Sow Nutrition and Piglet Viability

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Declaration of Authorship

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

Selection for highly prolific sows which produce large litter sizes has resulted in an increased number of piglets with low viability which not only show poor pre-weaning growth but display reduced lifetime performance. Over recent years there has been growing interest into the leucine metabolite, β-hydroxy β-methyl butyrate (HMB), with regards to livestock production due to its involvement in protein turnover, lipolysis and immune function. The limited available published studies on the effects of HMB supplementation to sows in gestation suggest possible beneficial effects on litter and piglet performance to weaning as well as on colostrum production. However, the results are inconsistent, the doses and timings of supplementation ambiguous and the replication is low. Therefore, this research set out to determine the effects of supplementing sows with HMB, over the transition period, on litter and piglet performance to weaning and on colostrum production. In addition, this research aimed to establish the optimum dose and duration of HMB supplementation required across the transition period to optimise any beneficial effects. Through three separate feeding trials this research found that HMB supplementation to sows improved the overall pre-weaning performance of piglets. In addition, this work found that supplementing HMB to sows improved immunoglobulin concentrations in colostrum (IgG, IgA and IgM) in a dose-dependent manner. Moreover, HMB supplementation increased sow colostrum yield and improved the colostrum intake of piglets which was reflected in improvements in early piglet growth. From this thesis it can be concluded that HMB supplementation to sows over the transition period improved overall piglet pre-weaning performance and the quality and quantity of colostrum produced. However, there were some inconsistencies between the results of individual trials, therefore more research is needed to establish reasons for this and to help further exploit the beneficial findings that were observed.

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Abbreviations

4E-BP1	4E binding protein 1
AA	Amino acid
ABN	Associated British Nutrition
ADFI	Average daily feed intake
ADG	Average daily gain
AHDB	Agriculture and Horticulture Development Board
ATP	Adenosine triphosphate
BCAA	Branched-chain amino acid
BW	Body weight
CF	Crude fibre
CLA	Conjugated linoleic acid
CP	Crude protein
CV	Coefficient of variation
d	Day (s)
DI	Deionised
DM	Dry matter
DNA	Deoxyribonucleic acid
eIF	Eukaryotic initiation factor
ELISA	Enzyme-linked immunosorbent assay
ESF	Electronic sow feeder
FI	Feed intake
GB	Great Britain
GH	Growth hormone

GLM	General linear model
h	hour (s)
HMB	β-hydroxy β-methyl butyrate
HMB-CoA	β-hydroxy β-methylbutyryl-coenzyme A
HMG-CoA	β-hydroxy β-methylglutaryl-coenzyme A
HRP	Horse radish peroxidase
IGF-1	Insulin-like growth factor 1
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IUGR	Intrauterine growth retardation
KIC	α-Ketoisocaproate
LD	Longissimus dorsi
LDL	Low-density lipoprotein
MC-CoA	β-Methyl crotonyl coenzyme A
MG-CoA	β-Methyl gluconly coenzyme A
mins	Minutes
MJ	Megajoules
mRNA	Messenger RNA
mTOR	Mammalian target of rapamycin
N_2	Nitrogen
NE	Net energy
NEFA	Non-esterified fatty acids
NF-κB	Nuclear-factor κB

NO_X	Nitrogen oxide
NRC	National Research Council
O_2	Oxygen
PIF	Proteolysis-inducing factor
PKC	Protein kinase C
PKR	Protein kinase R
RNA	Ribonucleic acid
S6	Ribosomal protein S6
S6K1	p70-S6 kinase 1
Secs	Seconds
SEM	Standard error of the mean
UK	United Kingdom
VFA	Volatile fatty acid
VS	Versus

1.

General Introduction

1.1. British pig industry

The British pig industry produced approximately 925,000 tonnes of pork in 2018 and is expected to produce 988,000 tonnes by 2021 (AHDB, 2019a). In 2018 over 71 % of the pork produced in the UK was consumed within the UK, the rest was exported to other countries including: Ireland, Germany and China (AHDB, 2019a, AHDB, 2019b). With the demand for pork increasing, the requirement to find ways of producing more pork, more efficiently is of vital importance. Selection for hyper-prolific sows which produce high litter sizes is one way of increasing sow productivity. In the UK alone the average number of piglets born per litter has increased from 12.5 pigs per litter in 2010, to 14.6 pigs per litter in 2018 with the top 10 % of sows producing over 17 pigs per litter (AHDB, 2019c). A result of this drive towards hyper prolificacy is that countries such as Demark can now wean up to 33 piglets per sow per year. However, Great Britain remains behind this figure by over seven piglets, weaning an average of 25.8 piglets per sow per year (AHDB, 2017a).

The effects of selection for hyper-prolific sows have not all been positive. Pre-weaning mortality levels are an ongoing concern for commercial industry, with levels in British herds ~ 12 % (AHDB, 2019c). In addition to this, the increase in litter sizes has come with additional negative side effects such as: increased number of still births, a reduction in birth weight, increased variation in birth weights within a litter (Canario et al., 2007), reduced viability, anoxia (Alonso-Spilsbury et al., 2005) and reduced individual colostrum consumption (Devillers et al., 2011). These factors not only affect piglet pre-weaning growth and survival but they can affect the piglets' overall lifetime performance (Douglas et al., 2013).

As the selection for hyper prolificacy is not expected to fall, there is a real need to find methods of alleviating the negative side effects that have been associated with it. Sow nutrition is one important area of research which may help to do this. Sow nutrition has previously been found to effect foetal growth and survival (Berard and Bee, 2010), number of piglets born alive (Gao et al., 2012), piglet birth weight (Rooke et al., 2001), pre-weaning growth (Mateo et al., 2007) and colostrum and milk production (Fritsche et al., 1993, Corino et al., 2009, Flummer

and Theil, 2012). Providing supplements to the sow's diet may therefore help meet the requirements for growth and survival of her offspring.

1.2. Factors affecting piglet pre-weaning mortality and growth

Approximately 80 % of pre-weaning mortality occurs within the first three days after birth. Whilst a large proportion of this is due to crushing by the sow, the underlying reasons are often starvation and chilling (Marchant et al., 2000, Herpin et al., 2002). Piglets experience a cold stress at birth which reduces their vigour and causes them to display less aggressive nursing behaviours. Therefore they receive less nutrients from colostrum for thermogenesis and less immunoglobulins which makes them susceptible to crushing (Herpin et al., 2002). Whilst attempts have been made to reduce mortality due to crushing, such as the use of farrowing crates, additional factors render certain piglets more susceptible to pre-weaning mortality. Understanding the factors which make some piglets more vulnerable is therefore essential in order to help prevent mortality and promote maximum pre-weaning growth.

1.2.1. Asphyxia

In sows on average 3 - 8 % of the litter is still born (van Rens et al., 2005, Vanderhaeghe et al., 2010). However in hyper-prolific sows the rate is even higher and still births have been found to account for as much as 10 - 15 % of the litter (Herpin et al., 2001, Baxter et al., 2008). A common cause of still births is asphyxia during the parturition process (Alonso-Spilsbury et al., 2005). Asphyxia is a condition caused by a reduction in the oxygen flow through the placenta to the foetus, which can lead to hypoxia and metabolic acidosis, and piglets are highly prone to this during birth (Alonso-Spilsbury et al., 2005). Piglets from larger litters and piglets born later in the birth order are more susceptible to asphyxia as the cumulative effects of successive contractions reduce the oxygen delivered to the new born piglets and increase the risk of damage or rupture to the umbilical cord, as well as increase the risk of early detachment of the placenta as parturition continues (English and Wilkinson, 1982). Prolonged farrowing durations and placental insufficiency are associated with asphyxia and increase the chances of mortality during delivery (Svendsen et al., 1986, as cited by Alonso-Spilsbury et al., 2005). Piglets which suffer from asphyxia but survive parturition often have reduced viability (Herpin et al., 1996); piglets can suffer irreversible brain damage if the umbilical cord breaks five minutes prior to birth

(Alonso-Spilsbury et al., 2005). Piglets with asphyxia have been found to display abnormal suckling, reduced intake of colostrum and limited passive transfer of immunity (Alonso-Spilsbury et al., 2005).

Oxytocin is commonly used in pig production to induce parturition and to reduce farrowing times (Alonso-Spilsbury et al., 2005) by stimulating uterine contractions (Mota-Rojas et al., 2005). However, this has been shown to increase the incidence of still births and the number of piglets born with ruptured umbilical cords, as well as increase the degree of meconium staining (Mota-Rojas et al., 2005) therefore oxytocin should be used with caution.

