

EVALUATION OF SUPPLEMENTATION OF THE LEUCINE METABOLITE, β -
HYDROXY- β -METHYL BUTYRATE (HMB), DURING GESTATION TO MOUSE
DAMS ON OFFSPRING BIRTH WEIGHT AND GROWTH VARIATION.

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Chapter 1

Introduction

Over the last twenty years, genetic companies have been increasing the genetic potential of sows to produce larger litters. The thought is that if the sow can produce more marketable pigs per year, then pork production will be more profitable for pork producers. However, although genetic selection for larger litters is beneficial, it also causes challenges for producers. An increase of one piglet per litter is associated with a 30 to 50 gram reduction in average piglet birth weight (Opschoor et al., 2010). Not only is there a reduction in average birth weight, but there is an increase in variability of birth weights in genetically superior, highly prolific sows (Wolf et al., 2008; Ashworth, 2013). Increasing litter size also increases the number of pigs born weighing less than 1 kg (Holl and Long, 2006; Kohler and Bierman, 2014). Piglets born below the average pig birth weight in a litter have a greater likelihood of not surviving through weaning. Piglets that weigh between 0.68 and 0.90 kg at birth have a 64% survival rate compared to piglets that weigh more than 1.36 kg at birth, which have a 95% survival rate (Holl and Long, 2006). System-wide benefits of increased litter size are limited due to these production losses and inefficiencies (Holl and Long, 2006) because they increase costs due to pigs not surviving to market weight, which include increased feed cost and medications, and the loss of pigs born that do not survive to market weight. Therefore, selection for increased litter size may not be beneficial unless measures are implemented to improve the survival of low-birth-weight piglets (Milligan et al., 2002a).

An analysis of sow farm productivity from 2005 through 2010 shows how genetic selection for larger litters has affected swine production (Knauer and Hostetler, 2013). From 2005 to 2010, total born increased by 1.21 pigs per litter and pre-weaning mortality increased from 13.7% to 14.8%. Similarly, from 2011 to 2016, total born continued to increase by 0.3 pigs per litter. Over this same period, pre-weaning mortality rose from 15.5% in 2011 to 17.3% in 2016 (Stalder, 2018). The increase in pre-weaning mortality observed during this time may explain why the number of pigs weaned has not increased as rapidly as the total number pigs born, with 10.2 pigs weaned per litter in 2011 and 10.3 pigs weaned per litter in 2017 (Stalder, 2013; Stalder 2018). If pre-weaning mortality could be reduced, then number of pigs weaned per litter could increase similar to the trend of increased total pigs born per litter.

Babcock Genetics conducted a trial to determine the effects of piglet birth weight on farm profitability (Kohler and Bierman, 2014). They found that large differences in profitability occur from only minimal changes in variability of birth weight. For example, a litter with 12 piglets has a mean birth weight of 1.45 kg and a litter with 10 piglets had a mean birth weight of 1.63 kg. The increase of 0.18 kg in mean piglet birth weights in the 10 piglet litter compared to the 12 piglet litter resulted in an increase in overall profitability of \$20.66 (Kohler and Bierman, 2014). In addition, profitability can increase if the number of pigs marketed/year increases, but only if within litter birth weight variation is unchanged (Kohler and Bierman, 2014). If birth weight variation increases, there may be more piglets born below the 1 kg birth weight threshold, resulting in a higher pre-weaning mortality. Therefore, variation in birth weights of large litters can cause producers to lose money.

A potential reason for elevated pre-weaning mortality and increased incidence of low birth weight pigs could be an increased incidence of intrauterine growth retardation in these large litters. Pigs exhibit the most extreme naturally occurring fetal growth restriction among litter bearing species (Wu et al., 2006). Many litters contain at least one piglet that is considerably smaller than its littermates with some of these piglets weighing as little as 30% of the largest littermate (Ashworth, 2013). This lack of uniformity in litter birth weights creates a challenge for producers. High piglet birth weight variation within litters is often associated with increased pre-weaning mortality, variable piglet weights at weaning, and a poorer post-weaning growth performance leading to economic losses and reduced production efficiency (Campos et al., 2012; Yuan et al., 2015). These piglets will also take longer to reach market weight compared to larger littermates which increases production costs.

Intrauterine growth retardation will remain a significant problem to the animal industry until interventions are researched and adopted by producers (Wu et al., 2006). Being able to understand the mechanisms behind this phenomena and potential solutions to counteract the effects of IUGR and low birth weight pigs in production systems is important. Researchers have begun to evaluate different nutritional options for potential solutions but have obtained varying results. This is why research on this matter needs to continue to be able to find a solution for swine producers to employ.

Chapter 2

Literature Review

Intrauterine growth retardation and low birth weights

Intrauterine growth retardation (IUGR) is defined as impaired growth and development of the mammalian embryo, fetus, or its organs during pregnancy (Wu et al., 2006). Intrauterine growth retardation may be considered a natural mechanism to protect the dam in cases of undernutrition. However, IUGR may not be beneficial for survival, optimal growth performance of the progeny, or efficiency of pork production. Intrauterine growth retardation affects many livestock species, but it is most severe in pigs (Ashworth, 2013).

In swine, uterine capacity limits fetal growth (Wu et al., 2006). As litter size increases, available nutrients for each fetus decrease due to increased fetal competition. This competition and inadequate supply of nutrients can cause suboptimal fetal development and consequently, lower birth weight and higher within-litter birth weight variation. A growth-retarded pig fetus is associated with a smaller placenta which has less blood flow due to less dense vasculature compared with placentae that support normal fetuses (Ashworth, 2013).

Often, IUGR piglets or low birth weight piglets are referred to as runt pigs. Runts have been defined in the scientific literature as piglets born weighing less than 1 kg or having a birth weight less than two-thirds of the litter's average birth weight (Perry and Rowell, 1969; Quiniou et al., 2002). These smaller piglets can weigh less than half of the body weight of their larger littermates. There is a positive correlation between piglet survival to weaning and body weight at birth (Leenhouders et al., 2002). This association

occurs because low birth weight piglets have difficulty competing with their heavier littermates for colostrum and milk (Leenhouders et al., 2002; Fix et al., 2010), which impairs their development.

Researchers found that IUGR and small birth weight fetuses can be identified as early as day 30 of gestation (van der Lende et al., 1990; Finch et al., 2002). Distribution of fetuses in body weight categories at 27 to 35 days post-mating is similar to body weight distribution of piglets at birth suggesting that disparities in birth weight are established very early in gestation (van der Lende et al., 1990). Restricted fetal growth early in gestation, before physical uterine capacity is reached, may suggest that fetal location in the uterine horn is not a determining factor in the occurrence of IUGR, but more likely involves placental and fetal development. Even though IUGR is apparent early in pregnancy, it worsens as fetuses develop and physical crowding can then become a factor that magnifies the differences in body weights among fetuses *in utero*.

There are conflicting ideas on whether birth weight and fetal development depend on location within the uterine horn. Perry and Rowell (1969) noted that smaller fetuses were generally located closer to the middle of the horn, with larger fetuses located near the ends of the horn. However, these observations were noted only when the uterine horn contained six or more fetuses. Other studies have shown that runts can be found at any position in the uterine horn (Wu et al., 2006), and gilts slaughtered during pregnancy demonstrated no relation between uterine location and weight of each fetus (van der Lende et al., 1990).

Effects of fetal growth retardation and low birth weight pigs

Fetal growth retardation can cause negative effects on neonatal survival, post-natal growth (Quiniou et al., 2002) and feed efficiency (Powell and Aberle, 1980), body composition of offspring (Hegarty and Allen, 1978; Powell and Aberle, 1980), meat quality of offspring (Gondret et al., 2005), and long-term pig health (Rooke and Bland, 2002; Le Dividich et al., 2005).

In terms of survival, small piglets have a disadvantage compared to their heavier littermates, and this disadvantage is exacerbated in large litters and litters from older sows (Milligan et al., 2002b). Litters with high variation in birth weight had more deaths, especially if the litter's mean birth weight was 1.05 kg or less (Milligan et al., 2002b). High neonatal mortality in livestock is associated with low birth weight. This is due to correlations found between birth weight and behavioral maturity of the animals (Ashworth, 2013). Piglets with birth weights below 1 kg are at a higher risk of pre-weaning mortality than piglets born weighing more than 1 kg (Zeng et al., 2018). 15 to 20% of piglets born do not survive to weaning (Quiniou et al., 2002), with low birth weight piglets representing 76% of pre-weaning deaths (Ashworth, 2013). In the last 10 years, pre-weaning mortality has increased with the increase in total numbers of pigs born alive (Stalder 2013; 2018; Figures 2.1 and 2.2). This increase in pre-weaning mortality is due to an increase in number of low birth weight pigs in each litter.

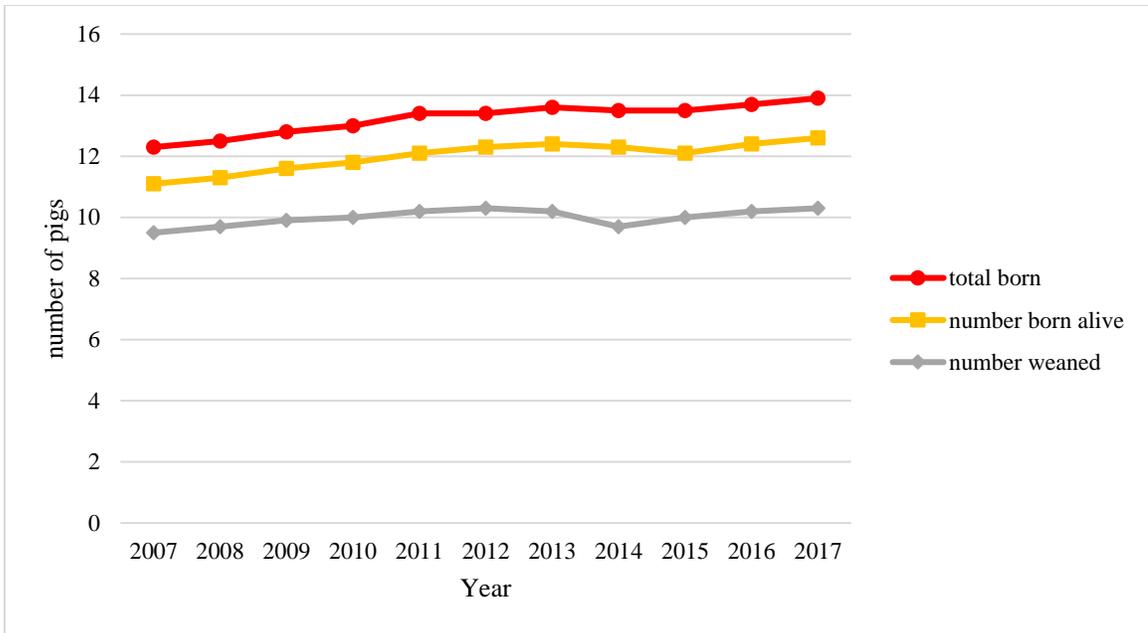


Figure 2.1 Sow farm productivity from 2007 to 2017. Data taken from Stalder (2013, 2018).

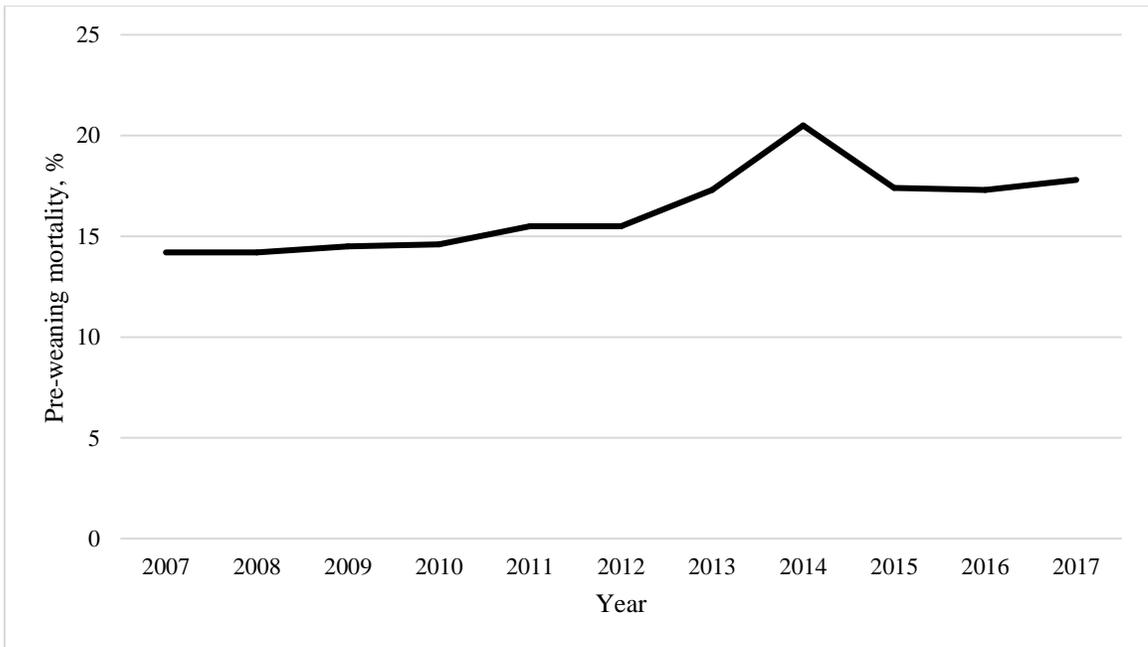


Figure 2.2 Pre-weaning mortality on farms from 2007 to 2017. Data taken from Stalder (2013, 2018).

While small piglets are at a greater risk of death than their larger littermates (Quiniou et al., 2002), survival of all piglets increases when there is reduced variation in

birth weights of piglets within a litter (van der Lende and de Jager, 1991). There is a negative linear relationship between piglet survival and coefficient of variation for within-litter birth weight, and a quadratic relationship between average birth weight and mortality of piglets is apparent (Fahmy and Bernard, 1971). Litters containing low-birth-weight piglets had more piglets born alive and had increased pre-weaning mortality, with the majority of deaths being low-birth-weight pigs (Milligan et al., 2002b). Therefore, even though these litters were initially larger, they did not produce more weaned piglets than litters that were smaller but had no low-birth weight piglets.

Intake of colostrum is delayed and inadequate in IUGR or low birth weight piglets (Leenhouwers et al., 2002). Low intake of colostrum leads to poor acquisition of passive immunity from the dam and poor nutritional status, thereby increasing pre-weaning mortality or resulting in poor growth performance during lactation (Yuan et al., 2015). In addition, IUGR neonates and low birth weight piglets have a higher susceptibility to infection due to an ineffective immune system compared to non-IUGR litter mates (Cromi et al., 2009).

Internal organs such as the liver and small intestine are smaller in proportion to body size in IUGR pigs compared to their larger littermates which leads to a lower metabolic capacity (Wu et al., 2006; Yuan et al., 2015). Reduction of metabolic function, such as absorptive capacity, due to insufficient growth *in utero* tends to cause higher death losses in these smaller fetuses *in utero* or of piglets postnatal. Decreased expression of proteins which regulate immune function, intermediary metabolism, protein synthesis, and tissue growth in the animal may be a major mechanism responsible for abnormal absorption and metabolism of nutrients, as well as reduced growth and impaired

development of the small intestine, liver, and muscle in IUGR piglets (Wang et al., 2008). Inefficient utilization of nutrients has been reported for pigs weighing less than 1.09 kg at birth (Rezaei et al., 2011), and appears to be caused by physiological immaturity at birth due to severe dysfunction of several organs such as the intestine (Wang et al., 2013), liver (Lui et al., 2013) and skeletal muscle (Wang et al. 2014). The small intestine of IUGR pigs has increased proteins and enzymes associated with oxidative stress and protein degradation and decreased abundance of proteins involved in maintenance of cell structure, nutrient absorption and transfer, and protein synthesis (Wang et al., 2014). Due to increased activity of glutamate oxaloacetate transaminase and decreased activity of lipoprotein lipase in the liver of IUGR fetuses, there is an altered metabolism of nutrients, abnormal ammonia utilization, and a reduced detoxification capacity (Liu et al., 2013). In skeletal muscles of pigs multiple proteins involved in energy supply and protein metabolism, structure and type of muscle fibers, proliferation and differentiation of muscle fibers, and nutrient transport are differentially expressed in IUGR pigs compared to their normal birth weight littermates (Wang et al., 2013). Therefore, these overall capacity differences in utilization efficiency of nutrients among piglets may partly explain why differences in body weight at birth are often maintained or magnified throughout the nursing period (Yuan et al., 2015).

Usually, pigs weighing less than 1 kg at birth have very little chance of surviving to weaning. However, when these pigs do survive, their post-weaning growth performance also is negatively affected (Powell and Aberle, 1980; Quiniou et al., 2002; He et al., 2016; He et al., 2018). Higher inefficiency was noted by Quiniou et al. (2002) when piglets weighing 0.6 kg at birth required an additional 3 weeks to reach 25 kg

compared to piglets weighing 2.6 kg at birth. Similarly, low birth weight pigs (< 1,000 g) grew slower than medium (1,300 to 1,400 g) or high birth weight pigs (> 1,600 g), but feed utilization was not significantly affected (Powell and Aberle, 1980). Low birth weight pigs required significantly more time (23 days) to reach 106 kg body weight than their control littermates (Hegarty and Allen, 1978). In a recent study, slow growing pigs were found to have lower concentrations of IGF-1, insulin, leptin, and circulating amino acids which is believed to contribute to or be associated with slow growing pigs (He et al., 2016). In a separate study, no behavior differences between fast growing and slow growing pigs were observed, except that slow growing pigs spent more time at the waterers than fast growing pigs (He et al., 2018).

Both muscle mass and meat quality at slaughter is impacted negatively in low birth weight pigs (Rehfeldt and Kuhn, 2006). The number of secondary and total muscle fibers is reduced in IUGR fetuses, which adversely affects growth rate and post-mortem quality of meat (Ashworth, 2013; Yuan et al., 2015). Low birth weight pigs were found to have significantly larger muscle fiber diameter than larger littermates in the *biceps brachii*, *psoas major*, and *semitendinosus* muscles, which can be associated with fewer muscle cells (Hegarty and Allen, 1978; Wigmore and Stickland, 1983; Gondret et al., 2005). The reduction in number of muscle fibers and the larger diameter of those fibers in IUGR piglets compared with normal birth weight piglets results in decreased meat tenderness and quality of meat and economic losses (Gondret et al., 2005). Low birth weight barrows produced fatter carcasses with less lean percentage, while low birth weight gilts were similar to large birth weight barrows in carcass composition (Powell and Aberle, 1980). Similarly, carcasses from low birth weight pigs (0.80 to 1.20 kg at

birth) had the highest amount of intermuscular fat when compared to larger littermates (Beaulieu et al., 2010).

