2], %	58.58	58.77	57.90	56.04	60.80	0.85	0.75	<0.01	0.82

[1]Hot Carcass Weight (HCW) includes leaf fat

[2]Standardized fat-free lean = ((8.588 + (0.465 x HCW, lb) - (21.896 x fat depth, in) + (3.005 x LTL area, in^2)) ÷ HCW) x 100,
(Burson and Berg, 2001).

Table 5. Effects of methionine source and sex on whole and trimmed primal cuts.

	Diet			Sex			*P*-values		
Item	LMet	DLM	MHA	Barrows	Gilts	SEM	Diet	Sex	Diet × Sex
Pens, n	20	20	20	30	30				
Chilled side wt, kg[1]	48.95	48.41	47.64	49.56	47.10	1.15	0.34	<0.01	0.78
Whole shoulder, kg	10.38	10.44	10.08	10.65	9.94	0.24	0.27	<0.01	0.58
% chilled side wt	21.21	21.56	21.17	21.50	21.13	0.18	0.20	0.06	0.50
Bone-in Boston, kg	4.17	4.14	3.98	4.23	3.95	0.17	0.21	<0.01	0.66
% chilled side wt	8.50	8.53	8.34	8.54	8.37	0.21	0.44	0.22	0.15
Bone-in picnic, kg	5.45	5.54	5.34	5.59	5.30	0.10	0.33	0.01	0.09
% chilled side wt	11.17	11.48	11.24	11.30	11.30	0.18	0.22	1.00	0.04
Whole loin, kg	14.39	14.02	13.93	14.60	13.64	0.38	0.32	<0.01	0.87
% chilled side wt	29.38	28.94	29.26	29.44	28.95	0.32	0.41	0.08	0.99
Trimmed loin, kg	11.63	11.45	11.30	11.56	11.36	0.25	0.39	0.30	0.93
% chilled side wt	23.81	23.69	23.75	23.34	24.15	0.24	0.91	<0.01	0.66
Whole ham, kg	11.57	11.71	11.42	11.68	11.45	0.16	0.45	0.23	0.40
% chilled side wt	23.70	24.22	23.97	23.58	24.35	0.44	0.14	<0.01	0.18
Trimmed ham, kg	9.85	10.04	9.75	9.92	9.84	0.16	0.39	0.66	0.43
% chilled side wt	20.15	20.74	20.44	20.01	20.87	0.53	0.08	<0.01	0.18
Natural fall belly, kg	7.86	7.52	7.58	7.91	7.40	0.39	0.13	<0.01	0.77
% chilled side wt	16.03	15.53	15.89	15.94	15.69	0.56	0.13	0.25	0.42
Spareribs, kg	1.74	1.77	1.68	1.76	1.70	0.04	0.12	0.08	0.70
% chilled side wt	3.55	3.67	3.54	3.55	3.62	0.05	0.15	0.23	0.56
Miscellaneous Cuts									
Standardized Trim	0.27	0.25	0.26	0.29	0.23	0.05	0.42	<0.01	0.70
Leaf Fat	0.89	0.83	0.89	0.95	0.79	0.05	0.53	<0.01	0.37
Front and back Foot	1.08	1.08	1.05	1.07	1.07	0.03	0.03	0.95	0.65
% chilled side wt	2.21	2.25	2.21	2.17	2.28	0.07	0.70	<0.01	0.77

[1]Chilled Side Weight is on the left side and excludes leaf fat and standardized trim

Table 6. Effects of methionine source and sex on ham primal cuts.

	Diet			Sex			*P*-values		
Item	LM	DLM	MHA	Barrows	Gilts	SEM	Diet	Sex	Diet × Sex
Pens, n	20	20	20	30	30				
Inside ham, kg	1.89	1.92	1.83	0.03	1.89	0.04	0.23	0.66	0.31
% chilled side wt	3.89	3.99	3.87	3.79	4.04	0.07	0.37	<0.01	0.23
Outside ham, kg	2.51	2.55	2.51	2.48	2.54	0.05	0.43	0.25	0.38
% chilled side wt	5.17	5.30	5.19	5.01	5.43	0.08	0.41	<0.01	0.17
Knuckle, kg	1.42	1.44	1.39	1.40	1.44	0.03	0.49	0.18	0.16
% chilled side wt	2.91	2.97	2.92	2.82	3.05	3.05	0.64	<0.01	0.09
Inner shank, kg	0.69	0.70	0.68	0.70	0.68	0.02	0.78	0.25	0.59
% chilled side wt	1.43	1.45	1.44	1.42	1.46	0.03	0.78	0.22	0.47
Lite butt, kg	0.24	0.25	0.26	0.24	0.26	0.04	0.53	0.27	0.34
% chilled side wt	0.49	0.53	0.54	0.49	0.55	0.09	0.31	0.04	0.42
Boneless ham, kg[1]	5.82	5.91	5.69	5.75	5.87	0.10	0.28	0.28	0.22
% chilled side wt	11.97	12.25	11.97	11.61	12.52	0.20	0.29	<0.01	0.07

[1]Boneless ham = inside ham (NAMP #402F), kg + outside ham (NAMP #402E), kg + knuckle (NAMP #402H), kg.