1.2.2. Piglet birth weight and birth weight variation within a litter

It is well established that litter size is negatively associated with average piglet birth weight (Milligan et al., 2002a). An increase in litter size does not result in the same percentage increase in total litter weight, therefore an increase in litter size often results in a decrease in the average weight of individual piglets (Quiniou et al., 2002). Piglets with lower birth weights have lower viability, growth and survival to weaning. Marchant et al. (2000) found that only 28 % of piglets weighing less than 1.1 kg at birth survived until d 7 post-partum. Tuchscherer et al. (2000) also found that piglets that survived to weaning were heavier than piglets that did not (1.37 vs 1.06 kg). Low birth weight piglets are more likely to get crushed by the sow than piglets with heavier birth weights. Smaller piglets stay closer to the sow; this was proposed to be due to their lower energy reserves and so gain warmth from huddling close to the sow (Theil et al., 2011). Not only do low birth weights impair piglet pre-weaning growth but it can reduce the piglets' performance to slaughter. Gondret et al. (2005) demonstrated that low birth weight piglets (0.8 - 1.1 kg) took an additional 12 d to reach slaughter weight when compared to heavier litter mates. Low birth weight piglets have been found to consume less milk per suckle than heavier piglets (King et al., 1997) and compete less aggressively so have less access to the sow (Gondret et al., 2005).

Low birth weight piglets result from inadequate placental transfer of nutrients during gestation. The placenta is a key organ involved in the growth of the foetus; it supplies oxygen to and removes metabolites from the developing foetus through the umbilical cord (Schneider, 1991). The placenta undergoes major growth and angiogenesis between d 20 and 60 of gestation and by d 70 it has reached its maximum development (Reynolds and Redmer, 2001, Wu et al.,

2005, Wu et al., 2010). Placental angiogenesis is important in order to maximise utero-placental blood flow so that the supply of oxygen and nutrients to the foetus meet the demands for development (Wu et al., 2004, Wu et al., 2010). If placental angiogenesis is insufficient, the supply of oxygen and nutrients to the foetus may be inadequate to meet the demand for growth and therefore lead to reduced development (Wu et al., 2006). If foetal development and growth is severely impaired it can result in intrauterine growth retardation (IUGR); pigs experience the most severe naturally occurring IUGR amongst domestic animals (Wu et al., 2006). Hales et al. (2013) devised a visual scoring system to define the degree to which piglets suffer from IUGR (from normal to severe) based on facial features. Using this scoring method, Amdi et al. (2013) found that piglets suffering with severe IUGR were not only lighter but had reduced colostrum intake compared with piglets that were not affected by IUGR. Not only do piglets with IUGR experience poor growth performance, but they often suffer from intestinal disorders such as necrotizing enterocolitis which impairs intestinal function and can be a major cause of death (Thornbury et al., 1993, Wu et al., 2006). Although birth weight alone cannot determine whether a piglet has suffered with IUGR during foetal development (Amdi et al., 2013), it is often used as a practical indicator for IUGR on farms (Wu et al., 2006).

A common problem in larger litters is a greater variation between the birth weights of individual piglets within the litter (Litten et al., 2003). Increased variation in piglet weights within a litter at birth often results in increased variation in weights at weaning within that litter (Milligan et al., 2002a). This is because large variations in piglet weights within a litter can make it difficult for low birth weight piglets to gain weight as they are at a competitive disadvantage, they are often less vigorous and have to compete with much heavier litter mates to gain access to the sow (Milligan et al., 2002a, Quesnel, 2011). Therefore low birth weight piglets, in particular those from litters with high weight variation, suffer more from reduced colostrum intake (Quesnel, 2011).

Cross-fostering between litters is often carried out to create a more uniform litter and ensure low birth piglets are competing with litter mates of similar size, thus increasing their chances of access to the sow (Milligan et al., 2001a, Milligan et al., 2002a). However, cross-fostering has not always been found to be beneficial (Milligan et al., 2001b, Heim et al., 2012, Huting et al., 2017). Milligan et al.

(2001b) found that cross fostering piglets at birth to minimise birth weight variation within a litter did not improve piglet pre-weaning survival. In addition, Huting et al. (2017) found that whilst cross-fostering to uniform litters improved the pre-weaning performance of low birth weight piglets (≤ 1.25 kg) it reduced the weaning weights of high birth weight piglets (1.50 – 2.00 kg) by ~ 1 kg compared with piglets of similar weights which were reared in non-uniform litters.

1.2.3. Muscle fibres

Postnatal growth of piglets is largely determined by muscle fibres. In pigs, muscle fibre hyperplasia is set by ~ d 90 of gestation (Wigmore and Stickland, 1983). Primary muscle fibres develop first by the fusion of primary myoblasts between d 35 and 55 of gestation (Lefaucheur et al., 1995). Secondary muscle fibres then develop around the primary muscle fibres from foetal myoblasts between d 50 and 90 of gestation (Wigmore and Stickland, 1983, Duxson and Usson, 1989, Dwyer et al., 1994). An additional population of myoblasts, called satellite cells, do not form fibres but stay close to the myofibres. These cells can divide and serve as the source of new myonuclei during postnatal growth (Moss and Leblond, 1971, Rehfeldt et al., 2000). The majority of primary fibres begin to express slow myosin heavy chain by d 75 of gestation and mature into slow twitch muscle fibres (Lefaucheur et al., 1995). The majority of secondary muscle fibres express fast myosin heavy chain and mature into fast twitch muscle fibres (Bee, 2004). The number of primary muscle fibres cannot be influenced by environmental factors; however the number of secondary muscle fibres can be influenced by prenatal factors during foetal development including nutrition (Wigmore and Stickland, 1983, Dwyer et al., 1994). Dwyer et al. (1994) demonstrated that increasing sow feed intake for ~ 30 d timed prior to muscle fibre hyperplasia increased secondary muscle fibre production by 9 - 13 % and this improved the postnatal average daily gain (ADG) from d 70 to d 130. As the total number of muscle fibres are set at birth, postnatal growth of skeletal muscle is mainly influenced through muscle hypertrophy, an increase in the length and girth of muscle fibres. This is joint by the proliferative activity of satellite cells which are the source of new nuclei incorporated into the muscle fibres (Rehfeldt et al., 2000). Therefore lean growth and muscle mass are largely determined by the total number of muscle fibres (Rehfeldt et al., 2000).

Low birth weight piglets have been found to have a lower total number of muscle fibres than higher birth weight litter mates. This has been attributed towards a lower number of secondary muscle fibres which is a result of foetal undernutrition during gestation (Wigmore and Stickland, 1983, Handel and Stickland, 1987, Dwyer et al., 1994, Gondret et al., 2005). The reduced number of muscle fibres in low birth weight piglets restricts their postnatal lean growth. This is because muscle fibres grow in size towards a plateau which is achieved earlier at lower fibre numbers and then available nutrients are preferentially used for fat deposition (Rehfeldt et al., 2000).

1.2.4. Colostrum intake

The piglets' ability to conserve heat when they are first born is very limited; they are born with very little hair or subcutaneous fat and are covered in foetal fluid (Herpin et al., 2002). Piglets are born with stores of glycogen in the liver and the skeletal muscles which provide them with the energy they need during parturition and the immediate period afterwards (Le Dividich et al., 2005). These glycogen stores are released from the liver directly into the blood stream as glucose which can then be used as energy, or they are released from skeletal muscles as lactate into the blood as the piglet generates heat through thermogenesis (Elliot and Lodge, 1977, Mellor and Cockburn, 1986). Piglets' glycogen stores range from 30 - 38 g/kg of the piglet's body weight (BW) (Le Dividich et al., 2005) therefore larger piglets have higher glycogen pools than small piglets (Theil et al., 2011). These glycogen stores are depleted quickly; 75 % of liver glycogen and 41 % of muscle glycogen is utilised in the first 12 hours post-partum (Elliot and Lodge, 1977).

Colostrum is the first source of nutrients a piglet receives after birth and it is essential for increasing the energy reserves of the piglet (Devillers et al., 2007); if the piglet does not consume enough colostrum it may not have sufficient energy to survive. New born piglets have very little immune protection; colostrum contains high concentrations of immunoglobulins which provide the neonates with a source of maternal antibodies. This gives them passive immunity against diseases until their immune systems fully develop (Quesnel, 2011). Colostrum is also important to stimulate intestinal development (Xu et al., 2000, Devillers et al., 2011) and for thermoregulation (Le Dividich et al., 2005, Devillers et al., 2011). Piglets born later in the birth order and piglets from larger litter sizes are more

likely to have reduced colostrum intake compared with piglets born earlier in the birth order and piglets from smaller litters (Devillers et al., 2007).

Colostrum intake is highly variable amongst piglets. In the first 24 hours post-partum the average colostrum consumption has been shown to range from 210 to 370 g/kg of birth weight (Farmer et al., 2006). Colostrum intake is not only important in the period immediately post birth but it also important for the piglets' future growth as it has been found to influence weaning and finishing weights (Decaluwé et al., 2014, Declerck et al., 2016). Declerck et al. (2016) found that for every 1 g increase in colostrum intake, by piglets with a birth weight of 1.27 kg, there was an increase in weaning and finishing weights of 3.5 and 17.0 g respectively. In the first two hours post-partum the rate of colostrum intake by piglets has been found to represent 5 - 7 % of the piglet's birth weight, after this it decreases (Fraser and Rushen, 1992, Le Dividich et al., 1997). A study by Castrén et al. (1991) found piglets gained approximately 90 g in the two hours after birth but only gained an average of 25 g in hours three to five after birth. This is because for the first two hours after birth colostrum is almost continuously available. However, in hours three to five after birth colostrum gradually becomes available in cyclic discrete injections caused by bursts of oxytocin which are separated by periods of very little or no colostrum (Špinka and Illmann, 2015). A review by Farmer et al. (2006) suggested that if there was an unrestricted supply of colostrum, consumption could amount to ~ 450 g/kg birth weight and this would make up for the piglets' weak energy reserves. Devillers et al. (2011) found that the pre-weaning mortality rate of piglets that consumed over 200 g of colostrum was 7.1 %, whereas the mortality rate of piglets that consumed less than 200 g was 43.4 %. It has been suggested that piglets should consume a minimum of 250 g of colostrum in order to achieve good growth and weights at weaning (Quesnel et al., 2012, Hasan et al., 2019).