Genetic selection over time has increased the total number of pigs born per litter, which has been accompanied with an increase in pre-weaning mortality. Intrauterine growth retardation or an increase in low birth weight pigs, pigs born weighing less than 1 kg, may be causing the increase in pre-weaning mortality. Intrauterine growth retardation will remain a significant problem to the animal industry because there is an incomplete knowledge of possible solutions for producers to employ (Wu et al., 2006). Smaller piglets that survive past weaning grow slower than their larger litter mates. This reduction in growth increases the cost of production for producers or can reduce the amount of profit a producer can obtain from these smaller and slower growing pigs. Therefore, understanding the mechanisms behind how this phenomenon occurs and potential solutions to counteract effects of IUGR is pivotal for swine production today.

Sow nutrition in gestation

Nutrition and pregnancy outcomes

Due to genetic selection of highly prolific sows, standards set by NRC may or may not be the optimal nutritional feeding program during gestation (Campos et al., 2012). Suboptimal maternal nutrition during gestation leads to insufficient nutrients to support metabolic needs of both sows and fetuses. A reason why the swine industry may have incorrect nutritional recommendations is that the current dietary guidelines are based on genetic lines that are less prolific than sows currently used in commercial production (Ball et al., 2008). The modern sow has an increased requirement for energy and amino acids that are needed for maintenance but also for reproductive purposes

compared to the less prolific sows of the past (Ball et al., 2008). Incorrect maternal nutrition along with increased energy and amino acid requirements due to larger number of fetuses in the uterus may potentially be associated with the increase in fetal growth retardation currently occurring in commercial swine production. When sows enter pregnancy, pre-mating maternal undernutrition may negatively affect growth and development of early embryos and fetuses (Wu et al., 2006).

A reason for high pre-weaning mortality could be that, as the number of fetuses increase *in utero*, the amount of available nutrients per fetus decreases due to a decrease in uterine blood flow per fetus (Campos et al., 2012). This is most important in the last third of gestation when rapid fetal growth is occurring. Thus, suboptimal maternal nutrition during gestation has been regarded as one of the main causes of within-litter birth weight variation in modern sow genotypes. An overarching goal of improving maternal nutrition is to increase the homogeneity of development of conceptuses to decrease variation and improve birth weights of newborn piglets (Yuan et al., 2015).

Studies observing effects of maternal nutrition on birth weight have focused on different aspects of nutrition. For example, two studies conducted altering energy intake of sows (either restricted or above requirements) showed no effect on number of progeny alive at birth, mean birth weight of piglets, or piglet weight at weaning (Bee, 2004; Lawlor et al., 2007). Contrary to the findings of Bee (2004), other researchers reported feeding extra feed or energy in the last 23 days of gestation improved birth weight of piglets, but only marginally (Cromwell et al., 1989). Maternal protein intake, when deficient, reduces amino acid availability to the conceptus which greatly affects

embryonic and fetal survival in pigs (Wu et al., 2010). This topic will be discussed in more detail later in this review.

Maternal nutrition can affect *in utero* muscle fiber development in the pig, thereby influencing early postnatal growth rate. Prenatal events during fetal development affect body composition by influencing formation of secondary muscle fibers (Dwyer et al., 1994; Bee, 2004). The difference in carcass lean content between larger and smaller littermates may be due to the smaller littermates having lower secondary-to-primary muscle fiber ratios (Bee, 2004). These differences in muscle fiber numbers between low birth weight piglets and their larger littermates can be a result of maternal undernutrition during gestation. Maternal undernutrition is also an important factor that influences fetal programming *in utero*.

Fetal programming

Maternal nutrition and metabolic state have an important impact on embryonic and fetal development. There is growing evidence that maternal nutritional status can alter the epigenetic state of the fetal genome. Epigenetics is the study of heritable phenotypic changes that do not involve alteration in the sequence of DNA. These factors (genetics, epigenetics, maternal maturity, environment) affect the size and functional capacity of the placenta, uteroplacental transfer of nutrients and oxygen from the mother to the fetus, nutrient availability to the conceptus, fetal endocrine milieu, and metabolic pathways (Wu et al., 2006).

There is extensive evidence for both direct and indirect effects of nutrition on the reproductive tract of pigs (Foxcroft et al., 2000). This is also known as fetal programming, which occurs during development of the embryo and fetus. During fetal

programming, important physiological parameters can be reset by environmental events and most importantly these changes can persist into adulthood. This is supported by the Barker Hypothesis where events that occur *in utero* can lead to intrauterine growth retardation, low birth weight, and premature birth, and have a causal relationship to the origins of hypertension, coronary heart disease and non-insulin dependent diabetes in middle aged human adults (Barker and Clark, 1997). However, not all changes due to fetal programming are negative. Fetal programming can be beneficial because the maternal nutritional state during gestation can prime the fetus for the environment it will experience after birth. This process gives the offspring its best chance to survive upon birth. Negative influences of fetal programming result when a fetus is subjected to an environment that is different than the one it will experience after birth. Understanding the mechanisms behind fetal programming is necessary for improving efficiency of pork production (Li et al., 2017).

Fetal programming can be identified in runt pigs as early as day 27 to 35 of gestation (Foxcroft et al., 2014). A considerable proportion of the variation in growth performance after birth is determined largely during fetal development in the uterus (Foxcroft and Town, 2004). In large litters where uterine crowding occurs, placental development can be affected negatively. Dziuk (1968) hypothesized that an inability to compensate for the negative effect of fetal crowding on placental development limits fetal growth later in gestation. Consequently, placental development of some fetuses may be compromised, resulting in IUGR and an increase in birth weight variation as a result of crowding in early gestation (Foxcroft et al., 2014). An underdeveloped placenta early in gestation due to uterine crowding offers a biological explanation for increased variability

in birth weight and postnatal growth performance observed in older sows where litter sizes are larger in earlier parities (Foxcroft et al., 2014). High subsequent feed intakes of low birth weight offspring in the growing period does not result in normal development during compensatory growth, implying that some form of intrauterine fetal reprogramming had occurred (Widdowson, 1976).

Maternal nutrition during gestation is very important. Suboptimal nutrition during gestation can lead to negative effects in the offspring. Fetal programming is the idea that during development of the embryo and fetus important physiological parameters can be reset by environmental events which can last into adulthood and be passed onto the next generation. However, maternal nutrition and fetal programming are not the only determinants of offspring growth. Placental development and function is another important factor of growth and development of offspring *in utero*.

Placental development and efficiency

Development

Development of the placenta differs among species. Because of these differences, placentae are divided into different classifications. Classification of the various types of placenta is based on the histologic nature of the maternofetal interface, and represents: epitheliochorial (horses and pigs), endotheliochorial (carnivores), synepitheliochorial (ruminants), and hemochorial types (primates and rodents). In all placental types regardless of species, three fetal layers of the chorioallantoic placenta are present which include: the endothelial lining of the allantoic blood vessels, the chorioallantoic mesodermal connective tissue, and the chorionic epithelial cell layer also known as the

trophoblast layer (Hafez, 2017). The chorioallantoic placenta is the permanent functional placenta in domestic mammals, such as mice, pigs, and humans (Hafez, 2017).

Implantation begins on day 15 of gestation in pigs (Geisert and Yelich, 1997), and the porcine placenta grows rapidly between days 20 to 60 of gestation, with maximal development occurring by day 70 (Knight et al., 1977; Wu et al., 2005). This precedes the period of rapid fetal growth. The placenta also undergoes rapid formation of new blood vessels throughout gestation (Reynolds and Redmer, 2001). However, insufficient placental vascularization may lead to progressive deterioration in placental function and decreased placental transfer of oxygen and nutrients to fetuses (Wu et al., 2006). Maternal undernutrition during gestation may disrupt placental angiogenesis leading to a reduction in nutrient transfer between maternal and fetal tissues. Functional capacity of placentae for provision of nutrients and the exchange of gases is vital to fetal survival, growth, and development (Reynolds et al., 2006).

Regulating the synthesis of molecules such as nitric oxide, polyamines, and proteins by functional amino acids, such as arginine and glutamine, help stimulate placental growth and transfer of nutrients from the dam to the embryos or fetuses, thereby promoting conceptus survival, growth, and development (Wu et al., 2006; Wu and Meininger, 2009). Functional amino acids are defined as amino acids that participate in and regulate key metabolic pathways to improve health, survival, growth, development, lactation and reproduction (Wu, 2013). Researchers found that rates of nitric oxide and polyamine synthesis, both products of arginine metabolism, in both porcine and ovine placentae were greatest during early gestation at the time when placental growth is most rapid (Kwon et al., 2004; Wu et al., 2005; Gao et al., 2009). Wu et al. (2004)

hypothesized that IUGR results from impaired placental growth, placental vascular growth, or placental function caused by a reduction in placental nitric oxide synthesis. Therefore, understanding the mechanisms that regulate placental growth, including vascular growth and placental function, is crucial for improving litter size and fetal growth in pigs (Wu et al., 2010).

Relationship of placental development to fetal growth

Fetal growth depends on placental growth and efficiency which are directly related to the placenta's functional efficiency of delivering nutrients and oxygen to fetuses (Salafia et al., 2005, Ashworth, 2013). The establishment of fetal and placental circulation is one of the earliest events during embryonic and placental development (Wu et al., 2004). Reynolds and Redmer (2001) evaluated placental angiogenesis and reported that the vascular endothelial growth factor, the fibroblast growth factor, and the angiopoietin protein families, and their respective receptors, are the major factors regulating angiogenesis. Angiogenic factors interact with the local vasodilator, nitric oxide, to coordinate placental angiogenesis and blood flow. Angiogenesis is crucial to support the metabolic demands of the fetus, mainly during the latter half of gestation when an exponential increase in fetal growth occurs (Reynolds and Redmer, 2001). Also, having a better match between uterine capacity and the number of conceptuses in the uterus should counteract the detrimental effects on placental development and embryonic growth that is measurable by the 28th day of gestation (Foxcroft et al., 2000).

Placental efficiency

Once formed, interfaces between maternal and fetal blood circulations do not remain static but change throughout gestation (Georgiades et al., 2002). The fact that

placental development is not static is thought to be due to increased placental efficiency as gestation progresses. Placental efficiency is the ratio of fetal weight to placental weight and is vastly different among species and even breeds within the same species. Placental efficiency is determined by placental weight or size, the surface area of contact with the maternal endometrium, placental blood flow, and the ability of the placenta to transport nutrients to the fetus (Ashworth, 2013). Several other factors, notably the species-specific degree of permeability of the various layers making up the maternofetal barrier, the actual thickness of these layers, and the spatial relationship between fetal and maternal vasculatures, may be more important determinants of the efficiency of transplacental exchange of nutrients and waste. Efficiency of nutrient and gas exchange may be influenced by the arrangement of the capillary bed between the maternal and fetal vasculatures which varies by species. Vallet et al. (2003) suggested that fetal growth rate is less sensitive to intrauterine crowding than placental growth rate. In prolific Meishan females, an increase in placental efficiency may initially protect the developing fetus from a limitation in placental size (Vallet et al., 2003).

During late gestation, the placenta increases its performance without a proportionate increase in size through increased efficiency (Georgiades et al., 2002). As gestation progresses, intrauterine competition among littermates for the establishment of adequate surface area for nutrient exchange between fetal and maternal circulations may act to limit total litter weight and increase variation within littermates. Because uterine space is a factor limiting fetal growth, establishing a more efficient placenta could increase birth weights and reduce weight variation that occurs *in utero*.

Evidence supporting this enhanced placental efficiency is present in both humans and mice where the increase in fetal weight during late gestation is disproportionately higher than the minor increase in placental weight (Georgiades et al., 2002). For example during the third trimester of human pregnancy, placental weight increases by only 15% but fetal weight increases about 200%. This phenomenon is also observed in mice during the last quarter of gestation when placental weight remains relatively constant but fetal weights increase about 200% (Georgiades et al., 2002). This increase in efficiency results from an increase in blood vessels of the human fetal placenta and mouse labyrinth consistent with an increased efficiency in physiological exchanges between fetal and maternal blood.

Differences in placental function among swine breeds

Placental development differs among swine breeds. Due to increased prolificacy of modern sows, fetal growth and development has been limited by decreased uterine blood flow to each fetus (Campos et al., 2012). When comparing Yorkshire and Meishan uteri with regards to fetal and placental development, an increase in vascular density of the placentae was observed in the Meishan while in the Yorkshire only an increase in surface area of the placentae was observed (Biensen et al., 1998). Meishan placentae are about 70% smaller than the American breed, Yorkshire, but they are more highly vascularized (twofold more than Yorkshire) and therefore more efficiently transfer nutrients to fetuses even though the placenta is smaller for each fetus (Wilson et al., 1998). Well-developed placental vasculature enables the Meishan fetus to obtain sufficient nutrients from a relatively small placenta (Bazer et al., 1988), resulting in an increased rate of prenatal survival (Wu et al., 2010). Size of the fetus and piglet is

determined by the size and vascularity of its placenta (Wilson et al., 1998). Placental weight and surface area are reduced by 40% in Meishan placentae, compared with Yorkshire (Biensen et al., 1999). Markedly larger placentae (weight and surface area) were recovered from Yorkshire than from Meishan uteri on days 90 to 110 of gestation. Fetal weight increased from days 90 to 110 of gestation, however placental weight remained constant in the Meishan. This led to an increase in placental efficiency of the Meishan from 2.9 to 4.8, respectively (Biensen et al., 1999).

The same group at Iowa State University used embryo transfer to allow Yorkshire and Meishan females to have both Yorkshire and Meishan conceptuses brought to term. This study was conducted to observe differences in placental development between the two breeds when the dam is not of the same breed as the conceptus. Placental surface area remained relatively constant during the last third of gestation for Meishan conceptuses regardless of breed in which the fetuses were gestated (Biensen et al., 1998). Regardless of sow breed, the vascular density of Meishan placentae increased progressively from 3.0% on day 70 to 6.0% on day 110 of gestation, reaching values more than twofold greater than the vascular density observed in Yorkshire placentae on day 110 (2.8%; Biensen et al., 1998). This increase in vascular density of Meishan placentae was through both increases in diameter and number of blood vessels in the placenta. Placental efficiency on day 110 of gestation was evaluated between breeds by taking the weight of each fetus and dividing it by its placental weight. The Meishan conceptuses demonstrated a greater fetal weight:placental weight ratio across both uterine environments over Yorkshire conceptuses. When Meishan conceptuses were gestated in a Meishan uterus, placental efficiency was markedly greater than when gestated in a

Yorkshire uterus. The size of the placenta is driven by the uterus in which it is gestated up to day 90 of gestation. After day 90, when rapid fetal growth occurs, the genetic differences between breeds start to play a role. At this time, an increase in placental size is observed in Yorkshire conceptuses and an increase in vascularity is seen in Meishan conceptuses in order to facilitate the increased nutrient and waste exchange that occurs after day 90 of gestation.

Development of the placenta is an important step in gestation. Different molecules such as nitric oxide and polyamines stimulate placental growth and development. A well-developed placenta helps ensure healthy growth and development of the fetus. The placenta constantly undergoes angiogenesis allowing for nutrient transfer to occur between maternal and fetal blood vessels. The efficiency of the placenta can differ. Some placentae such as for Yorkshire pigs tend to increase in size throughout gestation keeping the vasculature the same diversity whereas the placentae for Meishan pigs tend to be smaller and increase in vascularity throughout gestation.

Previous intervention studies to mitigate incidence of low birth weight pigs

Hormones

Possible interventions to improve placental and fetal development previously researched include administration of exogenous hormones. When administering porcine growth hormone (GH) to sows, an increase in fetal weight and placental weight was observed (Sterle et al., 1995). Others suggest that GH plays a critical role in early gestation by positively influencing nutrient transfer, placental growth, and selectively improving growth conditions for smaller piglets (Rehfeldt et al., 2001). Studies have indicated that a greater physiological maturity at birth (i.e. body and tissue composition

and metabolic and hormonal state) also increase the likelihood of piglet survival (Leenhouwers et al., 2002).

Bump feeding

One strategy to improve piglet birth weight is to increase feeding levels to sows during late gestation. This is often referred to as “bump feeding”. Numerous studies have been conducted to evaluate this practice. This strategy is used in both sows and gilts. In sows, increasing the feeding level has not shown any significant improvement in birth weights of the litters (Cromwell et al., 1989; Miller et al., 2000; Shelton et al., 2009; Gonçalves et al., 2016; Mallmann et al., 2018). Though the general consensus is that bump feeding in sows does not affect birth weight, many of these studies have only been conducted through one reproductive cycle. Cromwell et al. (1989) showed an increase in birth weight when increasing feed during late gestation over multiple cycles. This observation may be explained by the sow having time to increase their body nutrient stores. In contrast, gilts respond to elevated feeding levels differently than sows. In general, elevating the feed intake in late gestation for gilts improved litter birth weights in several studies (Cromwell et al., 1989; Shelton et al., 2009; Gonçalves et al., 2016), but not all studies (Mallmann et al., 2018). A potential reason for the increase in birth weight in gilts and not in sows could be due to the fact that gilts are still growing and developing. Nutrients and energy that a gilt consumes need to first cover body maintenance requirements and growth before being put towards fetal growth. By adding extra nutrients or energy, gilts can partition more nutrients toward fetal development.

Amino acids

Another approach to mitigate IUGR or low birth weights is to enhance placental function. This may be achieved by supplementing sows during gestation with the functional amino acid, arginine. There is strong evidence that members of the arginine family of amino acids (arginine, glutamine, glutamate, proline, aspartate, asparagine, ornithine, and citrulline) have an important role in placental vascularization and development, especially during the first half of pregnancy (Campos et al., 2012). All of these amino acids have an important role in placental angiogenesis and placental, embryonic, and fetal development. According to Wu et al. (2010), nitric oxide, polyamines, arginine, and other functional amino acids (glutamine, leucine, proline) may regulate embryonic and fetal muscle growth and development via cell signaling through the mammalian target of rapamycin (mTOR) pathway. Sows supplemented with arginine had heavier placentae (Gao et al., 2012), and a greater litter birth weight of all piglets born and of all piglets born alive (Li et al., 2015) compared to sows without supplementation. Dietary supplementation with 0.83% L-arginine to gilts on days 14 to 28 or days 30 to 114 of gestation increased the number of live-born piglets and litter birth weight of live-born piglets (Ramaekers et al., 2006). In addition, supplementing the gestation diet with 0.4% L-arginine plus L-glutamine enhanced efficiency of dietary protein utilization by the sow, reduced variation in piglet birth weight, and increased litter birth weight (Wu et al., 2010). Therefore, increasing the dietary provision of arginine beyond that from a typical corn-soybean meal diet may be an effective means to enhance circulating arginine concentrations and improve pregnancy outcomes in pigs (Wu et al.,

2007). Similar results were observed when rats were supplemented with arginine during early or mid-gestation (Zeng et al., 2008).