Table 7. Effects of methionine source and sex on shoulder primal cuts.

	Diet			Sex			*P*-values		
Item	LMet	DLMet	MHA	Barrows	Gilts	SEM	Diet	Sex	Diet × Sex
Pens, n	20	20	20	30	30				
Bonesless Boston, kg	3.85	3.83	3.69	3.92	3.66	0.14	0.26	<0.01	0.68
% chilled side wt	7.86	7.90	7.74	7.91	7.76	0.18	0.52	0.22	0.16
Boneless picnic, kg	4.03	4.06	3.93	4.13	3.88	0.08	0.49	0.01	0.23
% chilled side wt	8.26	8.39	8.27	8.35	8.27	0.14	0.64	0.54	0.16
Neckbones, kg	0.83	0.80	0.83	0.82	0.82	0.05	0.75	0.88	0.90
% chilled side wt	1.68	1.66	1.75	1.65	1.74	0.08	0.51	0.19	0.83
Jowl, kg	1.43	1.33	1.33	1.41	1.33	0.04	0.08	<0.05	0.78
% chilled side wt	2.93	2.76	2.81	2.84	2.82	0.11	0.13	0.80	0.90
Clear plate, kg	0.75	0.74	0.75	0.82	0.68	0.03	0.93	<0.01	0.74
% chilled side wt	1.53	1.53	1.58	1.65	1.45	0.06	0.67	<0.01	0.82
Boneless shoulder, kg[1]	7.89	7.89	7.89	8.06	7.54	0.19	0.27	<0.01	0.79
% chilled side wt	16.12	16.29	16.00	16.25	16.02	0.16	0.33	0.14	0.70

[1]Boneless shoulder = boneless Boston butt (NAMP # 406A), kg + boneless picnic (NAMP #405A), kg.

Table 8. Effects of methionine source and sex on loin primal cuts.

	Diet			Sex			*P*-values		
Item	LMet	DLMet	MHA	Barrows	Gilts	SEM	Diet	Sex	Diet × Sex
Pens, n	20	20	20	30	30				
Canadian Back, kg	3.75	3.68	3.60	3.65	3.70	0.08	0.38	0.51	0.54
% chilled side wt	7.69	7.64	7.59	7.38	7.90	0.12	0.84	<0.01	0.67
Tenderloin, kg	0.55[a]	0.52[b]	0.52[b]	0.53	0.53	0.02	0.05	0.62	0.26
% chilled side wt	1.14	1.08	1.08	1.08	1.12	0.05	0.08	0.04	0.16
Sirloin, kg	1.00	0.99	0.95	0.95	1.01	0.05	0.48	0.07	0.32
% chilled side wt	2.04	2.04	2.00	1.92	2.13	0.09	0.73	<0.01	0.09
Backribs, kg	0.85	0.87	0.84	0.90	0.81	0.02	0.65	<0.01	0.44
% chilled side wt	1.74	1.79	1.76	1.81	1.71	0.07	0.61	0.05	0.47
Backbone, kg	2.23	2.17	2.19	2.17	2.23	0.14	0.72	0.29	0.22
% chilled side wt	4.54	4.48	4.60	4.37	4.71	0.19	0.64	<0.01	0.10
Boneless loin, kg[1]	5.30	5.20	5.07	5.14	5.24	0.14	0.26	0.33	0.79
% chilled side wt	10.86	10.76	10.66	10.37	11.15	0.19	0.65	<0.01	0.77

[1]Boneless loin = Canadian back loin (NAMP #414), kg + tenderloin (NAMP #415A), kg + sirloin (NAMP #413D), kg.

Table 9. Effects of methionine source and sex on carcass cutability.