Birth weight and litter size are major factors determining colostrum intake; individual colostrum consumption increases by 26 - 37 g per 100 g increase in birth weight (Devillers et al., 2005 and Devillers et al., 2004 as cited by Farmer et al., 2006). Heavier piglets have been found to be better at stimulating the sows' teat than smaller piglets and so can drain a larger amount of colostrum from them (King et al., 1997, Quiniou et al., 2002). However, low birth weight piglets have a higher requirement for colostrum per kg of birth weight than higher birth weight

piglets due to their larger surface-to-volume ratio (Noblet et al., 1987, Declerck et al., 2016) which results in greater heat loss (Herpin et al., 2002). The beneficial effects of colostrum are also more pronounced in piglets with low birth weights than in those with high birth weights (Declerck et al., 2016).

1.3. Sow nutrition

The nutritional status of the sow can affect the survival and viability of her offspring (Kim et al., 2007). Sow productivity in terms of numbers of piglets born per litter has increased dramatically over recent years and these piglets possess the potential for rapid growth. Sow nutrition has not changed to the same extent, yet sows are still expected to rear piglets to the same standards as when they produced smaller litters (Craig et al., 2015). Therefore, it is important to focus research on improving sow nutrition in order to maximise the potential of the hyper-prolific sow and her progeny.

1.3.1. Gestation

Sow feeding during gestation should be optimised in order to establish good liveborn litter size, maximise piglet birth weight and support maternal lean tissue mass, as well as support the growth of mammary tissue and replenish maternal lean tissue mass lost during the previous lactation (Whittemore and Kyriazakis, 2006). However, high feeding levels over the whole of gestation will tend to support maternal gains over foetal gains (Whittemore and Kyriazakis, 2006). Restricted feeding regimes are normally applied in order to prevent the sow becoming excessively fat and to try and maintain a body condition score of 3 (Young et al., 2004, Craig et al., 2015). Sows with high levels of back-fat at farrowing have been found to have reduced feed intake in lactation which results in higher back-fat loss (Young et al., 2004). High energy intake of sows between d 75 and d 100 of gestation can lead to increased fat deposition in the mammary gland and lead to reduced milk production in lactation (Farmer and Sørensen, 2001, Young and Aherne, 2005). Higher levels of back-fat around farrowing can also have negative implications on the farrowing process. High back-fat levels have been associated with prolonged farrowing durations which can increase the incidence of still births (Oliviero et al., 2010). High fat levels can affect the progesterone: oestrogen ratio. In the last few days of pregnancy there is an increased ratio of oestrogen to progesterone which stimulates prostaglandin synthesis and upregulates oxytocin receptor activation which is needed for

parturition (McCracken et al., 1999, Russell et al., 2003, Oliviero et al., 2010). Problems with the oxytocin receptor activation can weaken the expulsion phase of pregnancy (Oliviero et al., 2010). Alternatively, sows which are too thin during gestation often do not have sufficient fatty tissue at parturition, therefore milk production and growth of the piglets is compromised (Whittemore and Kyriazakis, 2006). Thinner sows often lose more body condition during lactation which can increase the time between weaning and oestrus (Whittemore and Kyriazakis, 2006, Craig et al., 2015).

1.3.1.1. Transition period

Sows are often fed a diet low in energy and protein throughout the whole of gestation and then are moved onto a diet high in energy and protein for lactation (Theil, 2015). There is no definitive time period to describe the transition period however, it is from ~ 10 d prior to parturition to d 10 of lactation (Theil, 2015). The transition period of the sow is of critical importance for the sow and her progeny as this is when colostrum is synthesised (Schneider, 1991, Hansen et al., 2012), nutrients are relocated from the blood to the mammary tissue and milk production begins (Theil et al., 2006, Hansen et al., 2012). Although substantial mammary gland growth occurs in the last third of gestation, the growth rate is accelerated in the last 10 d of gestation (Theil, 2015). The last 10 d prior to parturition is also essential for foetal growth as nearly one third of weight is gained during this period (Noblet et al., 1985, Theil, 2015). The high foetal growth rate increases the protein and amino acid requirements of the sow and normally nutrients are prioritised for the foetus during this period (Theil et al., 2012). Therefore, the diet the sow receives during this period is of key importance for the farrowing process and for the subsequent survival of piglets (Theil, 2015).

1.3.2. Lactation

During lactation adequate feed allowance needs to be provided to maximise milk yield and to minimise maternal fat and protein losses (Whittemore and Kyriazakis, 2006). Whittemore and Kyriazakis (2006) reported that for each additional 1 % of body fat lost from the sow across lactation, there would be 0.1 less piglets born in the next litter. In early lactation sow feed intake is normally restricted to support the change to the new lactation diet and to reduce the occurrence of agalactia (Noblet et al., 1998, Eissen et al., 2000) and then increased slowly by ~ 0.5 kg/d to appetite (Whittemore and Kyriazakis, 2006, Craig et al., 2015). High feed intake

in lactation can increase litter weights as sows have more energy for the production of milk (Eissen et al., 2003, Craig et al., 2017). However, voluntary feed intake is often too low to meet the demands for maintenance, growth and milk production, particularly in gilts (Noblet et al., 1990) and can be affected by many factors such as temperature, genotype, management and body size (Eissen et al., 2000). Therefore, the diet the sow receives in lactation is essential to maximise piglet growth and subsequent sow longevity.

1.3.3. Colostrum production

Colostrum production has been found to vary vastly amongst sows and very little is known about its synthesis or how long it is produced for (Theil et al., 2014a). The majority of colostrum is synthesised prior to parturition (Quesnel et al., 2015). The first component of colostrum, β-lactoglobulin, is synthesised from around d 80 of gestation (Dodd et al., 1994) and a major milk protein, β-casein, is first found at d 90 of gestation (Lee et al., 1993). Figure 1.1 displays the time points for the transition from colostrum to milk over lactation. The definition of colostrum that is generally accepted is that colostrum is mammary secreta ingested by the neonatal piglets until 24 hours after the birth of the first piglet (Devillers et al., 2004). Mammary secreta produced from 34 hours post-partum until d 4 of lactation is defined as transition milk, and milk secreted from d 10 of lactation is mature milk (Theil et al., 2014a). The periods between 24 and 34 hours post-partum and d 4 and 10 of lactation are described as transition periods. Gradually less colostrum and more transition milk are secreted between 24 and 34 hours post-partum and gradually less transition milk and more mature milk are secreted between d 4 and 10 of lactation (Theil et al., 2014a).

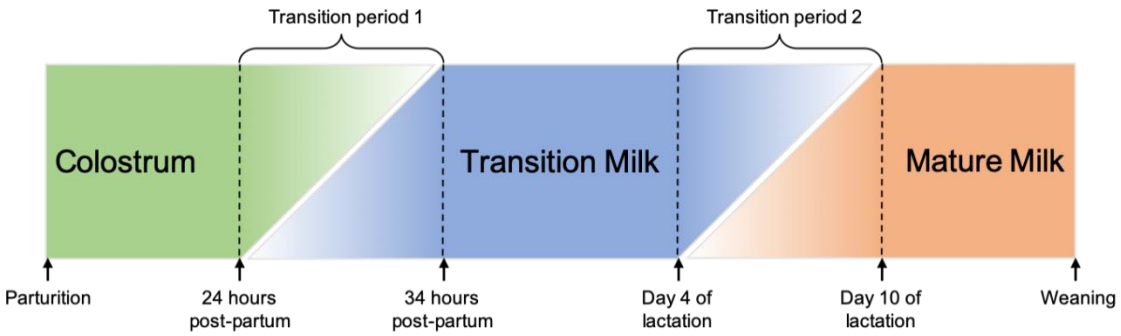

Figure 1.1. The transition from colostrum to milk during lactation

Transition period 1 = gradually less colostrum and more transition milk are produced. Transition period 2 = gradually less transition milk and more mature milk are produced.

1.3.3.1. Yield

The yield of colostrum produced has been found to range from 1.5 to 9.2 kg/sow (Devillers et al., 2007, Quesnel, 2011, Hasan et al., 2019). However, it is estimated that one third of sows do not produce enough colostrum to meet the recommended intake of 250 g/piglet (Quesnel et al., 2012). Devillers et al. (2004) devised an equation (Equation 1) to estimate colostrum yield based on the piglets' birth weight, time to suckle and 24 hour weight using bottle fed piglets.