Researchers have investigated different types of interventions to reduce the incidence of IUGR and low birth weight pigs. Exogenous growth hormone reduced incidence of low birth weight pigs but it is not approved for the use in swine by U.S. FDA. If an intervention can be identified that naturally increases endogenous growth hormone production, it may be promising. Bump feeding has been studied but has yielded inconclusive results regarding effects on birth weight where it is not shown to be effective in sows and may be effective in gilts. Supplementing diets with arginine increased birth weight but few trials have been conducted to evaluate effects on variability of birth weight.

β -hydroxy- β -methylbutyrate

Introduction to leucine and β -hydroxy- β -methylbutyrate

Amino acids are essential to biosynthesis of proteins. Some amino acids can be produced endogenously by the animal and others must be supplied through the diet. Amino acids that must be supplied in the diet are known as essential amino acids. Leucine, an essential amino acid in swine diets, plays a role in protein synthesis. In both *in vitro* and *in vivo* studies with rats, leucine has a direct effect on stimulating muscle synthesis (Anthony et al., 2002; Bolster et al., 2004) and inhibiting protein breakdown in muscle *in vitro* (Buse and Reid, 1975). β -hydroxy- β -methylbutyrate (HMB), a leucine metabolite, also plays a role in skeletal muscle development and reduction of proteolysis (Nissen and Abumrad, 1997; Slater and Jenkins, 2000; Wheatley et al., 2014). β -hydroxy- β -methylbutyrate is produced from α -ketoisocaproate (KIC), another metabolite of

leucine, via KIC dioxygenase enzyme (Nissen and Abumrad, 1997; Slater and Jenkins, 2000; Baxter et al., 2005). Approximately 2 to 10% of leucine oxidation proceeds to synthesis of HMB (Slater and Jenkins, 2000). *In vitro* data are consistent with the theory that HMB is responsible for the non-protein synthetic functions of leucine and KIC *in vivo* (Nissen and Abumrad, 1997).

In addition to its role in muscle synthesis, β -hydroxy- β -methylbutyrate can be converted to β -hydroxy- β -methylglutaryl coenzyme A (HMG-CoA) in some tissues and acts as the key carbon source for *de novo* cholesterol synthesis necessary for maintenance of maximal cell function (Nissen and Abumrad, 1997; Baxter et al., 2005). Cholesterol is also a precursor for many glucocorticoids, such as cortisol. β -hydroxy- β -methylbutyrate cannot fulfill the leucine requirement for protein synthesis.

β -hydroxy- β -methylbutyrate is used by humans but few studies have been conducted in animals to determine product safety. A toxicology study was conducted with 20-kg pigs fed 100 g HMB per day for 4 days (Nissen and Abumrad, 1997). This dose is approximately 100X higher than what is typically consumed by adult humans. None of the pigs exhibited untoward signs related to HMB consumption. There were no changes in blood cell parameters or percentages, organ weights, nor were there any histological lesions present in either control or HMB-fed pigs. Therefore, it seems that even at very high intakes there are no adverse effects, at least for a short amount of time. Another toxicology study was conducted to evaluate the toxicity of the calcium salt of HMB (CaHMB) when administered daily in the diet of rats for at least 90 days (Baxter et al., 2005). Administration of CaHMB in a basal diet for 91 days was tolerated well having no unscheduled sacrifices or deaths and no adverse effects on body weight gain.

Therefore, Baxter et al. (2005) concluded the no-observed-adverse-effect level for CaHMB was 5% of the daily diet under these experimental conditions. Also in a summary of nine clinical studies in humans, HMB had no adverse effects when supplemented at 3 g/d in men, women, young, and elderly subjects (Baxter et al., 2005).

Mode of action of leucine and HMB

Leucine and HMB may work through several proposed mechanisms. Increases in lean mass elicited by both leucine and HMB are thought to involve both decreases in muscle protein breakdown (Baxter et al., 2005) and increases in muscle protein synthesis simultaneously (Wheatley et al., 2014).

Leucine

Previous work utilizing the neonatal pig shows protein synthesis is acutely increased in neonates in response to supplementation of the branched chain amino acid, leucine (Wheatley et al., 2014). Leucine serves as a substrate for protein synthesis and as a nutrient signaling molecule that stimulates protein synthesis through activation of the intracellular signal transduction pathway that regulates mRNA translation (Wheatley et al., 2014).

In vitro observations clearly demonstrate that muscle protein turnover in rats and chicks is affected significantly by increasing protein synthesis with leucine and HMB at pharmacological levels exceeding 5 to 200 fold physiological concentrations (Ostazewski et al., 2000). Leucine was proved to significantly increase protein synthesis; however, it did not significantly inhibit proteolysis. The conclusion from this study is that leucine increases protein synthesis and HMB, but not leucine, significantly inhibited proteolysis (Ostazewski et al., 2000).

β-hydroxy-β-methylbutyrate

Based on many studies, researchers postulated that HMB supplementation would involve the following mechanisms: (1) upregulation of IGF-1 gene expression in skeletal muscles, (2) stimulation of protein synthesis by increasing the mTOR signaling pathway, and (3) suppression of proteolysis by the inhibition of the ubiquitin-proteasome system (Zanchi et al., 2011). β-hydroxy-β-methylbutyrate is claimed to increase strength and lean body mass by acting as an anti-catabolic agent, and minimizing protein breakdown and damage to cells that may occur with intense exercise (Slater and Jenkins, 2000). While it would appear that *in vivo* observations of HMB are clear in humans undergoing resistance-weight training (Ostazewski et al., 2000), further studies need to be conducted to determine what effect HMB might have on protein degradation in either normal or extremely catabolic humans as well as its effects on animals.

β-hydroxy-β-methylbutyrate effects on hormones (GH, IGF-1).

A few studies have been conducted to observe the effects of HMB on growth hormone (GH) and insulin-like growth factor 1 (IGF-1). Supplementation of HMB to sows increased GH and IGF-1 in the serum of offspring (Tatara et al., 2007; Tatara et al., 2012). Similarly, supplementing HMB to lambs during the first 21 days of life increased serum concentrations of GH and IGF-1 (Tatara, 2008). Data point out a stimulatory effect of HMB on the production of hepatic IGF-1 (Zanchi et al., 2011). IGF-1 is produced by both the liver and muscle in response to treatment with HMB and could act on skeletal muscle in an endocrine, paracrine, and autocrine fashion (Zanchi et al., 2011). IGF-1 exerts an anabolic action in skeletal muscle leading to hypertrophy of muscle fibers (Barton-Davis et al., 1998; Fiorotto et al., 2003). Chronic HMB treatment of rats

increased serum somatotrophic hormones as well as increased levels of pituitary GH mRNA and hepatic IGF-1 mRNA (Gerlinger-Romero et al., 2011). In human myoblasts, HMB increased IGF-1 mRNA expression (Kornasio et al., 2009). Bondine et al (2001) demonstrated that hypertrophy of myotubes *in vitro* induced by IGF-1 was dependent on the pathway initiated by PI3K and Akt, which leads to the activation of mTOR. IGF-1 promotes activation of protein synthesis by stimulating the process of initiation of mRNA translation. Further studies are needed to understand and determine if activation of this signaling pathway by HMB occurs as a result of increased expression of IGF-1.

β-hydroxy-β-methylbutyrate effects on the mTOR pathway

The enzyme known as mTOR is a protein kinase responsive to mechanical, hormonal, and nutritional stimuli and plays a central role in controlling cell growth, primarily by controlling mRNA translation efficiency. β-hydroxy-β-methylbutyrate acts upon the mTOR pathway by yet unknown mechanisms, increasing the phosphorylation of its protein substrates (4EBP-1 and p70S6K) and resulting in increased myofibrillar protein synthesis (Zanchi et al., 2011). β-hydroxy-β-methylbutyrate alone augments skeletal muscle protein synthesis in swine neonates in association with the activation of mTOR signaling (Wheatley et al., 2014). Increased protein synthesis in skeletal muscle occurs because of activation of the mTOR-dependent translation initiation factors (Wheatley et al., 2014). Overall, Wheatley et al. (2014) reported an increase in protein synthesis when supplementing HMB to swine neonates due to an increase in activation of the mTOR pathway with no differences in proteolysis.

In a cell culture model, incubation with 50 mM of HMB significantly stimulated muscle protein synthesis. This response was correlated positively with an increase in

phosphorylation of mTOR and two important substrates of mTOR (4EBP-1 and p70S6K), which are proteins involved in increased translation of mRNA and protein synthesis in muscle (Eley et al., 2007). Importantly, this stimulating effect was completely abolished in the presence of rapamycin, an mTOR inhibitor. Eley et al. (2008) found that HMB could attenuate the depression of protein synthesis by modulating protein synthesis inhibitors such as: lipopolysaccharides, tumor necrosis factor alpha, and angiotensin 2.

Expression of mTOR and other proteins involved in insulin signaling were investigated to better understand HMB-stimulated skeletal muscle hypertrophy (Pimental et al., 2011). In a one-month period, HMB treatment at 320 mg/kg BW induced a significant increase in weight of the extensor digitorum longus muscle and soleus muscles but did not change total body weight, food intake, or fat and liver weight in rats 3 to 4 months of age. Relative to the control group, the supplemented group demonstrated an increase in mTOR protein levels and activation of p70S6K, which are linked to increased skeletal muscle mass in the extensor digitorum longus muscle (Pimental et al., 2011). Supplementation of HMB increased skeletal muscle protein mass by directly inducing increased mTOR expression and activation of p70S6K and not via phosphorylation of Akt/PKB. Based on the results from this study, the mechanism of action for HMB appears to be related to increases in mTOR/p70S6K pathway signaling which leads to improved protein synthesis and muscle hypertrophy (Pimental et al., 2011).

β-hydroxy-β-methylbutyrate effects on the ubiquitin-proteasome system

The ubiquitin-proteasome system is a proteolytic system dependent on energy (ATP) and degradation of intracellular proteins whose activity is increased in conditions of exacerbated muscle catabolism, such as fasting, hypogravity, immobilization, and bed rest (Lecker et al., 2006). Smith et al. (2005) observed in mice implanted with MAC16 tumor cells that HMB supplementation was effective in reducing muscle proteolysis observed in cancer-induced cachexia, which was reflected in the attenuation of muscle mass loss. Importantly, this observation was correlated positively with a decrease in catalytic activity of the proteasome. Supplemental HMB can markedly decrease muscle damage as evidenced by reduced leaking of creatine phosphokinase (CK) out of muscle cells during strenuous exercise. Oral supplementation of HMB can slow or partially prevent muscle wasting by suppressing upregulation of the ubiquitin-proteasome system and by enhancing protein synthesis rates (Smith et al., 2005).

There are a few potential mechanisms in which HMB can act in the body. β-hydroxy-β-methylbutyrate has been connected to both increasing protein synthesis and decreasing proteolysis, increasing serum growth hormone and insulin-like growth factor 1 concentrations as well as mRNA expression for growth hormone and insulin-like growth factors 1, activation of the mTOR pathway and inhibition of the ubiquitin-proteasome system. Potential mechanisms of action presented suggest that HMB supplementation could influence animal production positively.

Effects of excess dietary leucine

Researchers have found that feeding the branched chain amino acid (BCAA) leucine in excess can have negative effects on animals. Although the branched chain

amino acids (leucine, isoleucine, and valine) are essential amino acids they are also toxic at high concentrations (Harris et al., 2004). The majority of research has been conducted on leucine. Some negative effects of excess dietary leucine can reduce levels of some amino acids in the circulating amino acid pool and reduce growth performance in animals.

All branched chain amino acids share the same regulatory enzyme in their catabolic pathway, α -ketoacid dehydrogenase complex (BCKDC). Activity of this enzyme is under tight control for both conserving and oxidizing all BCAAs (Harris et al., 2004). This is important because a lack of BCKDC activation could lead to an excess of BCAA in the blood leading to neurological dysfunction and brain damage (Harris et al., 2004).

High intakes of leucine in both humans and animals has been shown to decrease the concentrations of the other branch chain amino acids in both blood and muscle (Harper et al., 1984; Wiltafsky et al., 2010). This is believed to be due to the increase in activity of BCKDC from increased leucine. Excess leucine intake increases the activity of the shared enzyme which depletes the plasma and tissue stores of isoleucine and valine as well as their keto-acids (Harper et al., 1984). Similar observations were found when feeding excess leucine to weaned pigs where the increased intake of leucine increased plasma leucine and its keto-acid while decreasing plasma levels of isoleucine and valine and their keto-acids (Wiltafsky et al., 2010). However, branched chain amino acids are not the only amino acids in plasma influenced by extensive leucine. Plasma levels of histidine, threonine, and serine increased linearly; methionine and proline were influenced quadratically; and aspartic acid and glutamine decreased linearly with

increasing levels of leucine in the diet over 100% of requirement. This experiment also showed a reduction in growth performance in the animals when fed excess leucine. In two separate experiments increasing leucine concentrations over what they marked as 100% in the diet linearly decreased daily feed intake and gain (Wiltafsky et al., 2010).

Depressed feed intake in pigs fed excess dietary leucine has been shown in multiple studies (Wiltafsky et al., 2010; Wessels et al., 2016a; Wessels et al., 2016b). Reduction in food intake caused by excess leucine intake is thought to be due to a decrease in hypothalamic serotonin concentration in animals fed high leucine. Plasma leucine was negatively correlated with hypothalamic tryptophan, which is used in serotonin production (Wessels et al., 2016a; Wessels et al., 2016b).

Caution must be taken when supplementing leucine to humans or animals due to the negative effects of excess leucine discussed above. Since HMB and leucine act in similar ways, it is plausible that HMB can be used instead of leucine leaving behind the negative effects that leucine has on the body's amino acid pool and growth performance. At this point in time, it is unknown if high levels HMB has any effects, positive or negative, on the concentrations of plasma branched-chain amino acids.

Previous animal studies supplementing HMB

Swine

Maternal supplementation of HMB before and throughout lactation has yielded mixed results. For example, Nissen and coworkers (1994) found that HMB supplemented sows had elevated fat content in the colostrum whereas Flummer and Theil (2012) did not observe any changes in composition of colostrum but did report an increase in yield of colostrum. Pre-weaning mortality was lower in piglets from sows supplemented with

HMB (Flummer and Theil, 2012). This could potentially be due to the increase in colostrum production allowing all piglets to have a sufficient amount and gain the necessary nutrients and passive immunity from the sows. Over three trials, consistently improved pig weight gains were observed from HMB-fed sows. Feeding 2.0 g HMB/day starting 2 days before parturition through lactation increased weaning weight at day 21 by 7% (Nissen et al., 1994). HMB supplementation to sow diets throughout lactation increased concentration of HMB in milk and skeletal muscle of pigs 28 days after birth (Wan et al., 2017).

Maternal HMB supplementation provides benefits to growth performance of offspring in both pigs and sheep (Tatara et al., 2007). Piglets born from sows supplemented with HMB were significantly heavier at birth and expressed increased daily weight gain allowing them to reach market weight faster than pigs born to unsupplemented sows (Tatara et al., 2007). Offspring from sows supplemented with HMB had a greater body weight and lean percentage at market than did pigs from sows that were not supplemented (Wan et al., 2017). Tatara and others (2012) conducted another study following offspring to market after maternal supplementation during the last two weeks of gestation and supplementation of HMB to offspring pre- and post-weaning. Treatment with HMB reduced fattening time and increased body weight at birth, daily body weight gain, bone weight, volumetric bone mineral density, and geometrical parameters and mechanical endurance of the femur of the offspring (Tatara et al., 2012).

Supplemental HMB also enhances protein synthesis in skeletal muscle of swine neonates. Fetuses were programmed prenatally due to maternal supplementation of HMB in late gestation and had increased liver size (and probably metabolic capacity), improved

immune function, and increased lean tissue gain over pigs whose dams were not supplemented (Flummer et al., 2012). Intrauterine growth retarded piglets fed HMB at 800 mg/kg during days 7 to 28 after birth had a net body weight gain and average daily gain similar to that of normal birth weight piglets fed a control diet (Wan et al., 2016a). Not related to body weight, HMB supplementation markedly increased the cross-sectional area of type II muscle fibers and the mRNA expression of mTOR, IGF-1, and myosin heavy chain isoform IIB in the *longissimus dorsi* muscle of piglets (Wan et al., 2016a). These findings demonstrate that HMB supplementation during the early postnatal period could improve skeletal muscle growth and maturity by accelerating fast-twitch glycolytic fiber development in pigs.

Poultry

β -hydroxy- β -methylbutyrate has also been studied in poultry. A study was conducted using Arbor Acres broilers with three dietary treatments supplemented with HMB-Ca (0, 0.05%, 0.1%) during the starter (1-21 days) and grower (22-42 days) phases. At day 21, birds receiving 0.1% HMB-Ca had more breast muscle yield, less abdominal fat than the control, and a higher dressing percentage than birds fed the control or 0.05% HMB-Ca (Qiao et al., 2013). At day 42 of age, 0.1% HMB-Ca increased breast muscle yield than the control and decreased abdominal fat compared with the control or 0.05% HMB groups (Qiao et al., 2013). Overall dietary treatment of HMB-Ca did not affect serum growth hormone or insulin in any of the treatments. This study suggests that dietary supplementation of HMB-Ca improved growth performance, stimulated breast muscle development, and decreased abdominal fat deposition in broiler chickens.

Ruminants

Steers supplemented with HMB beginning 82 days before market expressed improved daily weight gain, feed intake, and feed efficiency when slaughtered at 105 days on feed (Van Koevering et al., 1994). However, a second group marketed at 147 days had an overall poorer performance than the earlier marketed group. The reason behind this reduction was that this group had a lower overall performance prior to supplementing HMB (Van Koevering et al., 1994). The steers from the HMB group in this study also had leaner carcasses than the un-supplemented steers (Van Koevering et al., 1994).

In animal production, HMB has potential to be a promising tool. β -hydroxy- β -methylbutyrate can increase birth weights and market weight in swine. β -hydroxy- β -methylbutyrate has also been shown to produce leaner carcasses in both swine and ruminants. More research is needed to fully understand all the effects that HMB may have when supplemented to animals and determine a range of doses that are most effective in each species.