	Diet			Sex			*P*-values		
Item	LMet	DLMet	MHA	Barrows	Gilts	SEM	Diet	Sex	Diet × Sex
Pens, n	20	20	20	30	30				
Bone-in carcass cutting yield, %[1]	79.65	79.95	79.65	79.13	80.38	0.66	0.66	<0.01	0.65
Bone-in lean cutting yield, %[2]	63.63	64.44	63.77	63.77	64.70	0.67	0.20	<0.01	0.59
Boneless carcass cutting yield, %[3]	54.97	54.82	54.52	54.17	55.36	0.73	0.56	<0.01	0.66

[1]Bone-in carcass cutting yield = [(trimmed ham, kg + bone-in Boston, kg + bone-in picnic, kg + trimmed loin, kg + natural fall belly, kg) ÷ left side chilled weight, kg] x 100.

[2]Bone-in lean cutting yield = [(trimmed ham, kg + bone-in Boston, kg + bone-in picnic, kg + trimmed loin, kg) ÷ left side chilled weight, kg] x 100.

[3]Boneless carcass cutting yield = [(inside ham, kg + outside ham, kg + knuckle, kg) + (Canadian back loin, kg + tenderloin, kg + sirloin, kg) + (boneless Boston, kg + boneless picnic, kg) + (belly, kg)) ÷ left side chilled weight] x 100.

Table 10. Effects of methionine source and sex on early loin and chop face quality and color[1]

	Diet			Sex			*P*-values		
Item	LMet	DLMet	MHA	Barrows	Gilts	SEM	Diet	Sex	Diet × Sex
Pens, n	20	20	20	30	30				
Loin									
Visual color[2]	3.08	3.25	3.08	3.13	3.13	0.11	0.41	1.00	0.14
Visual marbling[3]	1.58	1.60	1.58	1.70	1.47	0.10	0.98	0.05	0.53
Subjective firmness[4]	3.09	3.14	3.14	3.12	3.12	0.13	0.93	0.95	0.80
Lightness, *L**[5]	49.03	48.63	50.51	50.19	48.59	0.82	0.25	0.10	0.91
Redness, *a**[6]	9.40	9.97	10.35	10.32	9.50	0.77	0.25	0.08	0.06
Yellowness, *b**[7]	5.48	5.50	5.91	6.05	5.21	1.31	0.61	0.04	0.17
45min 10th rib pH	6.06	6.12	6.16	6.18	6.05	0.07	0.67	0.12	0.22
Loin pH	5.45	5.48	5.45	5.49	5.44	0.04	0.24	<0.01	0.26
Drip loss, %[8]	7.03	5.58	6.68	6.45	6.41	6.41	0.09	0.95	0.72
Chop									
Visual color	2.99	3.29	3.14	3.22	3.07	0.15	0.10	0.18	0.91
Visual marbling	1.67	1.82	1.62	1.92	1.49	0.17	0.52	<0.01	0.63
Subjective firmness	2.70	2.95	2.85	2.97	2.70	0.13	0.38	0.08	0.21
Lightness, *L**	52.89	52.34	54.30	53.35	53.00	1.99	0.36	0.77	0.27
Redness, *a**	8.59	8.63	9.64	9.23	8.68	1.39	0.19	0.30	0.47
Yellowness, *b**	5.70	5.40	6.58	6.19	5.60	1.67	0.18	0.28	0.31
Moisture, %	73.86	73.94	73.79	73.59	74.14	0.21	0.84	0.01	0.89
Extractable Lipid, %	2.44	2.41	2.39	2.75	2.08	0.15	0.97	<0.01	0.81
Warner-Bratzler shear force, kg[9]	3.03	3.06	3.10	2.96	3.16	0.09	0.85	0.06	0.72
Cook loss, %[10]	18.18	16.20	18.50	17.33	17.92	0.74	0.07	0.49	0.58

[1]Early postmortem traits were evaluated 1 d postmortem.
[2]NPPC color based on the 1999 standards measured in half point increments where 1 = palest, 6 = darkest.
[3]NPPC marbling based on the 1999 standards measured in half point increments where 1 = least amount 6 = greatest amount of marbling.
[4]NPPC firmness based on the 1991 scale measured in half point increments where 1 = softest, 5 = firmest.
[5]*L** measures darkness (0) to lightness (100; greater *L** indicates a lighter color).
[6]*a** measures redness (greater *a** indicates a redder color).
[7]*b** measures yellowness (greater *b** indicates a more yellow color).
[8]Drip loss = [(initial weight, kg - final weight, kg) ÷ (initial weight, kg)] x 100.
[9]Includes Warner-Bratzler shear force evaluated on chops cooked to 63° C
[10]Cook loss = [(initial weight, kg - cooked weight, kg) ÷ initial weight, kg] ×100

Table 11. Effects of methionine source and sex on fresh belly characteristics.