Equation 1

$$CI\ (g) = -217.4 + (0.217 \times t) + \left(1{,}861{,}019 \times \frac{BW24}{t}\right) + BWB \times \left(54.80 - \frac{1{,}861{,}019}{t}\right) \times (0.9985 - 3.7 \times 10^{-4} \times t_{FS} + 6.1 \times 10^{-7} \times {t_{FS}}^2$$

Where:

CI = colostrum intake, BWB: body weight at birth (kg), BW24: body weight at 24 hours (kg), t_{FS}: time between birth and first suckle (mins), t: time since first measure of birth weight (mins) (Devillers et al., 2004).

However, Theil et al. (2014b) found that the equation underestimated the colostrum intake of sow reared piglets by approximately 43 %, as sow reared piglets are more active than bottle fed piglets so require more colostrum to reach a certain live weight gain. Therefore, Theil et al. (2014b) devised the following new equation (Equation 2) using a mechanistic prediction model:

Equation 2

$$CI\ (g) = -106 + (2.26\ \times WG) + (200\ \times BWB) + (0.111\ \times D) - \left(1{,}414\ \times \left(\frac{WG}{D}\right)\right) + \left(0.0182 \times \left(\frac{WG}{BWB}\right)\right)$$

Where:

CI: colostrum intake, WG: piglet weight gain in 24 hours (g), BWB: piglet body weight at birth (kg), D: duration of colostrum suckling (mins) (Theil et al., 2014b).

The variability in colostrum yield can be attributed to many factors including sow, piglet and environmental factors (Devillers et al., 2007, Declerck et al., 2015, Hasan et al., 2019, Quesnel and Farmer, 2019). The effect of parity on colostrum yield is ambiguous. There is limited evidence to suggest that higher parity sows and gilts produce less colostrum than sows in their second or third parity. Devillers et al. (2007) found that sows of parities 2 or 3 produced ~ 25 % more colostrum than gilts and 18 % more colostrum than parity 4 sows. Decaluwe et al. (2013) confirmed this parity effect and found that sows of parity 4 to 7 produced 840 g less colostrum than sows of parity 1 to 3. In contrast, studies by Quesnel (2011), Declerck et al. (2015), Hasan et al. (2019) found no effect of parity on colostrum yield. It has generally been accepted that unlike milk production, litter size does not influence colostrum production (Farmer et al., 2006, Devillers et al., 2007, Quesnel, 2011). However, a recent study by Hasan et al. (2019) put this to question as it found that for each additional live born piglet in a litter the yield of colostrum increased by 93.6 g.

Little is known about how sow nutrition influences colostrum yield (Theil et al., 2014a, Quesnel and Farmer, 2019). A study by Loisel et al. (2013) looked at the effect of level and source of dietary fibre (DF) on colostrum yield. The level of fibre in the diet was increased from 13 % DF (low fibre group) to 23 % DF (high fibre group) from d 92 of gestation. In the high fibre group, wheat and barley were partly replaced by soybean hulls, wheat bran, sunflower meal and sugar beet pulp. Whilst there was no effect on colostrum yield, low birth weight piglets (< 900 g) from the high fibre group had a higher colostrum intake than low birth weight piglets from the low fibre group. The authors attributed this towards a numerically shorter period between birth and first suckling of low birth weight piglets in the high fibre than the low fibre group. Therefore, a greater ability to reach the teat

may have increased colostrum intake (Loisel et al., 2013). A study by Krogh et al. (2012) found that sows supplemented with 1.3 % conjugated linoleic acid (CLA) 7 d prior to parturition had a lower colostrum yield compared with the control group (409 vs 463 g/piglet, respectively) but higher levels of fat in colostrum (6.3 vs 5.2 %). These studies indicate that sow nutrition at the end of gestation is important for colostrum yield.

1.3.3.2. Immunoglobulins

In pigs, there is little or no placental transfer of antibodies therefore immunoglobulins in colostrum are essential for neonatal survival as they provide the piglets with immune protection (Curtis and Bourne, 1971, Quesnel, 2011). The three main immunoglobulins in sow colostrum are immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM). IgG is the most abundant immunoglobulin in colostrum and is the most prominent immunoglobulin in serum used to fight infections (Hurley and Theil, 2011). IgA is the second most prominent immunoglobulin in colostrum and its primary function is to help prevent mucosal infections in the intestinal and respiratory tracts by agglutinating microbes (Hurley and Theil, 2011). Finally, IgM is the least prominent immunoglobulin in colostrum and is involved in primary defence (Hurley and Theil, 2011). The relative abundance of these immunoglobulins changes as colostrum turns to milk and IgA becomes the most abundant immunoglobulin in mature sow milk (Hurley, 2015). All of IgG and the majority of IgM (80 %) in colostrum is derived from sow serum, whereas the majority of IgA is synthesised in the mammary gland and only 40 % of it is derived from sow serum (Bourne and Curtis, 1973).

The levels of immunoglobulins are highest in the first couple of hours post-partum and they decline with increased time following parturition. At parturition, the levels of IgG, IgA and IgM in sow colostrum have been found to range from 51-102, 5.5-24 and 1.3-10.7 mg/ml, respectively. However, by 24 hours post-partum the levels of IgG, IgA and IgM range from 6-20, 1.9-6.6 and 0.9-2.4 mg/ml, respectively (Hurley, 2015). Therefore, piglets must consume colostrum early in order to obtain adequate levels of immunoglobulins. Devillers et al. (2011) found that there was no close relationship between colostrum intake and IgG concentration in piglet plasma. After piglets consumed ~ 200 g of colostrum plasma concentration did not increase anymore which is most likely due to gut

closure. In pigs, the transfer of intact immunoglobulins to the circulation can only occur prior to gut closure which can happen as early as 24 hours. After this uptake of immunoglobulins is into enterocytes from the gut (Le Dividich et al., 2005).

Not only do concentrations of immunoglobulins in colostrum change drastically with time from parturition but they can show high levels of variance between sows on the same unit (Klobasa and Butler, 1987, Farmer and Quesnel, 2009). Many factors can affect colostrum immunoglobulin concentrations including: parity, season and genotype (Farmer and Quesnel, 2009). It has been suggested that immunoglobulin concentrations in sows increase as parity increases (Klobasa and Butler, 1987, Cabrera et al., 2012). Although Quesnel (2011) found no effect of parity on IgG concentration at parturition, at 24 hours post-partum IgG concentration was higher in sows above parity 5.

Nutritional attempts have been made to increase concentrations of immunoglobulins in colostrum. Corino et al. (2009) supplemented sows with 0.5 % CLA from 7 d prior to parturition until 7 d into lactation and found that this enhanced concentrations of IgG, IgA and IgM in colostrum by 26.8, 74.3 and 39.3 %, respectively. A study by Leonard et al. (2012) found that supplementing sows with seaweed extract at a dose of 10 g/d for 7 d prior to parturition increased the concentration of IgA in sow colostrum compared with the control (11.6 vs 8.0 mg/ml) with a tendency for an increase in the concentration of IgG compared with the control (84.6 vs 75.6 mg/ml). These studies demonstrate that maternal nutrition in late gestation is also important for immunoglobulin concentrations.

1.4. β-hydroxy β-methyl butyrate

β-hydroxy β-methyl butyrate (HMB) is a metabolite of the essential branched-chain amino acid (BCAA) leucine. It is a natural biologically active substance which is found in small quantities in common dietary products. The highest concentrations of HMB have been found at levels of 150, 123 and 25 μg kg^{-1} in catfish, grapefruit and alfalfa respectively, but it is also found in products such as asparagus, avocado and cauliflower (Zhang et al., 1994, Tatara, 2008, Qiao et al., 2013). In pigs, HMB is derived exclusively from leucine; plasma physiological levels have been found to range from 2 to 4 μM and increase to 15 to 30 μM after leucine administration at a dose of 50 g (Nissen and Abumrad, 1997).

The metabolic pathway for the formation of HMB from leucine and how it is further metabolised in mammals is presented in Figure 1.2. Initially, leucine is converted to α-ketoisocaproate (α-KIC) both in the cytosol and mitochondria of the muscles and liver. Following this, α-KIC may be metabolised by two different pathways (Nissen and Abumrad, 1997). The majority of α-KIC is oxidised in the mitochondria to isovaleryl-CoA via branched-chain ketoacid dehydrogenase (BCKAD). This then undergoes further catabolism within the mitochondria to yield different metabolites leading to the formation of β-hydroxy β-methylglutaryl-CoA (HMG-CoA) (Nissen and Abumrad, 1997, Szcześniak et al., 2015). However, approximately 5 % of leucine metabolism takes place via a second pathway in the cytosol. Here, HMB is produced from α-KIC via the KIC dioxygenase enzyme and serves as a carbon source for *de novo* cholesterol synthesis in tissues (Nissen and Abumrad, 1997, Tako et al., 2004, Szcześniak et al., 2015).