Mouse model for study of litter bearing species

Sow studies can be a challenge to conduct due to their long gestation length, large space requirements, and high expense. Utilizing another litter bearing species as a model for sows could be beneficial. Mice are a litter bearing species that also exhibit intrauterine growth retardation. A colony of genetically identical mice housed in a controlled environment on the University of Minnesota's St. Paul Campus have a within-litter birth weight coefficient of variation of 10% (Clarke et al., unpublished). This is only

slightly lower than the 15% to 25% that has been reported in sows (Wu et al., 2010; Quesnel et al., 2014).

Lifespan and housing

The most common mouse used in a research setting is the C57Bl/6j strain also known as Black 6. These mice have an average lifespan of two years. The average mature body size for this strain is around 25 grams (Bachmanov et al., 2002). On average, adult mice can drink up to 5 ml of water per day and eat between 4 and 5 grams of chow per day. Feed intake increases depending on the physiological state of the animal and ranges from 5 grams per day, for maintenance, to up to 20 grams per day during lactation (Speakman, 2008).

Mice are social animals, so they are typically housed in groups but can be housed individually under special circumstances. Under group housing situations in a standard size cage, females of the same litter are housed in the same cage with up to 5 adult mice per cage. Males from the same litter are housed together with up to 4 adults per cage. Males housed with non-littermate males will fight so this is avoided by housing one litter of males per cage.

Reproduction

Mice reach sexual maturity between 4 and 6 weeks of age. However, typical breeding age is 6 to 8 and 8 to 10 weeks of age for females and males, respectively. Breeding exhaustion for mice is typically 5 to 6 litters over a span of 8 to 12 months. Mice can be mated individually (one male and female per cage), in trios (one male with two females) or as a harem (one male with three or more females). Timed mating can be accomplished by observing presence of a copulatory plug, but usually pregnancy can be

visually determined by abdominal distension by 12 to 14 days into gestation and by a noticeable increase in dam body weight.

The average gestation length for a mouse ranges between 18 and 22 days. Mice of the C57/Bl6 strain have an average gestation length of approximately 19.2 days (Murray et al., 2010). Litter sizes in the mouse are 4 to 12 pups with an average of 6 pups per litter. Lactation for a mouse can last for 4 weeks. Normally, mouse pups are weaned from the dam at 21 days of age but can be weaned at 28 days of age if the pups are deemed too small to survive on their own at 21 days of age. Average weaning weight for Black 6 mice is approximately 9 grams for both males and females. Mice weighing under 7 grams at weaning are less likely to survive than if they weighed over 7 grams. These small pups are weaned at 28 days to increase their body weight (personal observations).

Comparison of placental development between mice and swine

Mouse

The first part of placental development after fertilization is decidualization (Malassiné et al., 2003). In the mouse, this process is induced by implantation. An interesting fact about the mouse placenta is that it is actually not fully formed until halfway through gestation. The mouse placenta initially possesses a chorioviteline structure but changes into a chorioallantoic structure at 11.5 days of gestation (Malassiné et al., 2003). By 12.5 days of gestation, the definitive chorioallantoic placenta is clearly subdivided into: i) layer of maternal decidua, ii) junctional zone, and iii) labyrinth zone, which can be seen below in Figure 2.3.

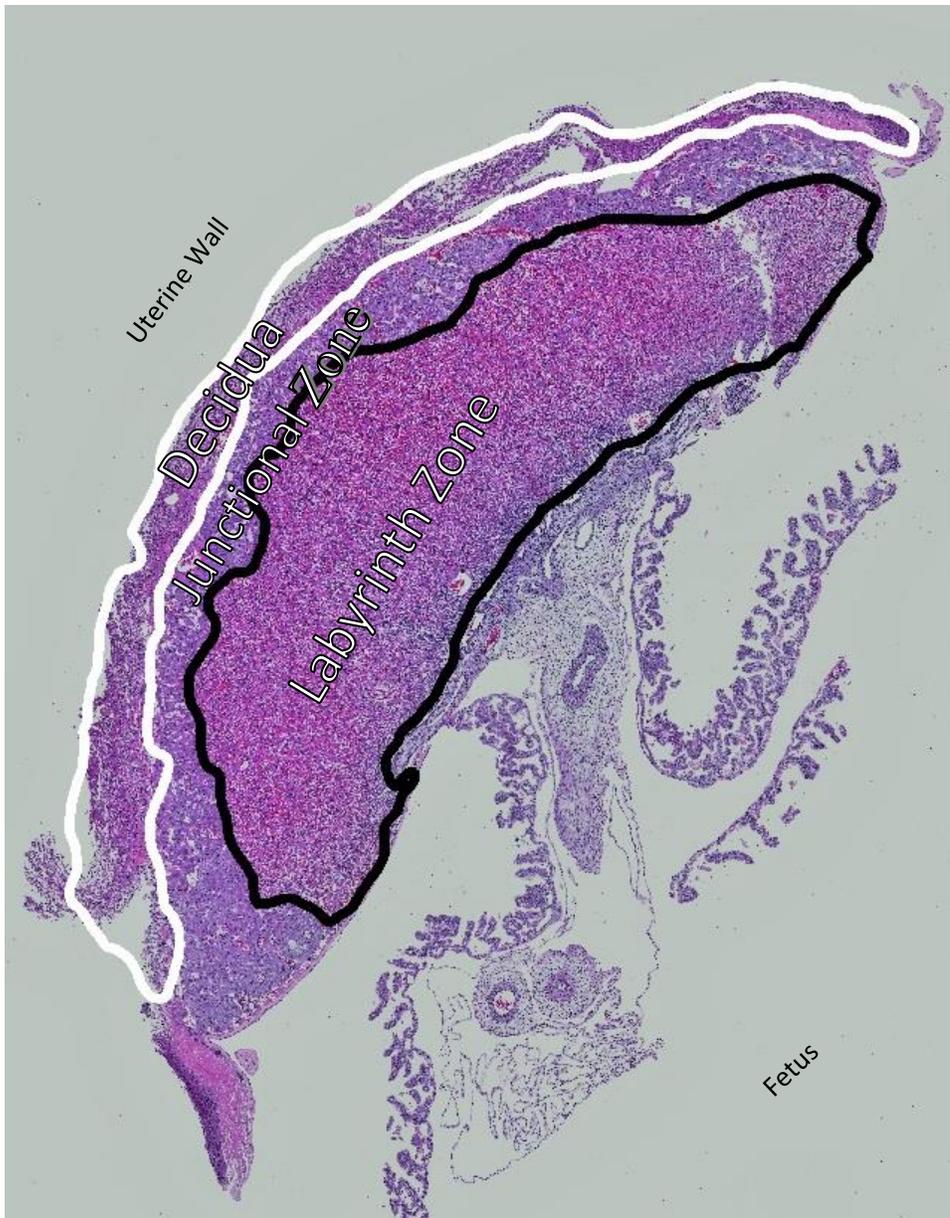


Figure 2.3. Histological section of mouse placenta documenting the three main layers. Photo credit Clarke, 2019.

The mouse labyrinth structure allows countercurrent exchanges between maternal and fetal capillaries arranged in parallel to each other (Malassiné et al., 2003). In the labyrinth, the trophoblast begins to differentiate into three layers; 2 syncytiotrophoblast layers in contact with the fetal endothelium, and only one cytotrophoblast in contact with maternal blood. The maternal blood then enters the labyrinth and bathes the fetal

trophoblast, allowing exchanges with fetal blood. A labyrinth placenta is the most structurally elaborate type and is found in rodents. In these species, the chorion is penetrated by a web-like arrangement of channels (Hafez, 2017).

Swine

The placental type of swine is diffuse. A diffuse placenta is when maternal and fetal tissues interdigitate over the entire surface of contact. This is also known as a folded type placenta which is the simplest form that describes the geometrical pattern of the maternal and fetal tissues (Hafez, 2017). Another type of classification of the porcine placenta is epitheliochorial. The epitheliochorial type is the most superficial placenta, lacking significant invasion of the uterine tissues. Pigs possess a variant form of placental circulatory system termed a crosscurrent system (Figure 2.4). The crosscurrent system is an intermediate with respect to the efficiency of exchange of nutrients and gases between an exclusively concurrent (not documented in any mammal to date) and exclusively countercurrent arrangement (guinea pig). Concurrent systems are the least efficient for nutrient transfer and countercurrent systems are the most efficient and ideal for nutrient transfer (Ahokas and McKinney, 2008).

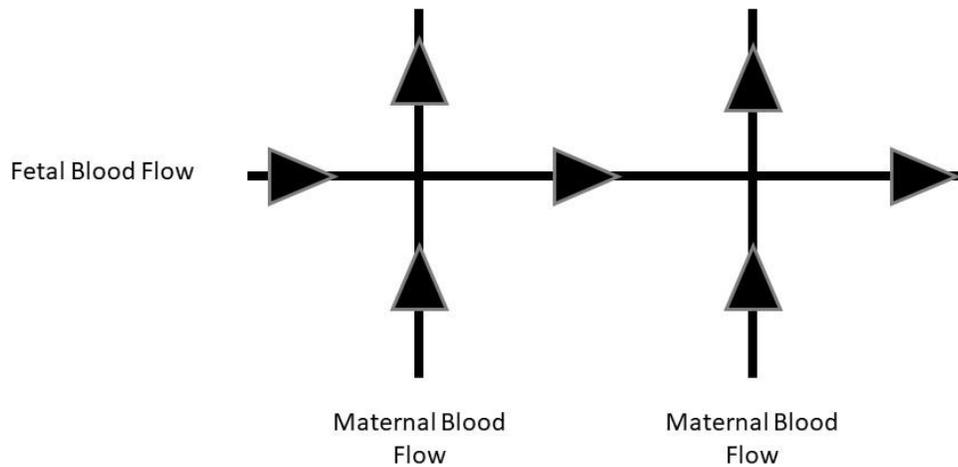


Figure 2.4. Nutrient exchange flow in swine placenta, Crosscurrent system. Diagram adapted from Leiser R. and P. Kaufmann, 1994.

Sow studies can be difficult to perform for multiple reasons leading to the benefit of finding a cost-effective model for the sow. Mice are litter bearing species that are also affected by intrauterine growth retardation and varying birth weights. The gestation length of a mouse is much shorter at 19-21 days compared to approximately 115 days for the sow. Other benefits of mice are that they can be housed in a relatively small space, are inexpensive, and have the same issue of variation in birth weights even when genetically identical and housed in a controlled environment. When using the mouse as a model for the pig some precautions need to be taken when considering dosing between the two species. To ensure correct dosing, allometric scaling of the doses should be performed.

Allometric scaling

Allometry is the study of size and its consequences. The scaling of biologically functional systems can be studied and described mathematically by using allometric equations (Sharma and McNeill, 2009). Allometric scaling is a tool used to extrapolate doses of medication or nutritional supplements among species. Obviously, an elephant is not the same size as a mouse and therefore they need different doses of a drug to elicit the same effects. To understand allometry, it helps to understand the relationship between the metabolic rate of an animal and its size. This relationship is known as Kleiber's Law. Kleiber's Law was developed in 1932 and states that the metabolic rate for all organisms follows exactly the $\frac{3}{4}$ power-law of body mass. Although the $\frac{3}{4}$ -power law is valid, as research continues to be conducted and technology improves, researchers are finding that this idea is not exactly as straight forward as it appeared when first discovered in the 1930's (Glazier, 2005).

There are multiple ways, other than using metabolic body size, that dosing using scaling can be determined and there is much debate about which method is best. For drug dosing there is the "dose by factor" method which applies an exponent for body surface area (Equation 1).

Equation 1:

$$\text{HED}^* (\text{mg/kg}) = \text{Animal NOAEL}^\dagger (\text{mg/kg}) \times (\text{weight}_{\text{animal}} [\text{kg}] / \text{weight}_{\text{human}} [\text{kg}])^{1-0.67\#}$$

*Human equivalent dose

†No observed adverse effect level

#Range of superscripts that can be used for this equation

Equation taken from Nair and Jacob (2016)

Equation 1 above is based on body weight of the test animals and humans. Basing doses directly by body weight however has resulted in gross over or underestimations of doses. For example, the direct extrapolation of a dose established in mice or rats to an adult human may actually be wrong by a factor of 10 or more (Rucker and Storms, 2002). To address this inaccuracy, correction factors (K_m) were calculated and equation 1 was updated to equation 2 with K_m factors located in Tables 2.1 and 2.2.

Equation 2:

$$\text{HED (mg/kg)} = \text{Animal NOAEL (mg/kg)} \times (\text{animal } K_m / \text{human } K_m)$$

Equation taken from Nair and Jacob (2016).

A third equation (Equation 3) can be used to calculate the animal equivalent dose (AED*) from human doses. This equation uses a K_m ratio which is the human K_m divided by the animal K_m and vice versa. Values of the K_m ratios are also denoted in Tables 2.1 and 2.2.

Equation 3:

$$\text{AED}^* \text{ (mg/kg)} = \text{Human dose (mg/kg)} \times K_m \text{ ratio}$$

*Animal Equivalent Dose

Equation taken from Nair and Jacob (2016).

Table 2.1. Human equivalent dose (HED) calculation based on body surface area.

Species	Reference body weight, kg	Working weight range, kg	Body surface area, m ²	K _m value ¹	K _m Ratio ²	K _m Ratio ³
Human	60	-	1.62	37	-	-
Mouse	0.02	0.011-0.034	0.007	3	12.3	0.081
Hamster	0.08	0.047-0.157	0.016	5	7.4	0.135
Rat	0.15	0.08-0.27	0.025	6	6.2	0.162
Ferret	0.30	0.16-0.54	0.043	7	5.3	0.189
Guinea pig	0.40	0.208-0.700	0.05	8	4.6	0.216
Rabbit	1.8	0.90-3.0	0.15	12	3.1	0.324
Dog	10	5-17	0.50	20	1.8	0.541
Monkey	3	1.4-4.9	0.25	12	3.1	0.324
Marmoset	0.35	0.14-0.72	0.06	6	6.2	0.162
Squirrel monkey	0.60	0.29-0.97	0.09	7	5.3	0.189
Baboon	12	7-23	0.60	20	1.8	0.541
Micro pig	20	10-33	0.74	27	1.4	0.730
Mini pig	40	25-64	1.14	35	1.1	0.946

Table adapted from Nair and Jacob (2016)

¹ Convert dose in mg/kg to dose in mg/m², multiply by K_m

² Convert animal dose in mg/kg to HED in mg/kg by dividing animal dose by K_m ratio

³ Convert animal dose in mg/kg to HED in mg/kg by multiplying animal dose by K_m ratio

Table 2.2. Animal equivalent dose (AED) calculation based on body surface area.

Species	Reference body weight, kg	K _m Value ¹	K _m Ratio ²	K _m Ratio ³
Human	60	37	-	-
Mouse	0.02	3	12.3	0.081
Hamster	0.08	5	7.4	0.135
Rat	0.15	6	6.2	0.162
Ferret	0.30	7	5.3	0.189
Guinea pig	0.40	8	4.6	0.216
Rabbit	1.8	12	3.1	0.324
Dog	10	20	1.8	0.541
Monkey	3	12	3.1	0.324
Marmoset	0.35	6	6.2	0.162
Squirrel monkey	0.60	7	5.3	0.189
Baboon	12	20	1.8	0.541
Micro pig	12	27	1.4	0.730
Mini pig	40	35	1.1	0.946

Table adapted from Nair and Jacob (2016)

¹ Convert dose in mg/kg to dose in mg/m², multiply by K_m

² Convert human dose in mg/kg to AED in mg/kg by multiplying animal dose by K_m ratio

³ Convert human dose in mg/kg to AED in mg/kg by dividing animal dose by K_m ratio

Another way to extrapolate a dose required for a given response between species is through feed intake. Most of the time this is done on the basis of kcal of energy ingested. This process is commonly used and is very straight forward. For example, to calculate the necessary dose using this method, one needs to know the energy concentration of the diet and the amount of diet consumed on a daily basis. Then, a simple calculation reveals the dose based on the kcal of energy ingested. This method allows one to use a swine trial to extrapolate an effective dose to a mouse trial or vice versa even though mice are very different in size from pigs. This method of scaling doses allows researchers to use more cost-effective animal models such as mice to evaluate concepts on products intended for larger, more expensive animals.

Different approaches may be taken to scale doses among different species.

Kleiber's Law or the $\frac{3}{4}$ Power Law was the first approach founded which focuses on metabolic body weight. The "dose by factor" approach is similar to Kleiber's Law where they use body surface area but instead of using body weight, correction factors known as K_m values were developed. A third approach is to calculate doses by the amount of calories the animal consumes. This route the simplest and most straightforward approach to allometric scaling.

Summary

An intervention is needed to reduce the number of low birth weight and IUGR pigs in modern swine production. Low birth weight piglets are becoming an increasingly important issue with the selection of highly prolific sows and larger litter sizes. Low birth weight piglets (< 1 kg) have a very low likelihood of surviving to weaning. If these piglets do survive past weaning, they usually are slow growing and have poor meat quality compared with their normal birth weight littermates. This lower efficiency in the animal depresses production efficiency and increases cost of production for producers.

Mice are a litter bearing species that are affected by IUGR, have short gestation lengths, small stature, and are cost effective. Genetically identical mice housed in environmentally controlled rooms still express IUGR and birth weight variation is still seen. Rats, which have the same placenta as mice, when supplemented with arginine during gestation had the same outcomes, of increased litter size and weight, that are recorded in sows supplemented with the same thing, even with differences in placentae of swine and rodents (i.e. attachment sites and nutrient exchange mechanisms). It is these

reasons we believe the mouse can be used as a model for the sow as long as the dose is scaled and calculated correctly due to their differences in size to ensure valid results.

A nutritional solution may be an effective way to improve the variation in litters and reduce the number of low birth weight pigs. Researchers have previously investigated different feeding practices (bump feeding) or supplements (arginine, glutamine) that can be fed to sows to improve their reproductive performance and increase birth weights of piglets. However, this research has yielded contradictory results and has not investigated within-litter weight variation. β -hydroxy- β -methylbutyrate is an option to increase birth weight due to its positive influences on protein synthesis, protein degradation, and hormone production. β -hydroxy- β -methylbutyrate has the potential to affect both fetal and placental development.