	Diet			Sex			*P*-values		
Item	LMet	DLMet	MHA	Barrows	Gilts	SEM	Diet	Sex	Diet × Sex
Pens, n	20	20	20	30	30				
Length	71.33	70.74	70.58	71.14	70.64	1.45	0.56	0.41	0.75
Width	29.44	29.13	29.19	29.33	29.18	0.89	0.87	0.78	0.98
Thickness, cm[1]	3.65	3.62	3.65	3.85	3.43	0.12	0.95	<0.01	0.77
Flop, cm	29.74	28.00	30.39	32.06	26.69	2.08	0.59	<0.01	1.00

[1]Thickness was an average of measurements from 8 locations from the anterior to posterior.

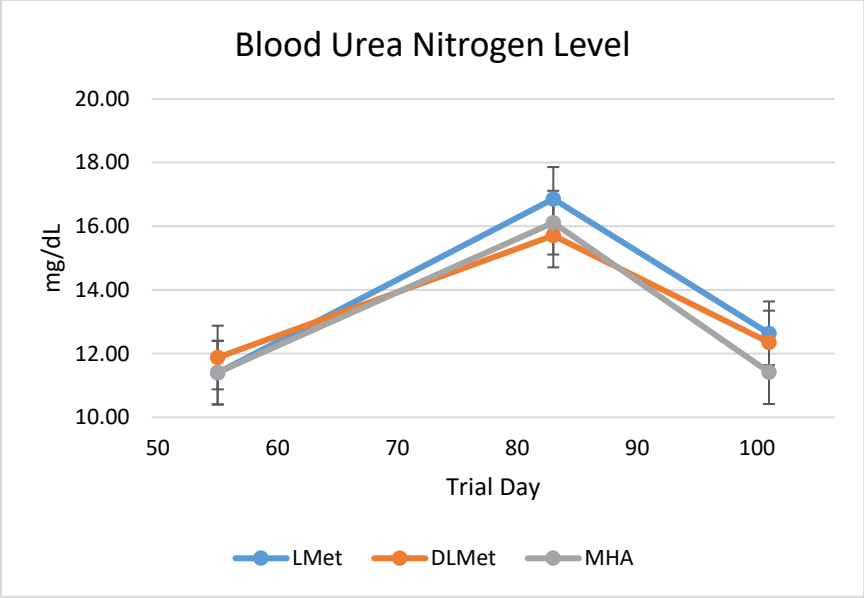

Figure 1. Effects of methionine source and sex on Blood Urea Nitrogen levels

Supplemental Table 1. Experimental diet composition and nutrient composition for Phases 1 and 2[1]

Ingredient, %	**Grower-1 (25-50 kg)**	**Grower-2 (50-75 kg)**
Ground corn	56.58	64.64
Sorghum	10	10
Soybean meal	30	22
Field peas	-	-
Soybean oil	1	1
Limestone	0.83	0.79
Dicalcium phosphate	0.92	0.8
L-Lysine	0.09	0.17
L-Threonine	0.03	0.05
L-Tryptophan	-	-
Salt	0.4	0.4
Vitamin-mineral premix[2]	0.15	0.15
Calculated values		
ME, kcal/kg	3,346	3,356
Crude protein, %	19.92	16.76
Ca, %	0.66	0.59
P[3], %	0.31	0.27
Total AA[4], %		
Arg	1.18	0.95
His	0.48	0.4
Ile	0.73	0.6
Leu	1.53	1.34
Lys	0.98	0.85
Met	0.28	0.24
Met + Cys	0.55	0.48
Phe	0.86	0.72
Thr	0.65	0.56
Trp	0.22	0.17
Val	0.79	0.66

[1]Diets for Phases 1 and 2 are formulated to meet nutrient requirements for 25-50 and 50-75 kg pigs, respectively.
[2]The vitamin-mineral premix will provide the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.
[3]Standardized total tract digestible P.
[4]Amino acids are indicated as standardized ileal digestible AA.

Supplemental Table 2. Analyzed diet composition and nutrient composition for Phases 1 and 2

Calculated values	**Grower-1 (25-50 kg)**	**Grower-2 (50-75 kg)**
CP	19.92	16.76
Met (total)	0.28	0.24
Met (supplemental)	<0.010	<0.010
Cys	0.27	0.24
Met+Cys	0.55	0.48
Arg	1.18	0.95
Ile	0.73	0.6
His	0.48	0.4
Leu	1.53	1.34
Lys	0.98	0.85
Phe	0.86	0.72
Trp	.	.
Thr	0.65	0.56
Val	0.79	0.66

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