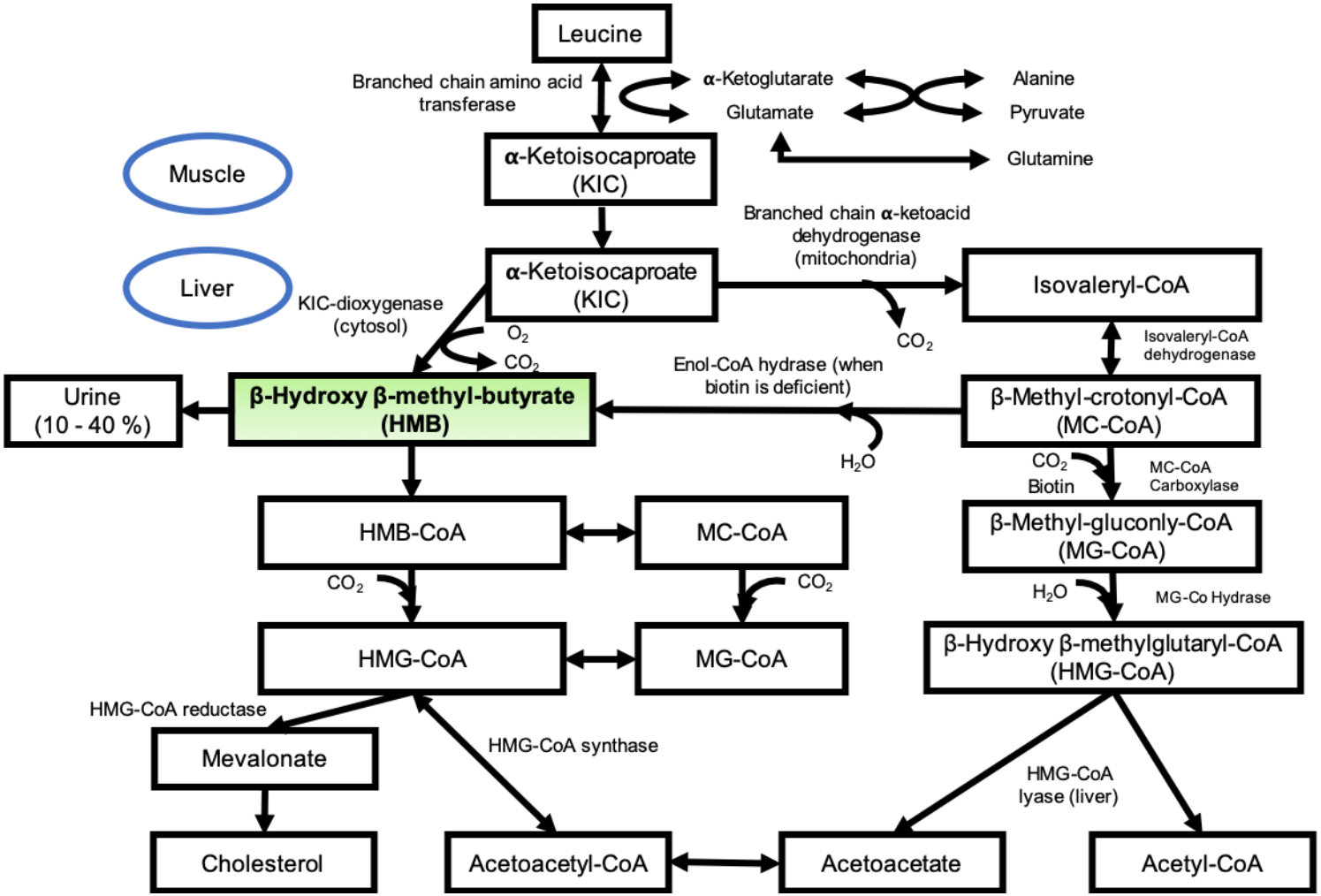

Figure 1.2. An overview of leucine/HMB metabolism in mammals. Adapted from Nissen and Abumrad (1997).

HMB has been found to have anti-catabolic (Ostaszewski et al., 2000), anabolic (Eley et al., 2007) and lipolytic effects (Nissen et al., 1994, Flummer and Theil, 2012). Therefore, HMB is often used as an ergogenic aid by body builders and athletes to promote exercise performance (Wilson et al., 2008). However, over the past 20 years there has been increased interest in the positive effects supplemental HMB may have in animal production. Positive effects on growth performance and health status have been found in several livestock species including: broilers, pigs and sheep (Nissen et al., 1994, Krakowski et al., 2002, Tatara et al., 2007, Tatara, 2008, Qiao et al., 2013). Supplemental HMB is commercially available in the more common form of calcium HMB monohydrate (Wilson et al., 2008) however, it is also available in a free-acid form (Wilson et al., 2013, Tinsley et al., 2018).

1.4.1. The role of HMB in cholesterol synthesis

Cholesterol is a zoosterol found in all mammalian cells. It has numerous functions in the body including serving as a precursor for the synthesis of vitamin D and bile acids, and is an important component of cell membranes (McDonald et al., 2011). The sarcolemma is a cell membrane which surrounds striated muscle fibre cells and these rely completely on *de novo* synthesis of cholesterol (Wilson et al., 2008, Szcześniak et al., 2015). HMB is converted to HMG-CoA by a carboxylation reaction in the cytosol; HMG-CoA can then be used for cholesterol synthesis (Nissen and Abumrad, 1997). Stressed or damaged muscle cells may not be able to produce sufficient HMG-CoA to support adequate cholesterol synthesis needed to maintain cell functions. Supplemental HMB may provide a source of HMG-CoA which can then be used to maintain adequate cholesterol synthesis and may result in decreased muscle damage and faster recovery (Nissen et al., 2000, Wilson et al., 2008).

On the other hand, in cases of hypercholesterolaemia, HMB has been found to lower LDL-cholesterol levels (Nissen et al., 2000). Nissen et al. (2000) analysed data from nine studies in which men and women (varying in age from 18 to 79 years) of different fitness abilities were supplemented with ~ 3 g/d HMB for three to eight weeks. This analysis found that HMB lowered the level of LDL-cholesterol by 7.3 % in individuals whose cholesterol levels at the start of the study were > 5.17 mmol/L. Whilst the reason for this is unclear, the most common form of HMB is calcium HMB monohydrate and it is thought that calcium supplementation

increases bile acid secretion and excretion, which reduces serum cholesterol through increased bile regeneration in the liver (Ditscheid et al., 2005, Wilson et al., 2008).

1.4.2. The role of HMB in protein turnover

The effect HMB has on animal growth may be due to the role it plays in the regulation of skeletal muscle protein turnover (Szcześniak et al., 2015). HMB has been found to enhance protein synthesis and attenuate protein degradation *in vitro* and *in vivo* (Ostaszewski et al., 2000, Smith et al., 2005). The role HMB plays in protein turnover is summarised in Figure 1.3.

1.4.2.1. HMB and protein synthesis

The role of leucine in protein synthesis is well characterised (Li and Jefferson, 1978, Anthony et al., 2000, Wang et al., 2018). Eley et al. (2007) suggested that HMB may stimulate protein synthesis in a similar way to leucine, through the activation of the mammalian target of rapamycin (mTOR). mTOR is a conserved serine/threonine kinase that has a main role in the regulation of cell growth and metabolism (Zoncu et al., 2011, Chi, 2012). The phosphorylation of mTOR leads to the phosphorylation of its downstream targets: eukaryotic initiation factor (eIF) 4E binding protein 1 (4E-BP1) and p70-S6 kinase 1 (S6K1). The role of 4E-BP1 is to regulate protein translation by preventing formation of the eIF4E × eIF4G complex which would normally permit recruitment of the 43S ribosomal subunit, ultimately resulting in protein translation. In its phosphorylated form, 4E-BP1 is unable to prevent formation of the eIF4E × eIF4G complex and thus protein translation is initiated (Anthony et al., 2000, Eley et al., 2007, Wang et al., 2018). Activation of S6K1 leads to phosphorylation of its target, ribosomal protein S6 (S6), part of the 40S ribosomal subunit, enabling protein translation (Anthony et al., 2000, Eley et al., 2007, Wang et al., 2018). Eley et al. (2007) demonstrated the link between HMB and this pathway through the increased phosphorylation levels of mTOR and its downstream targets including 4E-BP1, eIF4E and S6K1, when myotubes were treated with HMB. This effect was inhibited by rapamycin indicating the importance of the mTOR pathway in HMB treatment.