Chapter 3

Evaluation of beta-hydroxy-beta-methylbutyrate (HMB) supplementation to mouse dams in gestation on offspring birth weight and growth variation

Abstract

The objective of this study was to determine if supplementation of mouse dams with β -hydroxy- β -methylbutyrate (**HMB**) calcium salt throughout gestation would improve pup birth weight uniformity and improve growth performance of offspring. Data were collected from litters of 56 mouse dams and their offspring. Dams were assigned to one of 4 treatments; control (**CON**; n = 13), Low Level HMB (**LL**; n = 14), High Level HMB (**HL**; n = 15), and Low Level Pulse fed from gestational days 6 to 10 (**PUL**; n = 14). A randomly selected subset of 27 dams was euthanized on gestational day 18 to collect placentae and pup weights. The remaining dams gave birth and lactated for 28 days. Offspring were reared until 8 weeks of age. All mice were fed a corn-soy diet, with HMB supplementation provided only to dams during gestation. Supplementation of HMB had no effects on overall gestation and lactation performance of mouse dams. Dietary treatment during gestation did not affect total number of pups born per litter, birth weight of pups, or number pups weaned per litter. Variation, expressed as standard deviation and coefficient of variation, was not different among treatments for birth weight or weaning weight. Range of body weights within litter at birth, weaning, and 8 weeks of age were not affected by HMB supplementation. No differences were observed in placental weights and size of labyrinth area of the placenta due to dietary treatments. Placental efficiency was reduced in the placentae of the LL dams ($P < 0.05$) compared with CON. Overall, offspring growth performance measures such as average daily gain, average

daily feed intake and gain to feed were similar among all treatments. Offspring body weight at 5 and 8 weeks of age; however, was lower for offspring from the HL dams compared to offspring from LL dams ($P < 0.05$). Body composition of offspring at 5 and 8 weeks of age was similar regardless of dam HMB supplementation during gestation. In conclusion, dietary HMB supplementation of mouse dams during gestation had no effect on offspring birth weight, variation in birth weight, or growth performance of offspring.

Key Words: birth weight variation, growth performance, HMB, mouse

Introduction

Genetic selection of swine over the years has led to an increase in litter size. Over the past 10 years, total pigs born per litter has increased by about 2 pigs/litter from 12.3 pigs in 2007 to 13.9 pigs in 2017 (Stalder, 2013; Stalder 2018). During this same period of time, number of pigs weaned per litter has increased, but not as dramatically, from 9.5 pigs in 2007 to 10.3 pigs in 2017 (Stalder, 2013; Stalder, 2018). One of the reasons for this discrepancy is that there has also been an increase in pre-weaning mortality from approximately 14% to 18% in that 10-year span (Stalder, 2013; Stalder 2018). This increase in pre-weaning mortality could be a result of increased incidence of intrauterine growth retardation and low birth weight pigs. Intrauterine growth retardation (IUGR) is defined as the impaired growth and development of mammalian embryos and fetuses or its organs during pregnancy (Wu et al., 2006). As litter size increases, available nutrients for each fetus decrease due to increased fetal competition. This competition causes suboptimal fetal development and as a consequence, lower birthweight and higher within-litter birthweight variation (Campos et al., 2012). Fetal growth restriction, *in utero*, can cause permanent negative effects on neonatal survival, post-natal growth (Quiniou et al., 2002) and feed efficiency (Powell and Aberle, 1980), body composition of offspring (Hegarty and Allen, 1978; Powell and Aberle, 1980), meat quality of offspring (Gondret et al., 2005), and long-term health of pigs (Rooke and Bland, 2002; Le Dividich et al., 2005). Intrauterine growth retardation will remain a significant problem to the animal industry because there is incomplete knowledge of possible solutions for producers to employ (Wu et al., 2006).

Fetal growth depends on placental growth and efficiency which are directly related to the functional efficiency of delivering nutrients and oxygen to fetuses (Salafia et al., 2005). Placental efficiency is influenced by the weight or size of placentae, contact surface area with maternal endometrium, placental blood flow, and the ability of the placenta to transfer nutrients to fetuses (Ashworth, 2013).

Suboptimal maternal nutrition during gestation leads to inability to provide the correct amount of nutrients in support of metabolic demands of both sows and fetuses (Campos et al., 2012). Fetal growth requires accretion of protein, and when protein is low in the maternal diet, fetal weight, and ultimately birth weight can be affected. Therefore, researchers are beginning to focus on the use of amino acids or their metabolites as a potential dietary intervention to reduce the incidence of IUGR or low birth weight in swine. Leucine is an essential amino acid, and its metabolite, β -hydroxy- β -methyl butyrate (HMB), could potentially reduce incidence of IUGR and low birth weight pigs. In both *in vitro* and *in vivo* studies with rats, leucine stimulated muscle synthesis (Anthony et al., 2002; Bolster et al., 2004) and inhibited proteolysis in muscle *in vitro* (Buse and Reid, 1975). Similarly, β -hydroxy- β -methylbutyrate also enhanced skeletal muscle development and reduced proteolysis (Nissen and Abumrad, 1997; Slater and Jenkins, 2000; Wheatley et al., 2014), which suggests potential mechanisms of how HMB might reduce the effects of IUGR and incidence of low birth weight pigs.

Researchers have studied HMB supplementation of sows with various endpoints but none have focused on HMB use in gestation diets to increase uniformity of birth weight of pigs within litter. Maternal HMB supplementation has improved growth performance of offspring in both pigs and lambs (Tatara et al., 2007). Piglets born from

sows supplemented with HMB were heavier at birth and had increased daily weight gain allowing them to reach market weight faster than pigs farrowed by sows fed un-supplemented diets (Tatara et al., 2007). Others found that sows fed diets supplemented with HMB from 3 to 4 days before parturition through lactation had increased milk fat content and heavier piglet weights at weaning, compared to piglets from non-supplemented sows (Szcześniak et al., 2014). Supplemental HMB also enhanced protein synthesis in skeletal muscle of swine neonates (Wheatley et al., 2014).

Unfortunately, sow studies are difficult to conduct due to length of time, high cost, space available and large number of animals needed. Therefore, finding a suitable model for sows is helpful. Mice are litter bearing species that are also affected by intrauterine growth retardation (Wu et al., 2006). Preliminary data from the University of Minnesota showed a 10% coefficient of variation (CV) for birth weight within litter of genetically identical mice housed in a controlled environment. The within-litter CV for birth weight of pigs can range from 15% to 25% (Wu et al., 2010; Quesnel et al., 2014). Therefore, we hypothesized that the mouse could be a reasonable model for the sow to evaluate effects of dietary HMB on birth weight variation and growth performance of offspring.

Materials and Methods

The experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Animals and Experimental Design

This experiment was conducted in a Research Animal Resources Facility on the St. Paul campus of the University of Minnesota. Fifty-six post-pubertal, virgin, wild type

Agouti A^{vy} (93% C57bl/6 background, isogenic; Weinhouse et al., 2014) dams were used to conduct this experiment. Animals were assigned randomly to one of four treatments: Control (CON; n = 13), Low Level HMB (LL; n = 14), High Level HMB (HL; n = 15), and Pulse (PUL; n = 14). Dams assigned to the PUL treatment received the CON diet until day 6 of gestation when they were switched to the LL diet until day 10 of gestation then were switched back to the CON diet for the remainder of gestation. Dams in the PUL treatment were assumed to be pregnant until pregnancy was confirmed. Days 6 to 10 of gestation were used for the PUL treatment because formation of the labyrinth structure of the placenta occurs during this period in the mouse (Cross et al., 1994). The labyrinth structure is the location of nutrient transfer between dam and fetus in the mouse. In each treatment, half of the dams were euthanized on gestational day 18 to collect each pup and its associated placentae. All dams were housed individually throughout the experiment in standard 28 cm x 18 cm x 13 cm plastic cages with corn cob bedding and shredded paper for nesting material. Dams were provided *ad libitum* access to feed and water. During breeding, individual males were placed in cages with each female and were removed after 24 hours of exposure. Pregnancy was confirmed 14 days after male exposure based on increased body weight of dams. If dams were not pregnant, they were housed with a male again for another 24 hours. Dam body weight was collected weekly through the 3-week gestation period and subsequent 4-week lactation period. Feed disappearance was recorded weekly for all dams.

Diet and Treatments

Sires and dams received a diet based on corn and soybean meal before breeding. The diet was modeled after a sow lactation diet that is commonly used in the commercial

swine industry. Diets were blended offsite as a mash (Tables 3.1 and 3.2) and pelleted in the lab following standard laboratory procedures developed in Dr. Faulk's laboratory. In brief, 1 kg of diet was mixed with 1,600 mL of water to obtain a thick consistency. The diet-water mixture was then pelleted by using the food grinder and sausage stuffer kit for a KitchenAid mixer (Pro 600™ Series 6 Quart Bowl-Lift Stand Mixer, Model No. KP26M1XER) and placed on baking trays. Trays were then placed in a drying oven for approximately 12 hours at 26-32°C. Nutrient concentrations of the experimental diet compared to NRC nutrient requirements for mice are shown in Table 3.3. β -Hydroxy- β -methylbutyrate calcium salt (Ca-HMB; Hefei TNJ Chemical Industry Co., Ltd., Hefei, China) was mixed into the CON diet before pelleting. Concentration of HMB in experimental diets was determined by Eurofins Microbiology Laboratories, Inc. (Des Moines, IA) using an internal HPLC method for analysis.

At male exposure, diets fed to dams were switched to the experimental gestation diet that was assigned randomly. Dams designated for CON continued to receive the CON diet, but dams assigned to the LL and HL treatments were switched to a diet containing 3.5 or 35 mg HMB/g diet, respectively. Doses of HMB used in this experiment were extrapolated using allometric scaling from the dose used for sows by Tatara et al. (2007). Briefly, scaling for the low dose was determined by calculating the amount of HMB sows consumed per kcal NE in the study by Tatara et al. (2007). For the LL treatment, 4.15 mg Ca-HMB (85%) per gram diet was supplemented to provide 1.4 mg HMB/kcal NE which equalled the calculated dose used by Tatara et al. (2007). Supplementation for HL treatment was 10 times that of the LL treatment. Doses were based on energy intake instead of body weight because that scaling by energy intake

yields a more accurate dose than if calculated by body weight. Doses calculated by body weight can be wrong by a factor of 10 or more (Rucker and Storms, 2002).

Litter Performance

Individual birth weight of pups and total litter weight were recorded within 24 hours of birth. Following birth, litters were weighed weekly. Body weight was also recorded for pups recovered from dams that were euthanized on day 18 of gestation (one day before expected parturition).

Growth Performance of Offspring

At weaning, all pups were ear notched individually for identification and sexed. All pups were weaned and fed the CON diet and remained on this diet until 8 weeks of age. Weaned pups were caged by litter and sex. Individual body weight and feed disappearance for each cage were recorded weekly.

Body Composition

At five and eight weeks of age, all pups were transferred to the University of Minnesota Phenotyping Core (Minneapolis, MN) for measurement of body composition using magnetic resonance imaging (Echo MRI, Echo Medical System; Taicher et al., 2003). Body weight was recorded at time of scanning. Fat mass, lean mass, and total water of live pups were determined.

Histology

On gestational day 18, 28 dams were euthanized using CO₂ inhalation to excise uterine horns and collect placental tissues and fetuses. Tissue samples were immediately placed in a 10% formalin solution for no more than 24 hours to allow tissues to set. After 24 hours, all tissue samples were rinsed with a PBS solution, transferred to a 70% ethanol

solution, and stored at 4°C until submitted for staining. Tissue samples were embedded in paraffin using standard histological techniques, sectioned at a thickness of 4 µm, and stained with Hemotoxylin and eosin (H&E), which was performed at the Comparative Pathology Shared Resource, a core lab of the Masonic Cancer Center at the University of Minnesota (Minneapolis, MN). Stained slides were evaluated by light microscopy using an Olympus BX53 Microscope (Center Valley, NJ) at 4X power. The CellSense imaging software (Olympus, Center Valley, NJ) was used to outline the total labyrinth area. Area of the labyrinth was determined in two separate tissue sections for each placenta. The average measurement of two sections was recorded for the area of the labyrinth. Measurements were completed by the same individual that was blinded to treatments to reduce variation. This analysis was completed on 208 placentae collected.

Statistical Analysis

For all analyses, the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) was used. Breeding group was included in all the following statistical models as a random effect. Breeding group was designated as the contemporary group of dams that were successfully mated during a given 2-week breeding period. Results are reported as least squares means, and comparisons among treatments were performed using the PDIFF option of SAS with the Tukey-Kramer adjustment for multiple comparisons. Treatment effects were considered significant if $P < 0.05$ and a trend if $0.1 > P \geq 0.05$.

Dam and litter performance data were analyzed as a completely randomized design. Individual dam served as the experimental unit. The statistical model for dam and litter performance included dietary treatment as a fixed effect.

To further evaluate birth weight, birth weight categories were used to analyze individual birth weights of pups. The two heaviest and two lightest birth weights per litter were designated to either the high or low birth weight category, respectively. Birth weight, placental weight, placental efficiency (ratio of fetal weight:placental weight), and labyrinth area were analyzed using a statistical model that included fixed effects of dietary treatment, birth weight category, and their interaction. A second statistical model was used to analyze these same traits except that birth weight category was nested within dietary treatment.

To investigate differences in variation of birth weight, weaning weight, and body weight of offspring at 8 weeks of age, standard deviations of each litter, ranges (difference between lightest and heaviest birth weight), and the differences between the median and the lightest birth weight from each litter were calculated and compared among treatments. This analysis was repeated with litters that contained 8 pups or greater. Litters of 8 pups or more were considered to be large litters because they contained at least two more pups than the mean litter size for this mouse strain (Verley et al., 1967; Johnston et al., 2007).

Repeated measures analysis was used to determine effects of dam treatment during gestation on offspring performance and body composition from weaning to eight weeks of age. The statistical model for offspring performance and body composition included time (weeks), dietary treatment of dams, sex and the interactions of all three factors.

Results

Dam Performance in Gestation and Lactation

Dietary treatment had no effect on overall gestation or lactation performance of dams (Table 3.4). There was no effect of HMB on body weight during the first two weeks of gestation (Table 3.5); however, in the third week of gestation, body weight of dams assigned to PUL tended to be greater than body weight of dams assigned to HL ($P < 0.10$). Dietary treatment had no effect on dam ADG and ADFI. An interaction ($P < 0.01$) was found between treatment and stage of gestation for ADFI of dams. There were no differences in ADFI among treatments in the first two weeks of gestation but in the third week, dams assigned to HL had a lower ADFI than LL and PUL dams ($P < 0.01$). A decrease in ADFI was observed from week 2 to week 3 of gestation in dams assigned to HL while all other dams increased ADFI during this time (Figure 3.1). During the 4-week lactation period there were no differences among dietary treatments for body weight, ADG, and ADFI and no significant interactions among dietary treatments and stage of lactation (Table 3.6).

Litter Performance

There were no effects of dietary treatment during gestation on total number born alive per litter, number weaned per litter, average pup birth weight, litter birth weight or litter weight at weaning (Table 3.7). Supplementation of HMB to dams during gestation had no effect on placental weight or labyrinth area (Table 3.8). Placental efficiency, however, was lower ($P < 0.05$) in LL dams compared to CON dams while dams assigned to PUL and CON treatments tended to have a higher placental efficiency than the LL and HL dams ($P < 0.10$).

No differences were observed among treatments within birth weight categories for weight (combined fetal and birth), placental efficiency, or labyrinth area (Table 3.9). Placental weights in the low weight category tended to be higher in PUL dams compared to CON dams ($P < 0.10$). Similar results were observed when using a statistical model that nested birth weight category within dietary treatment (Table 3.10).

Statistical analysis of diet effects on birth weight and placental traits were repeated with uterine horn included in the model (Table 3.11). Weights (combined fetal and birth) of offspring from LL and HL dams were lower than weights from dams of CON and PUL treatments in the right uterine horn only ($P < 0.05$). Placental weights and labyrinth area were similar among treatments within each uterine horn. There was a tendency for placental efficiency in the right uterine horn of PUL and CON dams to be greater than LL dams ($P < 0.10$). Similar results were observed when the statistical analysis nested uterine horn within treatment (Table 3.12).

Pups born from HL dams that completed term had a lower birth weight than pups from CON dams ($P < 0.01$; Table 3.13). Pups retrieved from euthanized dams of the PUL group had greater weight than the pups of HL- and LL-supplemented dams ($P < 0.01$).

Variance in birth weight within litter expressed as either CV or SD was not affected by dietary treatment of dams during gestation (Table 3.14). Variance in weaning weight among litters expressed either as CV or SD was not affected by dam treatment during gestation. Ranges, calculated by the difference between the heaviest and lightest pup in each litter at birth and weaning, were not different among treatments. However, at 8 weeks of age, offspring of LL dams had a lower range in body weight per litter than offspring of HL dams ($P < 0.05$). The difference between the median weight of each litter

and the lightest pup of that litter, noted as lower half, were similar among treatments at birth, weaning, and 8 weeks of age. Measurements of variance including within litter birth weight CV, within litter wean weight CV, and values of SD, range, and lower half for birth weight, pup weaning weight, and body weight at 8 weeks of age in litters containing 8 or more pups were not influenced by dietary treatments (Table 3.15).

Growth Performance of Offspring

Dietary treatments fed to dams during gestation had no effects on overall performance of offspring (Table 3.16). In weeks 1 to 4 post-weaning, offspring from LL dams had a greater body weight than offspring of HL dams ($P < 0.05$; Table 3.17). There was no effect of dietary treatments during the 4 weeks post-weaning on average daily gain (ADG); however, there was a significant treatment by week post-weaning interaction ($P = 0.02$). During the first week post-weaning, offspring from PUL dams had greater ADG than those from all other treatments, but this advantage was lost during the second week when PUL offspring had a lower ADG than both CON and HL offspring. During the 4 weeks post-weaning, a dietary treatment trend ($P = 0.10$) and significant effect of time ($P < 0.01$) were observed for gain to feed ratio (G:F). Offspring from HL and PUL dams displayed a greater G:F than LL offspring during the first week post-weaning. However, during week two, G:F decreased for PUL offspring and was lower than that of HL offspring. There were no differences in G:F among treatments during weeks 3 or 4 post-weaning.

Males and females had similar body weights at weaning. However, in weeks 1 to 4 post-weaning, males had heavier body weights than females ($P < 0.05$; Table 3.18). Throughout the 4-week growth period post-weaning, males had greater ADG than female

offspring ($P < 0.01$). Average daily feed intake was similar between males and females in the first week post-weaning but became greater in males in weeks 2 to 4 ($P < 0.05$).

During weeks 1 and 3, male offspring expressed a greater G:F than female offspring ($P < 0.05$). There was no significant interaction between sex, dam dietary treatment and week post-weaning for body weight, ADG, ADFI, or G:F (Table 3.19).

Body Composition

Offspring from LL dams were significantly heavier at the 5-week body scan than offspring from CON and HL dams. At this time, CON offspring were also heavier than HL offspring ($P < 0.05$; Table 3.20). At the 8 week body scan, LL offspring continued to be heavier than those from all other dietary treatments ($P < 0.05$). Lean mass percentage at 5 weeks of age was greater in HL offspring than LL and PUL ($P < 0.05$). At 8 weeks of age, lean mass percentage in HL continued to be greater than LL offspring ($P < 0.05$). No differences were observed in fat mass at 5 weeks of age. However, at 8 weeks of age, offspring from LL group had a higher fat mass percentage than offspring from PUL dams ($P < 0.05$). Dam dietary treatment did not affect total water percentage of offspring at 5 weeks of age. At 8 weeks, offspring of PUL dams had a higher total water percentage than offspring of LL dams ($P < 0.01$). At both body scans, males had a heavier body weight than females ($P < 0.01$; Table 3.21). There were no sex differences in lean mass percentage at either body scan. Females had a greater fat mass percentage than males at the 5-week body scan; however, these results were reversed at the 8-week body scan ($P < 0.02$). No differences were observed in total water percentage between males and females at either body scan. No significant interaction among sex, dam dietary treatment and

week of body composition scan for body weight, lean mass percentage, fat mass percentage, and total water percentage was observed (Table 3.22).

Discussion

A comparison between the mouse and sow can be found in Table 3.23. These animals are both litter bearing species that are both affected by variation in litter size. The strain of mice that were used in this experiment have a within-litter birth weight variation of 10%. This is lower than the range that has been recorded in sows of 15% to 25% (Wu et al., 2010; Quesnel et al., 2014). The range for sows is considerably larger than the mouse, however the range of variation for the sow contains genetic variation, environmental variation, and inherent variation whereas the variation reported in the mouse strain is only the inherent variation due because the mice are genetically identical and were housed in a tightly controlled environment.

No adverse effects on sow performance during gestation, lactation, or subsequent reproductive cycles have been reported when supplementing HMB to sows during late gestation and lactation (Nissen et al., 1994). In the current study, the lack of a positive or negative response to dietary HMB supplementation on mouse dam performance during gestation and lactation observed in the present study was expected.

In the third week of gestation, we noted a decrease in feed intake of dams in the HL group. It is possible that the high HMB inclusion created an aversion to the diet that caused the decline in feed intake. However, if an aversion to a high concentration of HMB existed, we would have expected to see the decline in feed intake when diets were first introduced and not after 2 weeks of consumption. Baxter et al. (2005) fed rats 5% dietary HMB for 90 days and reported that 5% inclusion in the diet was the no-observed-

adverse-effect level. Therefore, we would not expect the HL dose at 4.3% of the diet to decrease feed intake. However, the rats used by Baxter et al. (2005) were not pregnant or lactating. As a result, there may have been an interaction between the high concentration of dietary HMB and pregnancy in the present experiment that resulted in depressed feed intake. Furthermore, body weights of the mouse dams were not different among dietary treatments during gestation until the third week when dams fed HL had a lower body weight. This reduction in body weight was most likely caused by the reduction in feed intake observed for HL dams during this same time period.

Birth weight of pups was not affected by maternal HMB supplementation during gestation. These findings are in agreement with previous research conducted in swine (Nissen et al., 1994; Flummer and Theil, 2012; Flummer et al., 2012). However, other studies have reported an increase in birth weight from maternal HMB supplementation in pigs (Tatara et al., 2007; Tatara et al., 2012; Wan et al., 2016b). Mixed results of HMB on birth weight may be due to the varying lengths of feeding HMB supplemented diets. Swine studies with positive results began supplementation two weeks before farrowing (Tatara et al., 2007; Tatara et al., 2012) or even as early as day 35 of gestation through farrowing (Wan et al., 2016b). In previous swine trials where no differences in birth weight were observed, dietary HMB supplementation was provided only 3 to 10 days before farrowing (Nissen et al. 1994; Flummer et al. 2012), leading to the thought that supplementation occurred too late in gestation for any effect on birth weight to be observed. In the current study, supplementation was either constant throughout the entire gestation period or only during a critical time in placental development. Therefore, we expected to observe a positive effect of maternal HMB supplementation on pup weight at

the end of gestation. No previous studies in mice have been published where mouse dams were supplemented with HMB during gestation to observe birth weight of pups.

Another potential reason for mixed results in previous trials may have been due to the wide range of doses for Ca-HMB that have been fed. In the studies where HMB supplementation had no effect on birth weight, sows were supplemented with 2 to 3 g HMB/day. This range of doses equates to 0.10 to 0.14% of the total diet. This level of supplementation is considerably lower than the low dose used in the current study. The swine studies showing positive results supplemented 4 grams per sow per day (Wan et al., 2016b) or about 7 grams of HMB per sow per day (Tatara et al., 2007; Tatara et al., 2012) which is 0.19% and 0.33% of the diets, respectively. In studies published previously, concentrations of HMB in the Ca-HMB products used were not reported. It is difficult to know the exact amount of HMB supplemented in the previous studies because concentrations of Ca-HMB products range from 80% HMB to 99% HMB. The product used in our study had a HMB concentration of 85%. This could have led to a lower dose of HMB than what has been supplemented in previous studies. This could also be a reason why no response to HMB supplementation was observed in the current study. With our product at 85% HMB, the LL dose in the present study provided 0.35% HMB in the diet. The 0.19% and 0.33% listed above is the percentage of total product that was used and not necessarily the amount of HMB that was supplemented. It is difficult to compare this dose to the doses used previously because no HMB concentrations of products were reported. Therefore, in the studies where positive results were reported, HMB levels could have differed from 0.19 or 0.33% of the diet that it was believed to be.

Supplementation of dam diets with HMB had no effect on within-litter variation of birth weight recorded as both standard deviation of birth weights and within-litter birth weight coefficient of variation (CV). These results are in agreement with the observations of Wan et al. (2016b). We hypothesized that HMB would increase uniformity of within-litter pup birth weights. β -hydroxy- β -methylbutyrate can increase endogenous levels of growth hormone (Tatara et al., 2007; Tatara et al., 2012). When providing sows with exogenous growth hormone, fetal and placental weights increased (Sterle et al., 1995). Growth hormone has also been shown to selectively improve the uterine environment for smaller pigs *in utero* by influencing placental nutrient transfer and placental growth (Rehfeldt et al., 2001). Supplementing HMB from day 35 of gestation through parturition reduced the percentage of piglets born weighing less than 1 kg body weight compared to unsupplemented sows (Wan et al., 2016b). β -hydroxy- β -methylbutyrate can increase the activity of the GH/IGF-1 axis (Tatara et al., 2007; Kornasio et al., 2009; Gerlinger-Romero et al., 2011; Tatara et al., 2012). If growth hormone selectively improves growth conditions for smaller piglets *in utero*, then this may be a potential mechanism to reduce the incidence of low birth weight pigs from sows fed diets supplemented with HMB. For these reasons, we hypothesized that HMB could potentially reduce the within-litter variation in pup weight at birth. However, we did not observe this response, which could potentially be due to using a non-efficacious dose of our HMB product. We did not measure growth hormone in this present study so it is unknown if dietary supplementation at the doses provided had any effect on endogenous growth hormone levels in the mice.

In the current study, birth weight was higher than fetal weight recorded on gestational day 18. Weights differed between gestational day 18 and birth at gestational day 19 by about 25-30%. We believe that this increase in weight between days is due to gestational gain during the last day of gestation and intake of colostrum after birth because birth weights were recorded after pups had suckled. However, all measurements of birth weight variation evaluated were not influenced by treatment no matter if they were from litters born on day 19 or litters removed from the dam at gestational day 18.

It is unknown if HMB can be transferred across the placenta to the fetus. However, if HMB does not cross the placenta and act directly on the fetus it may still have an indirect effect. One indirect effect would be to influence placental development by increasing the labyrinth area or placental weight. In the current study, we did not find any differences in placental development with HMB supplementation. Although placental development, labyrinth area, and placental weight, were not affected, this does not mean that placental function, such as increasing nutrient transporters in the placenta, could not have been influenced. The increase in the activity of GH/IGF-1 axis, caused by HMB has potential to affect placental function because an increase of IGF-1 expression increases amino acid transporters on the placenta (Vaughan et al., 2017). An increase in placental amino acid transporters allows more amino acids to be transferred to the fetus potentially leading to greater fetal growth. Tatara et al. (2012) observed increased circulating glutamine, glycine, valine, and tyrosine in newborn piglets from HMB-supplemented sows. In the current study, the crude measurement of placental efficiency (fetal weight:placental weight ratio) was not improved in HMB-supplemented dams compared with CON dams. However, this does not mean that placental function was not affected.

Placental amino acid transporters and nutrient flux were not measured in this study.

Future research may explain any potential effects of HMB on placental function such as increasing placental nutrient transporters.

Weaning weights of offspring were not different among treatments in this study. Previously, researchers noted that pigs from dams supplemented with HMB during late gestation through lactation had heavier weaning weights even if there were not differences in birth weights (Nissen et al., 1994). The increase in weaning weight with HMB supplementation may be a result of an increase in milk fat percentage in HMB supplemented sows (Nissen et al., 1994; Flummer and Theil, 2012; Wan et al., 2016b). The increase in milk fat could have increased the energy intake of the offspring leading to advanced growth over the offspring from un-supplemented sows that had a lower milk fat percentage. The mouse offspring in the present study may not have had different weaning weights because all dams were fed the same basal diet during lactation with no HMB supplemented.

No differences in overall growth performance measures, ADG, ADFI, or G/F, among treatments were observed. In the present study, body weight of the mice at 8 weeks of age was greatest in the LL group, but was not different among all other treatments. This response is different from the results observed by Wan et al. (2017) where pigs from sows supplemented with HMB during lactation displayed a greater weight at market, increased ADG, and a tendency for decreased F/G compared to pigs from unsupplemented sows. Other researchers have included HMB in diets of meat animals in the growing and finishing phases. In steers, supplementing HMB in diets fed 82 days before market, increased ADG and feed intake and improved feed efficiency

when steers were slaughtered at 105 days of age (Van Koevering et al., 1994). In broiler chickens, supplemental HMB at 0.1% increased ADG compared to birds on a control diet (Qiao et al., 2013). In pigs, offspring from sows fed HMB were heavier at slaughter than the control pigs (Wan et al., 2017). Broiler chickens fed diets supplemented with HMB also had an increase in final body weight (Qiao et al., 2013). In these studies, animals were fed HMB during the growth period. In our study, the offspring did not receive HMB after birth and therefore, this may be a reason why we did not see the same results in our experiment.

One of the mechanisms of action of HMB is increased protein synthesis and reduced proteolysis (Ostaszewski et al., 2000; Holecek et al. 2009; Pimentel et al., 2011; Wheatley et al., 2014; Wan et al., 2016a). Body composition of the mouse offspring was not altered by maternal HMB supplementation in this experiment. This is different than what researchers have previously reported in other species. Pigs from sows fed HMB had a higher lean meat percentage at slaughter than the pigs from control sows (Tatara et al., 2007; Wan et al., 2017). In broiler chickens, increased breast yield and reduced abdominal fat were observed at both 21 and 42 days of age with HMB supplementation (Qiao et al., 2013). In steers, less subcutaneous fat and improved marbling scores were observed when feeding HMB (Van Koevering et al., 1994). In all of these previous studies, with the exception of Tatara et al., 2007, animals received HMB supplementation during the growth period. The mice in the current study were not fed HMB after birth, which may be a reason why no effects on body composition were observed.

Prior to conducting this study, we believed that the mouse dam would be a suitable model for the sow. Mice are susceptible to IUGR and variation in birth weights,

even when they are genetically identical and housed under tightly controlled, constant environments. There are differences in placentae between the mouse and the sow. Mice have a discoidal placenta with a labyrinth system for maternal and fetal nutrient exchange, while the sow has a diffuse placenta and a cross-current system for maternal and fetal nutrient exchange. Even though they have different placental structures, the placentae are affected in a similar manner after GH/IGF-1 intervention. In both placental types, GH/IGF-1 increases the placental gene expression of Slc38a2, a gene for a neutral amino acid transporter in the tissue (Sferruzzi-Perri et al., 2007a, b; Tung et al., 2012). Therefore, the mouse dam may be a suitable model for the sow. However, more research needs to be conducted to confirm this model by determining nutrient uptake transporters of both placental types and if both placental types are affected similarly by different interventions.

In this study, we may have not observed the anticipated results because the HMB dose may not have been adequate to observe potential responses, but none of the previous studies reported concentrations of the Ca-HMB product that was being used to serve as a reference. Therefore, we could have over- or underestimated the amount of HMB that those animals were receiving when calculating our dose of HMB. Another potential reason is that it is unknown if HMB can be passed through the placenta to allow HMB to directly affect the offspring *in utero*.

In conclusion, maternal diet HMB supplementation had no effects on mouse dam performance in gestation or lactation, pup birth weight, variation in weight at birth and weaning, overall growth performance, or body composition of offspring. These results are contrary to those in previous animal studies where β -hydroxy- β -methylbutyrate

improved birth weight, as well as offspring growth performance and carcass composition when supplemented during the growing period. Concentrations of Ca-HMB used in previous research needs to be confirmed to evaluate if proper dosing was applied in this experiment or if a different dose will give different results. Researchers also need to determine if HMB can be transferred through the placenta or only through milk to act directly on the offspring, or if HMB has an indirect effect on offspring growth by altering placental development during gestation.

Table 3.1. Diet Composition, as fed %

Nutrient	Diet		
	CON	LL	HL
Corn, Ground	63.19	62.92	60.47
Soybean meal, 46.5% CP	29.30	29.17	28.04
Mineral Mix ¹	3.50	3.48	3.35
Vitamin Mix ²	1.00	1.00	0.96
Calcium carbonate	0.27	0.27	0.26
Choline chloride	0.20	0.20	0.19
Soy Oil	2.55	2.54	2.44
Ca-HMB ³ , 85% HMB	0.00	0.43	4.30

¹Contained the following ingredients per kg of premix: calcium carbonate, 357 g; potassium phosphate, monobasic, 196 g; potassium citrate, H₂O, 70.78 g; sodium chloride, 74 g; potassium sulfate, 46.6 g; magnesium oxide, 24 g; ferric citrate, U. S. P., 6.06 g; zinc carbonate, 1.65 g; manganous carbonate, 0.63 g; cupric carbonate, 0.3 g; potassium iodate, 0.01 g; sodium selenite, 10.25 mg; ammonium paramolybdate, .4 H₂O, 7.95 mg; sodium metasilicate, .9 H₂O, 1.45 g; chromium potassium sulfate, .12 H₂O, 0.275 g; lithium chloride, 17.40 mg; boric acid, 81.50 mg; sodium fluoride, 63.50 mg; nickel carbonate, 31.80 mg; ammonium vanadate, 6.60 mg.

²Contained the following ingredients per kg of premix: niacin, 3.0 g; calcium pantothenate, 1.60 g; pyridoxine HCl, 0.70 g; thiamine HCl, 0.60 g; riboflavin, 0.60 g; folic acid, 0.20 g; biotin, 0.02 g; vitamin E acetate, 7500 IU; vitamin B₁₂, 0.1%, 2.50 g; vitamin A palmitate, 400,000 IU; vitamin D₃, 100,000 IU; vitamin K₁, 75 mg.

³Sourced from Hefei TNJ Chemical Industry Co.,Ltd., Hefei, China. HMB was mixed with control diet before pelleting.

Table 3.2. Calculated nutrient composition of experimental diets (as fed)

Nutrient	Diet		
	CON	LL	HL
Dry matter, %	87.87	87.50	84.21
NE, kcal/kg	2,492.30	2481.60	2385.10
Crude protein, %	18.00	17.92	17.23
Crude fat, %	5.00	4.98	4.89
Amino acids:			
Lys, total %	1.05	1.04	1.00
Thr, total %	0.69	0.68	0.66
Met + Cys, total %	0.57	0.56	0.54
Trp, total %	0.24	0.24	0.23
Ile, total %	0.81	0.80	0.77
Val, total %	0.90	0.90	0.87
Arg, total %	1.15	1.14	1.10
His, total %	0.48	0.48	0.46
Leu, total %	1.59	1.59	1.53
Phe + Tyr, total %	1.16	1.15	1.10
Minerals:			
Calcium, total %	0.76	0.82	1.42
Phosphorus, total %	0.54	0.54	0.52
Sodium, %	0.12	0.12	0.12
Chlorine, %	0.19	0.19	0.18
Magnesium, %	0.18	0.18	0.18
Potassium, %	1.17	1.16	1.12
Copper, mg/kg	12.00	12.00	12.00
Iron, mg/kg	101.00	100.00	97.00
Manganese, mg/kg	25.00	25.00	24.00
Zinc, mg/kg	57.00	57.00	54.00
Vitamins:			
A, IU/kg	5,079	5,076	4,874
D, IU/kg	1,001	1,001	961
E, IU/kg	89	89	86
K, mg/kg	0.75	0.75	0.72
Riboflavin, mg/kg	7.46	7.45	7.15
Niacin, mg/kg	49.71	49.62	47.66
Pantothenic acid, mg/kg	16.00	15.95	15.73
Choline, mg/kg	2,530.05	2,523.46	2,414.32
Biotin, mg/kg	0.34	0.34	0.33
B-12, mg/kg	0.02	0.02	0.02
Folic acid, mg/kg	2.28	2.28	2.28
Pyridoxine, mg/kg	6.99	6.99	6.99

Thiamin, mg/kg	8.14	8.13	8.14
HMB, mg/g	0.00	3.50	35.00
<hr/>			
Analyzed nutrients:			
HMB, mg/g	0.00	6.12	36.80
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Table 3.3. Comparison of experimental diet to nutrient requirements of mice (as fed)

Nutrient	Diet	
	CON	NRC ¹
Dry matter, %	87.87	-
NE, kcal/kg	2,492.30	-
Crude protein, %	18.00	18.00
Crude fat, %	5.00	5.00
Amino acids:		
Lys, total %	1.05	0.40
Thr, total %	0.69	0.40
Met + Cys, total %	0.57	0.50
Trp, total %	0.24	0.10
Ile, total %	0.81	0.40
Val, total %	0.90	0.50
Arg, total %	1.15	0.30
His, total %	0.48	0.20
Leu, total %	1.59	0.70
Phe + Tyr, total %	1.16	0.76
Minerals:		
Calcium, total %	0.76	0.50
Phosphorus, total %	0.54	0.30
Sodium, %	0.12	0.05
Chlorine, %	0.19	0.05
Magnesium, %	0.18	0.05
Potassium, %	1.17	0.20
Copper, mg/kg	12.00	6.00
Iron, mg/kg	101.00	35.00
Manganese, mg/kg	25.00	10.00
Zinc, mg/kg	57.00	30.00
Vitamins:		
A, IU/kg	5,079	2,398
D, IU/kg	1,001	998
E, IU/kg	89	31
K, mg/kg	0.75	0.99
Riboflavin, mg/kg	7.46	7.05
Niacin, mg/kg	49.71	14.99
Pantothenic acid, mg/kg	16.00	15.87
Choline, mg/kg	2,530.05	1,999.59
Biotin, mg/kg	0.34	0.20
B-12, mg/kg	0.02	0.009
Folic acid, mg/kg	2.28	0.51
Pyridoxine, mg/kg	6.99	7.94

Thiamin, mg/kg	8.14	5.07
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¹Nutrient Requirements of Laboratory Animals: Fourth Revised Edition, 1995. Washington (DC): National Academies Press (US); 1995. 3, Nutrient Requirements of the Mouse.