These results have been confirmed in recent studies with neonatal piglets. In a study by Wheatley et al. (2014), 5 - 7 d old fasted neonatal piglets were infused with HMB at 0, 20, 100 or 400 µmol kg $BW^{-1}h^{-1}$ for 1 hour (HMB 0, HMB 20, HMB 100 and HMB 400 respectively). The rate of protein synthesis, as measured by a

radioactive labelled amino acid which was injected into the piglets 30 minutes before euthanasia, was increased in the longissimus dorsi (LD), gastrocnemius, soleus and diaphragm muscles of piglets in the HMB 20 group compared with piglets in the HMB 0 group. Protein synthesis rate in the LD was also greater in the HMB 100 than the control group. HMB 400 had no effect on the rate of protein synthesis. This study also found that eIF4E × eIF4G complex formation and S6K1 and 4EBP1 phosphorylation increased in the LD, gastrocnemius and soleus muscles with HMB 20 and HMB 100 and in the diaphragm with HMB 20. Again, HMB 400 had no effect on mTOR signalling. The lack of effect of HMB 400 suggests that HMB may be ineffective at very high doses (Wheatley et al., 2014). Whilst the reason for this is unknown, stimulation of protein synthesis by HMB decreases the release of certain amino acids from muscles to the blood (Holeček, 2017). Glutamine has many physiological functions in the body including an involvement in cell growth and differentiation, immune system modulation and nutrient metabolism (Wu et al., 2011) and its deficiency decreases protein synthesis in skeletal muscle (Holecek and Sispera, 2014). HMB supplementation has been found to decrease plasma levels of glutamine (Holecek et al., 2009). Therefore, it is possible that the higher dose of HMB (HMB 400) interfered with the metabolism of other amino acids such as glutamine which are needed for protein synthesis. In addition, Kao et al. (2016) found increased protein synthesis and translation initiation in muscles of 5 - 7 d old fasted neonatal piglets which were given an enteral supplementation of HMB. This study also found that HMB supplementation increased the numbers of fast twitch muscle fibres in the LD suggesting that HMB may stimulate satellite cell proliferation and differentiation in neonatal pigs (Kao et al., 2016).

Combined these results provide evidence that HMB enhances skeletal muscle protein synthesis by stimulating translation initiation through the activation of mTOR signalling, however, very high doses of HMB may be ineffective at stimulating this process. It is also unclear whether HMB interacts directly with mTOR or whether it is through either the leucine or insulin-like growth factor-1 (IGF-1) specific pathways.

1.4.2.2. HMB and proteolysis

It has been suggested that HMB may attenuate proteolysis through down regulation of the ATP-dependent ubiquitin-proteasome pathway (Smith et al.,

2004). The ubiquitin-proteasome pathway targets proteins for degradation via conjugation of ubiquitin to targeted proteins, marking them for disassembly in the proteasome (Wyke and Tisdale, 2005). Proteolysis-inducing factor (PIF), a sulphated glycoprotein that has previously been shown to induce degradation of skeletal muscle (Lorite et al., 1998), increases proteasomal activity via translocation of nuclear-factor κB (NF-κB) from the cytoplasm to the nucleus and inhibition of its inhibitor IκBα, in a manner dependent on protein kinase C (PKC) (Li and Reid, 2000). HMB has been shown to inhibit the action of PKC, thus preventing the PIF mediated translocation of NF-κB into the nucleus and the subsequent up-regulation of proteins associated with the ubiquitin-proteasome pathway in murine myotubes which had been treated with PIF (Smith et al., 2004). However, studies by Wheatley et al. (2014) and Kao et al. (2016) found no effect of HMB supplementation on protein degradation via the ubiquitin-proteasome pathway in neonatal piglets given supplemental HMB. Therefore, it is plausible that HMB is only able to prevent protein degradation in stressed cells due to its inhibitory mechanism, meaning it can only prevent muscle wastage from happening once it is occurring.

HMB can also attenuate the inhibition of protein synthesis. Studies have shown PIF can inhibit protein synthesis in muscles through a reduction in translation efficiency (Smith et al., 1999, Eley et al., 2007). PIF has been shown to inhibit protein synthesis in myotubes through the activation of double-stranded RNA-dependent protein kinase (PKR) resulting in the phosphorylation of eIF2, which inhibits protein synthesis (Eley et al., 2007). Eley et al. (2007) demonstrated that HMB can block the activation of PKR by PIF, therefore preventing the phosphorylation of eIF2 resulting in failure to block protein synthesis.

In combination these studies suggest that HMB may influence protein turnover by stimulating protein synthesis, decreasing proteolysis and attenuating the depression of protein synthesis, which may help to promote growth when used as a supplement in animal production.

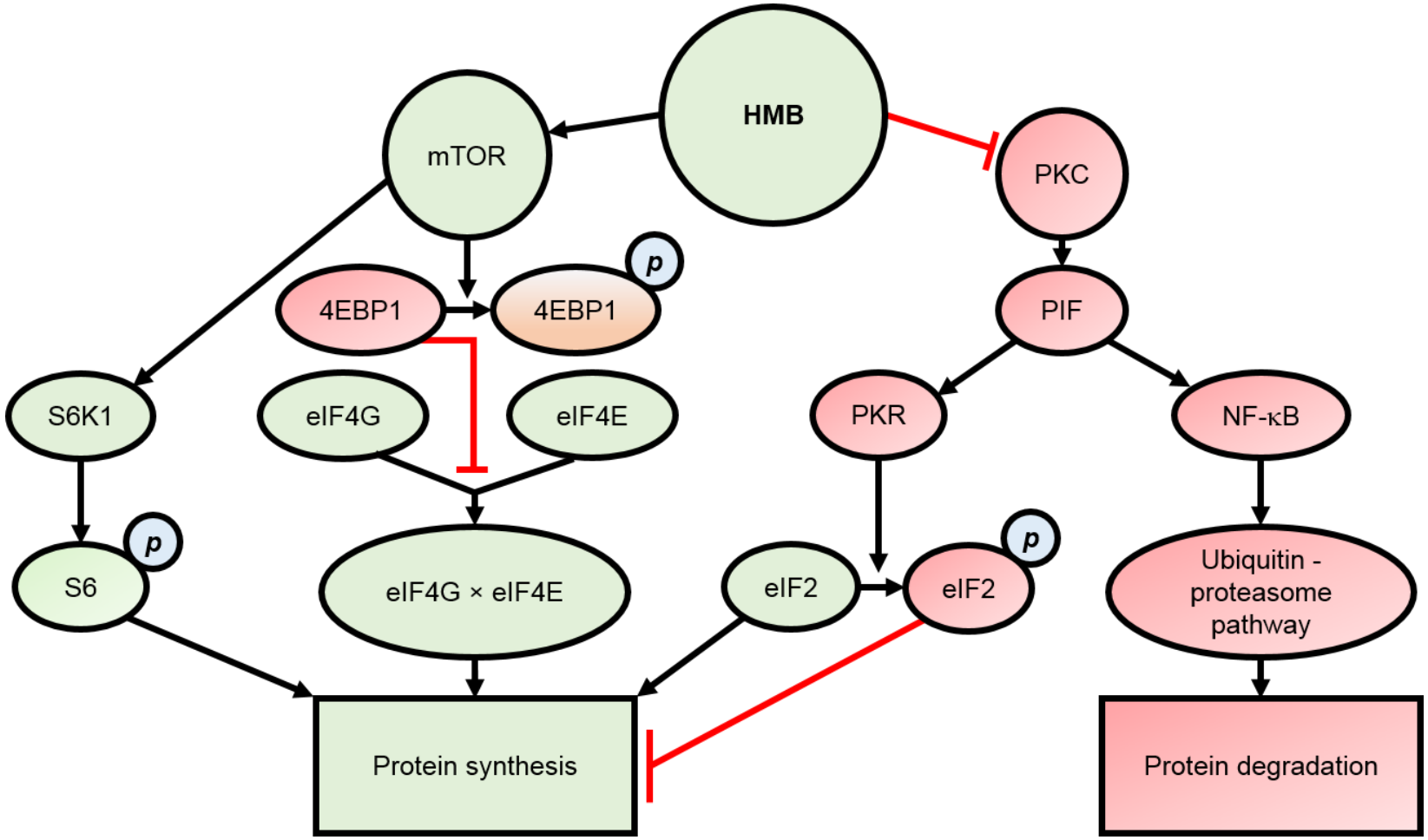

Figure 1.3. The consensus for the role of β-hydroxy β-methyl butyrate in protein turnover

Abbreviations: HMB = β-hydroxy β-methyl butyrate; mTOR = mammalian target of rapamycin; PKC = protein kinase C; 4EBP1 = 4E binding protein 1; *p* = phosphorylated form; PIF = proteolysis-inducing factor; S6K1 = p70-S6 kinase 1; eIF4G = eukaryotic initiation factor 4 G; eIF4E = eukaryotic initiation factor 4 E; PKR

= double-strand RNA-dependent protein kinase; NF-κB = nuclear factor-κB; S6 = ribosomal protein S6; eIF4E × eIF4G = eIF4E × eIF4G complex; eIF2 = eukaryotic initiation factor 2

Green represents positive mediators of protein synthesis, red represents inhibitors of protein synthesis or mediators of protein degradation and orange is a component that plays no further role in protein turnover. β-hydroxy β-methyl butyrate (HMB) activates the mammalian target of rapamycin (mTOR) either directly or indirectly, which leads to the phosphorylation of downstream targets resulting in protein synthesis. HMB blocks activation of protein kinase C (PKC) which in turn prevents activation of downstream targets including those involved in the ubiquitin - proteasome pathway preventing protein degradation. HMB also prevents the phosphorylation of eukaryotic initiation factor 2 (eIF2) which can inhibit protein synthesis in its phosphorylated form.