Table 3.4. Overall dam performance during gestation and lactation by treatment

Trait	Treatments				SE	<i>P</i> Value
	CON	LL	HL	PUL		
No. of dams	13	14	15	14	-	-
No. of cages	13	14	15	14	-	-
BW at mating, g	20.64	20.04	20.08	20.39	0.49	0.81
BW after birth ¹ , g	25.61	25.41	23.39	24.76	0.83	0.16
BW at weaning ¹ , g	27.58	27.02	26.72	27.14	0.87	0.89
Gestation:						
ADG, g	0.25	0.28	0.16	0.27	0.05	0.21
ADFI, g	4.40	4.27	4.12	4.38	0.28	0.76
Lactation ² :						
ADG ¹ , g	0.05	0.06	0.12	0.09	0.04	0.61
ADFI ¹ , g	9.09	9.15	8.98	10.34	0.74	0.27

¹Number of dams per treatment used in analysis: CON, n = 6; LL, n = 7; HL, n = 8; PUL, n = 7.

²28 day lactation period.

Table 3.5. Effect of dietary treatment on gestation performance of dams

Trait	Treatments				SE	P value	
	CON	LL	HL	PUL		Trt ¹	Trt*Time ²
No. of dams	13	14	15	14	-	-	
Avg. body weight, g					1.32	0.01	0.60
Week 1	21.07	20.53	20.44	21.05			
Week 2	24.55	23.84	22.61	24.82			
Week 3	32.03 ^{xy}	30.99 ^{xy}	29.27 ^x	33.66 ^y			
Avg. daily gain, g					0.12	0.18	0.94
Week 1	0.07	0.03	0.06	0.13			
Week 2	0.50	0.47	0.34	0.54			
Week 3	1.24	1.09	1.07	1.32			
Avg. daily feed intake, g					0.37	0.75	< 0.01
Week 1	4.31	4.16	4.37	3.56			
Week 2	4.07	3.70	4.14	3.61			
Week 3	4.20 ^{ab}	4.70 ^a	3.46 ^b	4.88 ^a			

¹Dietary treatment.

²Dietary treatment by stage of gestation interaction.

^{ab}Means within a row with different superscripts differ ($P < 0.05$).

^{xy}Means within a row with different superscripts differ ($P < 0.10$).

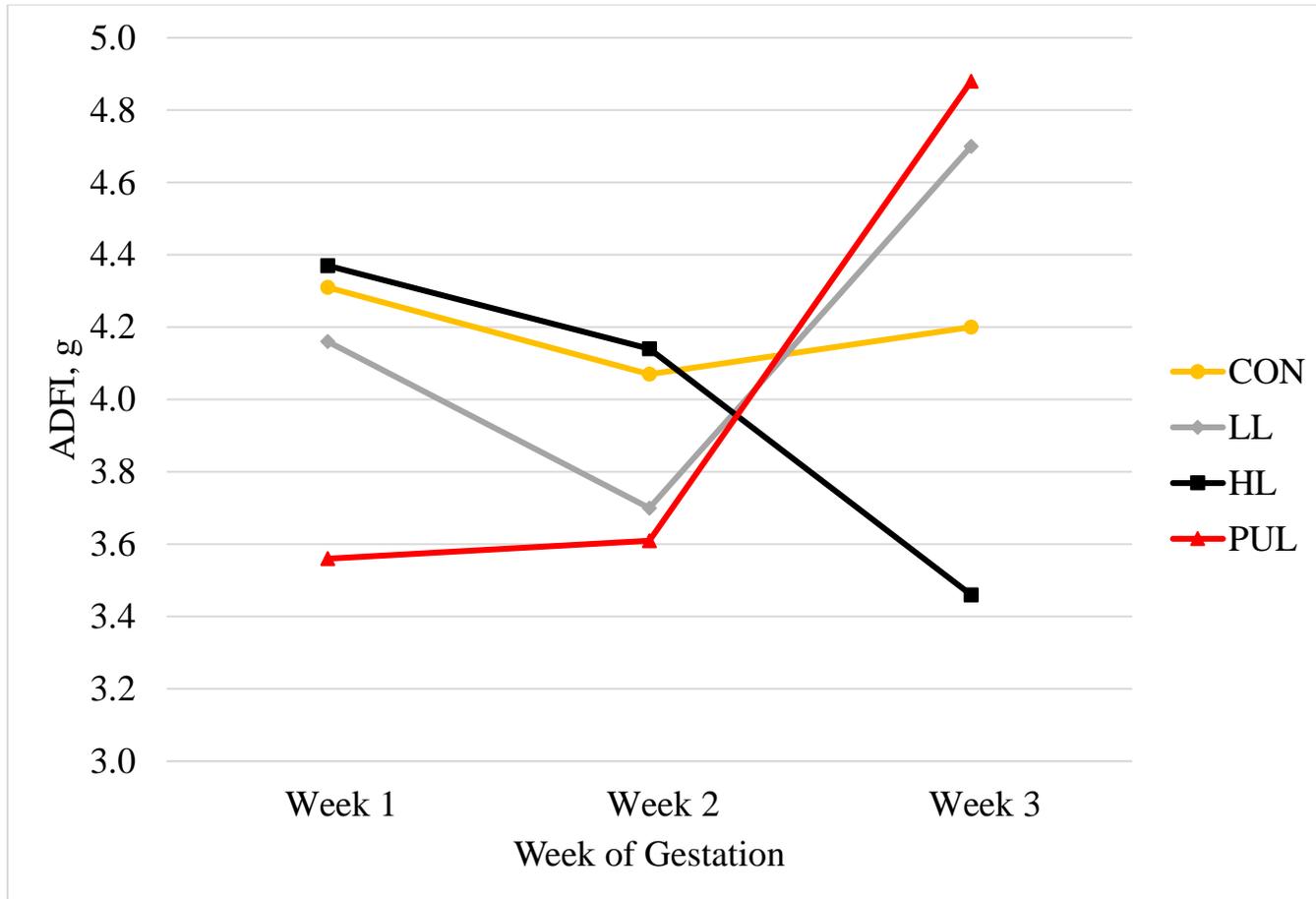


Figure 3.1 Interaction of dietary treatments and stage of gestation for ADFI of dams

Table 3.6. Effect of dietary treatment on lactation performance of dams

Trait	Treatments				SE	<i>P</i> value	
	CON	LL	HL	PUL		Trt ¹	Trt*time ²
No. of dams	6	7	8	7	-	-	-
Avg. body weight, g					1.33	0.12	0.66
Week 1	27.10	28.12	25.23	27.75			
Week 2	29.02	29.22	26.72	28.02			
Week 3	27.44	27.86	27.75	26.28			
Week 4	28.56	29.04	27.97	27.52			
Avg. daily gain, g					0.21	0.54	0.42
Week 1	-0.02	0.33	0.78	0.40			
Week 2	0.28	0.17	0.20	0.03			
Week 3	-0.22	-0.18	0.14	-0.25			
Week 4	0.26	0.30	0.02	0.31			
Avg. daily feed intake, g					1.08	0.44	0.79
Week 1	6.36	7.89	6.90	7.94			
Week 2	8.63	9.88	9.32	9.69			
Week 3	11.45	10.70	11.01	11.30			
Week 4	10.41	11.23	13.16	12.58			

¹Dietary treatment.

²Dietary treatment by stage of gestation interaction.

Table 3.7. Effect of dietary treatment on pre-weaning performance of litters

Trait	Treatments				SE	P value
	CON	LL	HL	PUL		
No. of litters, total	13	14	15	14	-	-
No. of litters, weaned	6	8	8	7	-	-
No. of pups, total	99	102	111	112	-	-
No. of pups, weaned	40	55	61	53	-	-
Total born alive per litter	7.55	7.16	7.51	7.82	0.51	0.71
Number weaned per litter	7.55	6.85	7.34	7.77	0.55	0.42
Avg. pup birth weight, g	1.18	1.16	1.13	1.19	0.07	0.81
Avg. pup wean weight, g	11.11 ^{xy}	11.89 ^x	11.15 ^{xy}	10.44 ^y	0.58	0.11
Litter birth weight, g	8.80	8.42	8.40	9.44	0.59	0.52
Litter wean weight, g	78.40	79.72	82.38	80.34	6.77	0.95

^{xy}Means within a row with different superscripts differ ($P < 0.10$).

Table 3.8. Effect of dietary treatments on placental characteristics

Trait	Treatments				SE	<i>P</i> value
	CON	LL	HL	PUL		
No. of litters	7	6	7	7	-	-
No. of pups	59	43	48	58	-	-
Placental weight, g	0.24	0.24	0.24	0.25	0.008	0.47
Placental efficiency ¹	4.415 ^{a,x}	3.967 ^{b,y}	3.971 ^{ab,y}	4.353 ^{ab,x}	0.20	< 0.01
Labyrinth area, mm ²	5.10	5.17	5.06	5.03	0.16	0.89

¹Placental efficiency = (fetal weight / placental weight).

^{ab}Means within a row with different superscripts differ (*P* < 0.05).

^{xy}Means within a row with different superscripts differ (*P* < 0.10).

Table 3.9. Effect of dietary treatment on fetal weight and placental characteristics across birth weight categories¹

Trait	Treatments				SE	P value	
	CON	LL	HL	PUL		Trt ²	Trt*BirthWtCat ³
No. of litters ⁴	13	14	15	14	-	-	-
Weight ^{4,5} , g					0.07	0.11	0.98
High	1.28	1.28	1.22	1.32			
Low	1.11	1.07	1.03	1.12			
Placental weight ⁶ , g					0.01	0.17	0.13
High	0.259	0.256	0.249	0.258			
Low	0.222 ^y	0.233 ^{xy}	0.234 ^{xy}	0.257 ^x			
Placental efficiency ^{6,7}					0.35	0.22	0.62
High	4.40	4.03	4.19	4.50			
Low	4.32	3.86	3.80	3.90			
Labyrinth area ⁶ , mm ²					0.25	0.65	0.37
High	5.21	4.92	4.95	4.98			
Low	4.87	4.85	5.21	4.87			

¹Birth weight categories were determined by selecting two heaviest and two lightest weights of pups within each litter for the high and low categories, respectively.

²Dietary treatment.

³Interaction of dietary treatment and birth weight category.

⁴Contains birth weights and fetal weights from pups euthanized at gestational day 18.

⁵No. of litters: CON = 13; LL = 14; HL = 15; PUL = 14.

⁶No. of litters: CON = 7; LL = 6; HL = 7; PUL = 7.

⁷Placental efficiency = (fetal weight / placental weight).

^{xy}Means within a row with different superscripts differ ($P < 0.10$).

Table 3.10. Effect of dietary treatment and weight category nested within diet on fetal weight and placental characteristics¹

Trait	Treatments				SE	<i>P</i> value BirthWtCat(trt) ²
	CON	LL	HL	PUL		
No. of litters ³	13	14	15	14	-	-
Weight ^{3,4} , g					0.07	0.98
High	1.28	1.28	1.22	1.32		
Low	1.11	1.07	1.03	1.12		
Placental weight ⁵ , g					0.01	0.13
High	0.259	0.256	0.249	0.258		
Low	0.221 ^y	0.233 ^{xy}	0.234 ^{xy}	0.257 ^x		
Placental efficiency ^{5,6}					0.35	0.62
High	4.40	4.03	4.19	4.50		
Low	4.32	3.86	3.80	3.90		
Labyrinth area ⁵ , mm ²					0.25	0.37
High	5.21	4.92	4.95	4.98		
Low	4.87	4.85	5.21	4.87		

¹Birth weight categories were determined by selecting two heaviest and two lightest weights of pups within each litter for the high and low categories, respectively.

²Weight category nested within diet.

³Contains birth weights and fetal weights from pups euthanized at gestational day 18.

⁴No. of litters: CON = 13; LL = 14; HL = 15; PUL = 14.

⁵No. of litters: CON = 7; LL = 6; HL = 7; PUL = 7.

⁶Placental efficiency = (fetal weight / placental weight).

^{xy}Means within a row with different superscripts differ ($P < 0.10$).

Table 3.11. Effect of dietary treatments and uterine horn on fetal weight and placental characteristics

Traits	Treatments				SE	<i>P</i> value		
	CON	LL	HL	PUL		Trt ²	Horn ³	Trt*Horn ⁴
No. of litters	7	6	7	7	-	-	-	-
No. of mice	59	43	48	58	-	-	-	-
Fetal weight, g					0.04	< 0.01	0.61	0.45
Right	1.02 ^a	0.94 ^b	0.95 ^b	1.08 ^a				
Left	1.01	0.95	0.97	1.02				
Placental weight, g					0.009	0.64	0.01	0.64
Right	0.234	0.243	0.235	0.243				
Left	0.246	0.245	0.253	0.253				
Placental efficiency ¹					0.23	< 0.01	0.03	0.53
Right	4.52 ^x	3.96 ^y	4.07 ^{xy}	4.59 ^x				
Left	4.24	3.97	3.83	4.12				
Labyrinth area, mm ²					0.20	0.82	0.71	0.06
Right	5.20	5.19	5.14	4.79				
Left	4.93	4.77	5.18	2.25				

¹Placental efficiency = (fetal weight / placental weight).

²Dietary treatment.

³Uterine horn pup was removed from.

⁴Interaction of dietary treatment and uterine horn in which pup was removed from.

^{ab}Means within a row with different superscripts differ ($P < 0.05$).

^{xy}Means within a row with different superscripts differ ($P < 0.10$).

Table 3.12. Effect of dietary treatments and uterine horn nested within diet on fetal weight and placental characteristics

Trait	Treatments				SE	<i>P</i> value Horn(trt) ²
	CON	LL	HL	PUL		
No. of litters	7	6	7	7	-	-
No. of mice	59	43	48	58	-	-
Fetal weight, g					0.04	<0.01
Right	1.02 ^{ab}	0.94 ^a	0.95 ^a	1.08 ^b		
Left	1.01	0.95	0.97	1.02		
Placental weight, g					0.009	0.12
Right	0.234	0.243	0.235	0.243		
Left	0.246	0.245	0.253	0.253		
Placental efficiency ¹					0.27	<0.01
Right	4.51 ^x	3.96 ^y	4.07 ^{xy}	4.59 ^x		
Left	4.24	3.98	3.84	4.12		
Labyrinth area, mm ²					0.23	0.33
Right	5.19	5.19	5.14	4.79		
Left	4.93	4.77	5.18	5.25		

¹Placental efficiency = (fetal weight / placental weight).

² Effect of uterine horn nested within dietary treatment.

^{ab}Means within a row with different superscripts differ (*P* < 0.05).

^{xy}Means within a row with different superscripts differ (*P* < 0.10).

Table 3.13. Effect of dietary treatment and measurement day on pup weight

Trait	Treatments				SE	<i>P</i> value		
	CON	LL	HL	PUL		Trt ¹	Born ²	Trt*Born ³
No. pups on D19	40	55	61	53				
No. pups on D18	59	43	48	58				
Weight, g					0.03	< 0.01	< 0.01	0.03
D19	1.37 ^a	1.32 ^{ab}	1.26 ^b	1.33 ^{ab}				
D18	1.01 ^{ab}	0.94 ^{bc}	0.95 ^b	1.05 ^a				

¹Dietary treatment of dam.

²Day of measurement: D19 = within 24 hours after birth; D18 = gestational day 18.

³Interaction between dietary treatment of dam and day of measurement.

^{ab}Means within a row with different superscripts differ ($P < 0.05$).

Table 3.14. Effects of dam dietary treatment on measures of variation in each litter

Trait	Treatments				SE	P value
	CON	LL	HL	PUL		
Within litter birth weight CV ¹ , %	6.91	8.09	7.63	6.93	0.81	0.69
Within litter wean weight CV ² , %	12.97	11.61	10.56	9.90	1.15	0.33
Pup weight ¹ , g						
SD ³	0.080	0.094	0.087	0.084	0.011	0.83
Range ⁴	0.231	0.280	0.241	0.256	0.035	0.77
Lower half ⁵	0.136	0.165	0.142	0.138	0.029	0.87
Wean weight ² , g						
SD	1.52	1.25	1.30	1.02	0.15	0.24
Range	4.10	3.29	3.65	2.90	0.39	0.25
Lower half	2.04	1.63	1.93	1.75	0.27	0.61
Body weight of pups at 8 weeks ² , g						
SD	2.58	1.98	2.60	2.85	0.28	0.18
Range	6.47 ^{ab}	5.04 ^b	6.81 ^{ab}	8.13 ^a	0.70	0.04
Lower half	4.14	2.76	3.23	4.71	0.63	0.15

¹No. of litters: CON = 13; LL = 14; HL = 15; PUL = 14.

²No. of litters: CON = 6; LL = 7; HL = 8; PUL = 7.

³Standard deviation within litter.

⁴The difference between the heaviest and the lightest pup of each litter.

⁵The difference between the median and the lightest pup of each litter.

^{ab}Means within a row with different superscripts differ ($P < 0.05$).

Table 3.15. Effects of dam dietary treatment in gestation on measures of variation in litters with a minimum of 8 pups¹

Trait	Treatments				SE	P value
	CON	LL	HL	PUL		
Within litter birth weight CV ^{2,3} , %	7.12	8.42	8.94	7.21	1.33	0.71
Within litter wean weight CV ³ , %	10.47	9.71	13.30	10.53	1.00	0.13
Pup weight ³ , g						
SD ⁴	0.075	0.101	0.107	0.085	0.016	0.48
Range ⁵	0.229	0.332	0.312	0.279	0.056	0.59
Lower half ⁶	0.133	0.221	0.203	0.130	0.046	0.40
Wean weight ⁴ , g						
SD	1.10	1.07	1.41	1.04	0.11	0.10
Range	2.83	3.14	4.13	2.90	0.36	0.08
Lower half	1.02	1.62	2.03	1.98	0.42	0.23
Body weight of pups at 8 weeks ⁴ , g						
SD	2.27	2.17	2.74	2.62	0.35	0.61
Range	5.51	5.59	7.51	7.36	0.94	0.35
Lower half	3.71	3.80	3.80	4.24	0.66	0.92

¹Litters containing 8 pups or more were used for this analysis.