1.4.3. The role of HMB in satellite cell proliferation

As mentioned in Section 1.4.2.1, Kao et al. (2016) found that HMB supplementation may stimulate satellite cell proliferation. Kornasio et al. (2009) also demonstrated the effect HMB has on myogenic cell proliferation in *in vitro* studies with human and chick myoblasts. They added various doses of HMB to serum starved cells for 17 hours followed by thymidine labelling for 4 hours. HMB increased the mRNA level of the muscle regulatory factor (MyoD) which is upregulated in satellite cells which have been activated. It also increased the incorporation of thymidine into DNA and increased cell numbers. HMB increased protein levels of the muscle differentiation factors, myocyte enhancer factor 2 and myosin heavy chain. HMB also triggered increased expression of IGF-1 mRNA. Therefore, HMB may have had a direct effect on cell proliferation or may have acted indirectly through IGF-1 (Kornasio et al., 2009).

1.4.4. The role of HMB on the GH/IGF-1 axis

HMB may influence body and muscle growth through either a direct or indirect association with the growth hormone (GH)/ IGF-1 axis (Qiao et al., 2013). As mentioned above, HMB has been found to enhance expression of IGF-1 mRNA in human and chick myoblasts (Kornasio et al., 2009). Studies *in vivo* have also demonstrated the effect of HMB on circulating levels of IGF-1. Tatara et al. (2007) found that supplementing sows with HMB for the last two weeks of gestation, at a dose of 50 mg/kg BW, increased the levels of GH and IGF-1 in piglet serum at birth by 38 and 20 % respectively, when compared to the control piglets. They also found that growth rate from birth to slaughter and femur bone weight were increased in pigs from sows which had been supplemented with HMB. The increased growth rate of the pigs may be due to the increased IGF-1 levels in piglet serum at birth as IGF-1 promotes myoblast proliferation, differentiation and protein turnover in muscle (Tatara et al., 2007, Pallafacchina et al., 2013, Yu et al., 2015).

A further study by Blicharski et al. (2017) supplemented sows with HMB at a dose of 200 mg/kg BW from d 70 until d 90 of gestation and found that the IGF-1 level in new born piglet serum was enhanced by 215 %. The difference in percentage increase in IGF-1 levels between the Tatara et al. (2007) and the Blicharski et al. (2017) study could be a result of the dose and duration of HMB supplementation. In addition, Tatara (2008) fed neonatal lambs HMB at a dose of 0.1 g/kg BW for

three weeks and found HMB enhanced both GH and IGF-1 concentrations by 71 % in serum at 21 d. However, HMB had no effect on the growth rate of the lambs.

However, some studies have found no effect of HMB on the GH/IGF-1 axis. Qiao et al. (2013) provided supplemental HMB to broiler chicks at doses of 0.05 or 0.1 % for 21 d and found no effect on GH. However, the ADG of the chicks was increased by 9 % and mortality reduced by 25 %. Interestingly they found that HMB increased serum levels of thyroid hormones, thyroxine and triiodothyronine, in chicks at d 21. The authors suggested that the increased growth rate of chicks was due to the increased levels of thyroid hormones which may have induced proliferation of satellite cells and myofiber protein accretion (Qiao et al., 2013). Foye et al. (2006) also injected turkey eggs with HMB on d 23 of incubation but found no effect of HMB on plasma IGF-1 at hatch, d 3 or d 7 post-hatch.

1.4.5. The role of HMB in lipolysis

Many studies in humans have found that HMB promotes lean body mass (Wilson et al., 2008). Wilson et al. (2008) suggested that this may be due to the role HMB has in protein turnover which could increase the body's ability to mobilise fat. A study by Duan et al. (2018) supplemented HMB at a dose of 0.62 % to grower pig diets for 45 d. They found that HMB reduced the weight of total fat mass and reduced the level of serum leptin, which is positively proportional to fat mass, in pigs at d 45. They also found that HMB supplementation promoted fatty acid catabolism in the perirenal adipose tissue through the increased gene expression of hormone sensitive lipase and adipose triglyceride lipase, which are genes involved in lipolysis (Duan et al., 2018).

Studies in sows have also demonstrated HMB to have a lipolytic effect enhancing fat metabolism. Nissen et al. (1994) supplemented sows with HMB at a dose of 10 mg/kg BW for 3 - 4 d prior to parturition and found that the fat level in colostrum was increased by 41 %. HMB may have increased fat mobilisation and elevated plasma levels of non-esterified fatty acids (NEFA) which may be used as energy for colostrum fat synthesis (Wilson et al., 2008, Theil et al., 2014a). In addition, HMB is a precursor for cholesterol synthesis (Nissen et al., 2000, Wilson et al., 2008), this may spare energy and result in increased fat synthesis (Nissen et al., 1994). However, whilst this study used 68 sows in total (34 per treatment), it was across three trials which were performed on different farms. Studies by Flummer and Theil (2012) and Wan et al. (2015) found no effect of maternal

supplementation with HMB on colostrum fat, however, both studies found that the levels of fat were increased in milk by an average of 14 and 21 % respectively, compared with the control groups. However, the number of sows used in each of these studies was also very low, 16 (8 per treatment) and 20 sows (10 per treatment) in the Flummer and Theil (2012) and the Wan et al. (2015) studies respectively.

Flummer and Theil (2012) also found that HMB supplementation increased the colostrum yield per piglet by 18 % compared with the control. Plasma levels of 3-hydroxy butyrate were lower in sows which had been supplemented with HMB after the colostrum period (6 vs 31 µM in the control group). This supports the idea that HMB affects fat mobilisation (Flummer and Theil, 2012) as 3-hydroxy butyrate is a metabolite produced by free fatty acid oxidation as a result of lipolysis (Vigili de Kreutzenberg and Avogaro, 2017). As mentioned in Section 1.3.3.1, colostrum yield varies drastically amongst sows; it has been found to range from 1.5 to 9.2 kg per sow (Devillers et al., 2007, Quesnel, 2011, Hasan et al., 2019). Therefore, 8 sows per treatment is a rather low number when determining colostrum yield.

1.4.6. HMB as an immunostimulant

The role of HMB as an immunostimulant has been demonstrated in many *in vitro* and *in vivo* studies (Peterson et al., 1999a, Siwicki et al., 2000, Krakowski et al., 2002, Siwicki et al., 2004). However, the exact mechanisms behind its effects are still unclear. Nissen and Abumrad (1997) examined the effect of leucine metabolites on blastogenesis of isolated sheep lymphocytes. They found that the only direct metabolite of leucine which affected lymphocyte blastogenesis was HMB. Furthermore, in an *in vitro* study with a chicken macrophage cell line, Peterson et al. (1999a) found HMB induced macrophage proliferation and enhanced macrophage effector functions such as nitrite production and phagocytosis. This was followed up by an *in vivo* study by Peterson et al. (1999b) in which HMB at doses up to 0.10 % of feed, were fed to broiler chicks for a 21 d period. The study found that nitrite levels in the macrophage culture supernatant were enhanced by HMB feeding at levels of 0.01 and 0.05 %. They also found that when chicks were injected with a sheep red blood cell suspension, those fed 0.10 % HMB had increased IgG levels in blood and increased total anti-sheep red blood cell antibody levels compared with the control.

Studies in fish have also demonstrated the immunostimulatory effect of HMB. Siwicki et al. (2003) supplemented rainbow trout with HMB at doses of either 0, 10, 25 or 50 mg HMB per kg^{-1} BW day^{-1} for 8 weeks. HMB increased phagocyte and lymphocyte T and B activity, lysosome activity in plasma and total serum immunoglobulin levels in a positive dose-dependent manner. HMB also reduced mortality by 62 % in a disease-challenged model, indicating that HMB supplementation may have a practical and economic impact (Siwicki et al., 2003). In an additional study by Kunttu et al. (2009), HMB was supplemented to the feed of rainbow trout at doses of 0, 25 and 50 mg kg^{-1} BW day^{-1} for 21 d. The production of reactive oxygen species in whole blood was enhanced by HMB treatment and was dose-dependent suggesting that HMB is effective in enhancing immune function.

Krakowski et al. (2002) looked at the effect of supplementing HMB to sows in late gestation on immune parameters in colostrum. They supplemented sows with HMB at a dose of 15 mg/kg BW for three weeks, from six weeks prior to parturition and found that sow serum IgG level was enhanced by 10.5 % compared with the control, and that the IgG level in colostrum was enhanced by 27 % compared with the control. Piglets from sows supplemented with HMB were also heavier at birth and weaning compared with piglets from sows that were not supplemented with HMB. However, this study only used five sows per treatment and as mentioned in Section 1.3.3.2, concentrations of immunoglobulins show high levels of variability amongst sows on the same unit (Klobasa and Butler, 1987, Farmer and Quesnel, 2009). Combined these studies provide evidence for the use of HMB as an immunostimulant. The study by Krakowski et al. (2002) highlights the potential positive effects of HMB supplementation on colostrum quality, which may be highly beneficial in the pork industry.

1.5. HMB supplementation to sows

Research into the effect of HMB on pig performance and in particular, the effect it may have on sow and litter performance is extremely limited. Positive effects have been found in terms of piglet birth weight, colostrum production and growth (Krakowski et al., 2002, Tatara et al., 2007, Flummer and Theil, 2012) when supplementing HMB to sows in gestation. To our knowledge at the time of writing, there are only nine published studies involving supplementing HMB to sows in gestation, the majority of which use eight or fewer sows per treatment, a very low

replication number. Morris (1999) devised the following power analysis equation to determine the number of replications needed:

Equation 3

$$n = \frac{2t^2(CV)^2}{d\%^2}$$

Where:

n = number of replications, t = t value for a chosen probability and degrees of freedom appropriate to the error variance (a constant value of 2 was used for a 0.05 probability), CV = coefficient of variation and d = standardised difference.