²Contains birth weights and fetal weights from pups euthanized at gestational day 18.

³No. of litters: CON = 8; LL = 5; HL = 6; PUL = 8.

⁴No. of litters: CON = 2; LL = 3; HL = 5; PUL = 4.

⁵Standard deviation.

⁶The difference between the heaviest and the lightest pup of each litter.

⁷The difference between the median and the lightest pup of each litter.

Table 3.16. Effects of dam gestational dietary treatment on overall post-weaning growth performance of offspring

Trait	Treatments				SE	<i>P</i> value
	CON	LL	HL	PUL		
No. of litters	6	8	8	7	-	-
No. of mice	40	55	61	53	-	-
No. of cages	13	17	20	17	-	-
ADG, g	0.34	0.31	0.32	0.34	0.02	0.12
ADFI, g	2.75	2.73	2.53	2.81	0.17	0.24
G:F	0.12	0.12	0.12	0.12	0.01	0.54

Table 3.17. Effect of dam dietary treatment on post-weaning performance of offspring

Trait	Treatments				SE	P value		
	CON	LL	HL	PUL		Trt ¹	Time ²	Trt*Time ³
No. of litters	6	8	8	7	-	-	-	-
No. of mice	40	55	61	53	-	-	-	-
No. of cages	13	17	20	17	-	-	-	-
Avg. body weight, g					0.50	< 0.01	< 0.01	0.08
Weaning	10.87 ^{ab}	11.37 ^a	11.27 ^a	10.16 ^b				
Week 1	13.37 ^{ab}	14.35 ^a	12.53 ^b	14.06 ^a				
Week 2	17.34 ^{ab}	17.71 ^a	16.74 ^b	17.19 ^{ab}				
Week 3	19.19 ^{ab}	19.61 ^a	18.50 ^b	18.73 ^{ab}				
Week 4	20.65 ^{ab}	20.91 ^a	19.73 ^b	20.24 ^{ab}				
Avg. daily gain, g					0.07	0.94	< 0.01	0.02
Week 1	0.61 ^b	0.68 ^b	0.59 ^b	0.75 ^a				
Week 2	0.62 ^a	0.53 ^{ab}	0.62 ^a	0.47 ^b				
Week 3	0.31	0.32	0.27	0.24				
Week 4	0.21	0.24	0.20	0.24				
Avg. daily feed intake, g					0.16	0.14	< 0.01	0.68
Week 1	2.45	2.51	2.21	2.59				
Week 2	3.25	3.14	3.01	3.11				
Week 3	2.98 ^{ab}	3.32 ^a	2.94 ^b	3.27 ^{ab}				
Week 4	3.39	3.16	3.16	3.18				
G:F					0.04	0.10	< 0.01	0.29
Week 1	0.27 ^{ab}	0.22 ^b	0.34 ^a	0.33 ^a				
Week 2	0.19 ^{ab}	0.16 ^{ab}	0.22 ^a	0.14 ^b				
Week 3	0.12	0.08	0.10	0.06				
Week 4	0.07	0.06	0.07	0.06				

¹Dietary treatment of dam

²Week post-weaning

³Dam dietary treatment by week post-weaning interaction

^{ab}Means within a row with different superscripts differ ($P < 0.05$).

Table 3.18. Effect of sex on post-weaning performance of offspring

Trait	Sex		SE	P value		
	Male	Female		Sex	Time ¹	Sex*Time ²
No. of mice	125	84	-	-	-	-
No. of cages	38	29	-	-	-	-
Avg. body weight, g			0.43	< 0.01	< 0.01	< 0.01
Weaning	11.15	10.69				
Week 1	14.26 ^a	12.89 ^b				
Week 2	18.45 ^a	16.05 ^b				
Week 3	20.89 ^a	17.13 ^b				
Week 4	22.71 ^a	18.06 ^b				
Avg. daily gain, g				< 0.01	< 0.01	0.35
Week 1	0.74 ^a	0.52 ^b	0.07			
Week 2	0.63 ^a	0.49 ^b				
Week 3	0.38 ^a	0.19 ^b				
Week 4	0.28 ^a	0.17 ^b				
Avg. daily feed intake, g			0.13	< 0.01	< 0.01	0.04
Week 1	2.38	2.48				
Week 2	3.27 ^a	2.98 ^b				
Week 3	3.25 ^a	3.00 ^b				
Week 4	3.47 ^a	2.97 ^b				
G:F			0.03	< 0.01	< 0.01	0.25
Week 1	0.34 ^a	0.24 ^b				
Week 2	0.20	0.16				
Week 3	0.12 ^a	0.06 ^b				
Week 4	0.07	0.05				

¹Dietary treatment of dam

²Dam dietary treatment by sex interaction

^{ab}Means within a row with different superscripts differ ($P < 0.05$).

Table 3.19. Interaction of sex, dam dietary treatment during gestation, and weeks post-weaning on offspring growth performance

Trait	Treatment								SE	Trt ¹	Sex	P value		T*S*Time ³
	CON		LL		HL		PUL					Time ²	T*S*Time ³	
	Male	Female	Male	Female	Male	Female	Male	Female						
No. of mice	25	15	37	18	30	31	33	20	-	-	-	-	-	-
No. of cages	7	5	11	6	10	10	10	7	-	-	-	-	-	-
Avg. body weight, g									0.60	< 0.01	< 0.01	< 0.01		0.95
Wean	11.20 ^{ab}	10.55 ^b	11.32 ^{ab}	11.44 ^{ab}	11.97 ^a	10.57 ^b	10.12 ^b	10.20 ^b						
Wk 1	14.28 ^{ab}	12.46 ^{cd}	14.86 ^a	13.84 ^{abc}	13.50 ^{bc}	11.56 ^d	14.43 ^{ab}	13.70 ^{abc}						
Wk 2	18.59 ^a	16.08 ^{cd}	18.65 ^a	16.77 ^{bc}	18.09 ^{ab}	15.39 ^d	18.45 ^a	15.94 ^{cd}						
Wk 3	21.17 ^a	17.21 ^{bc}	21.23 ^a	18.00 ^b	20.44 ^a	16.56 ^c	20.70 ^a	16.75 ^{bc}						
Wk 4	23.05 ^a	18.25 ^{bc}	22.81 ^a	19.00 ^b	21.99 ^a	17.49 ^c	22.98 ^a	17.50 ^{bc}						
Avg. daily gain, g									0.08	0.94	< 0.01	< 0.01		0.79
Wk 1	0.74 ^{ab}	0.48 ^{cd}	0.65 ^{bc}	0.52 ^{cd}	0.75 ^{ab}	0.44 ^d	0.84 ^a	0.66 ^{abc}						
Wk 2	0.67 ^a	0.57 ^{ab}	0.59 ^{ab}	0.47 ^{bc}	0.66 ^a	0.58 ^{ab}	0.60 ^{ab}	0.34 ^c						
Wk 3	0.42 ^a	0.21 ^{bcd}	0.42 ^a	0.23 ^{bcd}	0.34 ^{abc}	0.20 ^{cd}	0.35 ^{ab}	0.14 ^d						
Wk 4	0.26 ^{ab}	0.16 ^{ab}	0.28 ^{ab}	0.19 ^{ab}	0.25 ^{ab}	0.15 ^b	0.34 ^a	0.15 ^b						
Avg. daily feed intake, g									0.21	0.14	< 0.01	< 0.01		0.15
Wk 1	2.22 ^{bc}	2.69 ^{ab}	2.71 ^{ab}	2.31 ^{abc}	2.45 ^{abc}	1.98 ^c	2.21 ^{bc}	2.97 ^a						
Wk 2	3.41 ^a	3.09 ^{ab}	3.18 ^{ab}	3.09 ^{ab}	3.17 ^{ab}	2.85 ^b	3.31 ^{ab}	2.91 ^{ab}						
Wk 3	3.04 ^{ab}	2.92 ^b	3.48 ^a	3.15 ^{ab}	3.09 ^{ab}	2.79 ^b	3.51 ^a	3.02 ^{ab}						
Wk 4	3.54 ^{ad}	3.23 ^{abcd}	3.39 ^{abd}	2.94 ^{abc}	3.42 ^{ad}	2.89 ^{bc}	3.53 ^d	2.83 ^c						
G:F									0.05	0.10	< 0.01	< 0.01		0.74
Wk 1	0.36 ^a	0.17 ^d	0.22 ^{cd}	0.22 ^{cd}	0.35 ^{ab}	0.32 ^{ac}	0.41 ^a	0.25 ^{bcd}						
Wk 2	0.19 ^{ab}	0.18 ^{ab}	0.18 ^{ab}	0.14 ^{ab}	0.23 ^a	0.21 ^a	0.18 ^{ab}	0.10 ^b						
Wk 3	0.16 ^a	0.07 ^{ab}	0.10 ^{ab}	0.06 ^{ab}	0.13 ^{ab}	0.08 ^{ab}	0.09 ^{ab}	0.03 ^b						
Wk 4	0.08	0.06	0.07	0.06	0.08	0.06	0.08	0.04						

¹Dietary treatment of dam

²Week post-weaning

³Interaction of sex, dam dietary treatment during gestation, and weeks post-weaning

^{abcd}Means within a row with different superscripts differ ($P < 0.05$).

Table 3.20. Effect of dam dietary treatment on body composition of offspring

Trait	Treatments				SE	P value		
	CON	LL	HL	PUL		Trt ¹	Time ²	Trt*Time ³
No. of litters	6	8	8	7	-	-	-	-
No. of mice	40	55	61	53	-	-	-	-
No. of cages	13	17	20	17	-	-	-	-
Avg. body weight, g					0.41	< 0.01	< 0.01	0.65
5 weeks	15.21 ^b	15.93 ^a	14.45 ^c	15.42 ^{ab}				
8 weeks	20.38 ^b	21.29 ^a	20.03 ^b	20.45 ^b				
Lean mass, %					0.74	< 0.01	< 0.01	0.45
5 weeks	91.81 ^{ab}	90.79 ^b	92.10 ^a	90.92 ^b				
8 weeks	87.58 ^{ab}	86.86 ^b	88.58 ^a	87.95 ^{ab}				
Fat mass, %					0.31	0.03	0.02	0.44
5 weeks	7.81	7.86	7.34	7.32				
8 weeks	7.91 ^{ab}	8.28 ^a	8.07 ^{ab}	7.52 ^b				
Total water, %					0.74	0.05	< 0.01	0.68
5 weeks	70.21	69.36	70.35	70.26				
8 weeks	65.85 ^{ab}	65.36 ^b	66.43 ^{ab}	67.03 ^a				

¹Dietary treatment of dam

²Week post-weaning

³Dam dietary treatment by week post-weaning interaction

^{ab}Means within a row with different superscripts differ ($P < 0.05$).

Table 3.21. Effect of sex on body composition of offspring

Trait	Sex		SE	Sex	P value	
	Male	Female			Time ¹	Sex*Time ²
No. of mice	125	84	-	-	-	-
No. of cages	38	29	-	-	-	-
Avg. body weight, g			0.24	< 0.01	< 0.01	< 0.01
5 Weeks	15.99 ^a	14.51 ^b				
8 Weeks	22.98 ^a	18.10 ^b				
Lean mass, %			0.68	0.68	< 0.01	0.20
5 Weeks	91.29	91.53				
8 Weeks	87.51	87.98				
Fat mass, %			0.17	0.98	0.02	< 0.01
5 Weeks	7.27 ^b	7.89 ^a				
8 Weeks	8.25 ^a	7.64 ^b				
Total water, %			0.71	0.53	< 0.01	0.63
5 Weeks	70.22	69.87				
8 Weeks	66.19	66.14				

¹Dietary treatment of dam

²Dam dietary treatment by sex interaction

^{ab}Means within a row with different superscripts differ ($P < 0.05$).

Table 3.22. Interaction of sex, dam dietary treatment during gestation, and week of age on body composition of offspring

Trait	Treatment								SE	<i>P</i> value			
	CON		LL		HL		PUL			Trt ¹	Sex	Time ²	T*S*Time ³
	Male	Female	Male	Female	Male	Female	Male	Female					
No. of mice	25	15	37	18	30	31	33	20	-	-	-	-	-
No. of cages	7	5	11	6	10	10	10	7	-	-	-	-	-
Avg. body weight, g									0.51	< 0.01	< 0.01	< 0.01	0.83
5 Wks	15.86 ^{abc}	14.56 ^d	16.55 ^a	15.32 ^{bcd}	15.66 ^{bc}	13.23 ^e	15.91 ^{ab}	14.93 ^{cd}					
8 Wks	22.70 ^a	18.06 ^{bc}	23.47 ^a	19.10 ^b	22.84 ^a	17.22 ^c	22.90 ^a	18.01 ^c					
Lean mass, %									0.84	< 0.01	0.68	< 0.01	0.12
5 Wks	91.75 ^{ab}	91.88 ^{ab}	91.29 ^b	90.29 ^b	91.31 ^b	92.90 ^a	90.80 ^b	91.05 ^b					
8 Wks	87.46 ^{bc}	87.71 ^{abc}	87.52 ^{bc}	86.21 ^c	89.22 ^a	87.94 ^{ab}	87.72 ^{abc}	88.19 ^{ab}					
Fat mass, %									0.44	0.03	0.99	0.02	0.54
5 Wks	7.43 ^{bc}	8.19 ^{ab}	7.35 ^{bc}	8.36 ^a	7.00 ^c	7.68 ^{abc}	7.31 ^{bc}	7.34 ^{bc}					
8 Wks	8.26 ^a	7.57 ^{ab}	8.12 ^a	8.44 ^a	8.21 ^a	7.93 ^a	8.42 ^a	6.62 ^b					
Total water, %									0.91	0.05	0.53	< 0.01	0.19
5 Wks	70.64	69.77	70.03	68.69	70.35	70.35	69.77	70.66					
8 Wks	65.45 ^b	55.25 ^{ab}	65.42 ^b	65.31 ^b	67.36 ^a	65.50 ^b	66.55 ^{ab}	67.51 ^a					

¹Dietary treatment of dam

²Week post-weaning

³ Interaction of sex, dam dietary treatment during gestation, and weeks post-weaning

^{abcd}Means within a row with different superscripts differ (*P* < 0.05).

Table 3.23. Comparisons between the mouse and the sow

Trait	Mouse	Sow
Age of sexual maturity	6-8 weeks of age	160-220 days of age
Length of gestation	19-21 days	113-115 days
Length of lactation	21-28 days	21 days
Litter size	4-12	12-14
Within-litter birth weight CV,%	10% ¹	19% ²
Placental type	Discoid ³	Diffuse ⁴
Nutrient exchange mechanism	Counter-current system ⁵	Cross-current system ⁶
Number of teats	10 (5 pairs)	12-14 (6-7 pairs)

¹Value is inherent variation only, genetically identical mice housed in environmentally controlled rooms.

²Value includes genetic, environmental and inherent variability.

³A placenta where chorionic villi are arranged in a circular plate.

⁴A placenta made up of villi diffusely scattered over almost the whole surface of the chorion.

⁵The maternal and fetal blood flows in opposite directions with vessels parallel to each other and is the most efficient nutrient exchange system.

⁶The maternal and fetal blood vessels cross each other creating one point of contact for nutrient exchange.

Chapter 4

Conclusion

Genetic selection has resulted in an increase in litter size in sows. Increased litter size has also been accompanied with an increase in pre-weaning mortality. The increase in pre-weaning mortality that has been observed on sow farms today can potentially be due to an increase in within-litter birth weight variation by increasing the incidence of low birth weight pigs as a result of intrauterine growth retardation (IUGR). Intrauterine growth retardation has permanent negative effects on animals such as increased pre-weaning mortality, slower growth, reduced feed efficiency, fatter carcasses and poorer meat quality, and impaired long-term health. The increase in IUGR and low birth weight pigs increases production cost compared with their normal birth weight littermates and therefore, reduces the production efficiency of farms.

Many factors can affect birth weight, but the two main factors are placental development and maternal nutrition during gestation. Fetal growth depends on placental growth and efficiency which is directly related to the functional efficiency of the placenta in delivering nutrients and oxygen to the fetuses. Suboptimal maternal nutrition during gestation leads to the inability to provide the correct amount of nutrients. Researchers have evaluated interventions that may increase birth weight, but few have investigated approaches to potentially reduce within-litter birth weight variation. Interventions that have already been researched include exogenous growth hormone administration, a feeding practice known as “bump feeding”, and supplementation of amino acids, such as arginine or glutamine, during several periods of gestation. These interventions have had mixed effects in sows and gilts for increasing litter weight, birth weight, and reducing

variation in birth weight. Administration of growth hormone appeared to improve fetal weight, placental growth, and selectively improved uterine environment for smaller piglets. However, growth hormone administration is not an approved practice in the U.S. swine industry. Therefore, if an intervention can be found that naturally increases endogenous growth hormone, it may be effective in combating the incidence and effects of IUGR and low birth weight pigs.

The leucine metabolite, β -hydroxy- β -methylbutyrate (HMB), has been studied briefly in pigs. Three sow studies reported increases in birth weight with HMB supplementation during various stages of gestation with one report that documented HMB reduced the percentage of low birth weight pigs. β -hydroxy- β -methylbutyrate can increase endogenous levels of growth hormone in animals as well as increase weaning weights, market weights, and improve carcass quality in swine. Similar results have been observed in other species. It was our assumption that HMB acts directly on the fetus, however, it is unknown if HMB can act directly on the fetus by crossing the placenta or indirectly by affecting placental development and function.

The goal of the research presented in this thesis was to determine if HMB could reduce within-litter birth weight variation and improve growth performance in mice. Mice were chosen to serve as a less expensive surrogate model for sows. We found that diet supplementation of HMB to mouse dams during gestation had no effects on birth weights, within-litter birth weight variation, weaning weight, or growth performance and body composition of offspring after weaning. Maternal diet supplementation of HMB during gestation also had no effects on placental weight and labyrinth area but reduced placental efficiency ratio at the low level when fed to dams at 3.5 mg/g diet. More

research is needed to determine if HMB is effective in reducing variation in birth weight and growth performance of offspring in food animals. One of the first areas that needs to be explored is whether HMB crosses the placenta to determine if the observed effects are from a direct response of HMB on the fetus or indirectly by affecting placental development and function. Other areas of research that need to be explored are determining the most effective dose of HMB to administer and during which time points during gestation it should be supplemented to obtain positive results.

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