Previous data from our farm suggests that the CV for average piglet birth weight is ~ 15 - 20 %. If the standardised difference was predicted to be 10 % and the CV 18 %, Equation 3 suggests that 26 sows would be needed per treatment. Reducing the predicted standardised difference or increasing the CV would increase the replication needed.

In addition to the low replication, the results produced between these studies are inconsistent and the doses and timings of supplementation vary vastly. The dose of HMB supplemented to gestating sows has been found to vary from 10 mg/kg BW (Nissen et al., 1994) to 200 mg/kg BW daily (Blicharski et al., 2017), which is the equivalent of 2 and 40 g/d for a 200 kg sow, respectively. The timing of supplementation has been found to vary for sows in gestation from 3 d prior to parturition (Nissen et al., 1994) to 80 d prior to parturition (Wan et al., 2015). Whilst the majority of studies which have supplemented HMB to sows in gestation have provided it up until parturition, studies by Krakowski et al. (2002) and Blicharski et al. (2017) supplemented sows for ~ 3 weeks from ~ d 70 of gestation. Table 1.1 summarises the current literature regarding the dosage and timings which sows have been supplemented with HMB for and shows the main effects found.

As HMB influences protein turnover, the regulation of the GH/IGF-1 axis and satellite cell proliferation, it is plausible that providing sows with HMB in late gestation may enhance piglet birth weight. However, the results regarding this effect are inconsistent. Tatara et al. (2007) supplemented sow diets with HMB at a dose of 50 mg/kg BW for two weeks prior to parturition and found that the birth

weight of piglets was increased by 24 %, compared with piglets from control sows. In another study, Tatara et al. (2012) found that supplementing sow diets with HMB, again, at a dose of 50 mg/kg BW for two weeks prior to parturition, increased piglet birth weight by 23 % compared with the control. In these studies pigs also had an increased growth rate from birth to slaughter. The authors attributed this towards the increased levels of GH and IGF-1 observed in the piglets at birth. However, both these studies only used six sows per treatment which is very low replication for determination of sow performance data. In agreement with these studies, Blicharski et al. (2017) also found a positive effect of supplementing sow diets with HMB on piglet birth weight. They provided HMB to sows at a dose of 200 mg/kg BW for ~ 3 weeks from d 70 until d 90 of gestation and found that average piglet birth weight was increased by 81 %, however, this was only observed in male piglets. This study also had a very low replication of only six sows per treatment.

On the other hand, studies by Nissen et al. (1994), Flummer and Theil (2012) and Flummer et al. (2012) found no effect of supplementing sow diets with HMB on piglet birth weight. Nissen et al. (1994) supplemented sow diets with HMB at a dose of 10 mg/kg BW for 3 to 4 days prior to parturition and throughout lactation and found no effect on piglet birth weight. Similarly, studies by Flummer and Theil (2012) and Flummer et al. (2012) found no effect of supplementing sows with HMB on piglet birth weight when provided at doses of 2500 mg/d from 7 days prior to parturition, and 15 mg/kg BW for 10 days prior to parturition respectively. Again, these studies are all poorly replicated. The study by Flummer et al. (2012) only had two sows in the HMB treatment group and whilst the study by Nissen et al. (1994) had 34 sows in the HMB treatment group, they were combined from three different trials on three different farms.

Some of the discrepancies between the results of the studies may be due to the different doses and timings that HMB was supplemented for. The studies by Tatara et al. (2007), Tatara et al. (2012) and Blicharski et al. (2017), which all found positive effects of supplementing HMB to sows on piglet birth weight, all used much higher doses and for a longer duration of time (50 - 200 mg/kg BW, for 2 - 3 weeks) than the studies by Nissen et al. (1994), Flummer and Theil (2012) and Flummer et al. (2012) (10 - 15 mg/kg BW, for 2 - 10 d). Therefore, it would seem that the effect of supplementing sows with HMB on piglet birth weight

is dose and time dependent. However, with such low replication in each study it is impossible to determine the effect of HMB on piglet birth weight.

There is some evidence to suggest that supplementing sow diets with HMB in gestation affects colostrum production. As previously mentioned in Section 1.4.5, studies have found that HMB can enhance colostrum and milk fat concentration and colostrum yield measured per piglet, which may be due to the lipolytic effect of HMB (Nissen et al., 1994, Flummer and Theil, 2012, Wan et al., 2015). Furthermore, as mentioned in Section 1.4.6, Krakowski et al. (2002) demonstrated that maternal supplementation with HMB at a dose of 15 mg/kg BW for three weeks, from six weeks prior to parturition, enhanced the level of IgG in sow colostrum. However, as previously mentioned all of these studies are poorly replicated. The studies by Krakowski et al. (2002), Flummer and Theil (2012) and Wan et al. (2015) used five, eight and ten sows per treatment respectively. To our knowledge none of these results have been confirmed in larger scale studies or found with supplementing HMB at any other doses.

The low replication number of sows used in these studies makes the results unreliable. However, if the positive effects on piglet birth weight and colostrum production are real, then HMB may offer a solution to the negative effects observed with the increase in litter size. The dose and duration of HMB supplementation could also be key to the inconsistences observed in its effects on litter performance. Therefore, determining the effect of supplementing sow diets with HMB on litter performance in a large scale study and determining the optimum inclusion level and duration could improve piglet birth weight, colostrum production and the future performance of piglets.

Table 1.1. Summary of literature regarding β-hydroxy β-methyl butyrate use in sow studies

Reference	Breed and replication	Doses and duration	Quantity of HMB (g/sow/d)[1]	Effect[2]
Nissen et al. (1994)	70 Large White × Landrace sows (35 per treatment)	10 mg/kg BW 3-4 days prior to parturition until day 21 of lactation	2.0	No effect on birth weight ↑ milk fat ↑ weaning weights ↓ sow back-fat at weaning
Krakowski et al. (2002)	20 Polish Landrace sows (5 per treatment)	15 mg/kg BW 3 weeks, from 6 weeks prior to parturition	3.0	↑ birth weight ↑ IgG in colostrum and sow serum ↑ weaning weights
Tatara et al. (2007)	12 Polish Landrace sows (6 per treatment)	50 mg/kg BW 2 weeks prior to parturition	10.0	↑ birth weights ↑ growth rate from birth to slaughter ↑ levels of GH and IGF-1 in piglets at birth ↑ bone mineral density
Flummer and Theil (2012)	16 Danish Landrace × Yorkshire sows (8 per treatment)	2500 mg daily 7 days prior to parturition until 28 days post-partum	2.5	No effect on birth weight ↑ colostrum yield per piglet, milk fat and energy ↓ sow back-fat at weaning ↓ piglet weaning weights
Flummer et al. (2012)	5 Danish Landrace × Yorkshire sows (2 in HMB treatment)	15 mg/kg BW 10 days prior to parturition	3.0	No effect on birth weight No effect on weaning weight ↑ liver, spleen, kidneys and caecum weights of piglets at d 28

Table 1.1 continued

Reference	Breed and replication	Doses and duration	Quantity of HMB (g/sow/d)[1]	Effect[2]
Tatara et al. (2012)	24 Polish Landrace sows (6 per treatment)	50 mg/kg BW For 2 weeks prior to parturition	10.0	↑ birth weights ↑ piglet growth weight to 6 months old ↑ bone alkaline phosphatase activity and IGF-1 in piglet serum at birth and d 90 ↑ GH in piglets at birth ↑ bone weight and bone mineral density of piglets at 6 months
Wan et al. (2015)	20 Landrace × Yorkshire sows (10 per treatment)	4000 mg daily Day 35 until parturition	4.0	↓ still births ↑ litter birth weights, week 1 growth rate and average d 7 weights ↑ weight and protein content of longissimus dorsi of piglets at birth ↑ fat in milk on d 14 and d 21 Trend for ↑ piglet weaning weight
Blicharski et al. (2017)	12 Large White Polish sows (6 per treatment)	200 mg/kg BW Day 70 until day 90 of gestation	40.0	↑ birth weights of male piglets ↑ IGF-1 and leptin of piglets at birth ↑ levels of FSH, LH, estradiol and testosterone in piglet at birth ↑ bone weight and length
Wan et al. (2017)	20 Landrace × Yorkshire sows (10 per treatment)	2000 mg daily Throughout lactation	2.0	↑ plasma leucine and glucose levels of piglets at d 28 ↓ sow feed intake ↑ mTOR mRNA expression in skeletal muscle of piglets at d 28 No effect on weaning weight ↑ finishing weight of piglets (d 180)

Table 1.1 continued

Reference	Breed and replication	Doses and duration	Quantity of HMB (g/sow/d)[1]	Effect[2]
Hułas-Stasiak et al. (2019)	12 Pulawska gilts (6 per treatment)	200 mg/kg BW Day 70 until day 90 of gestation	40	↑ birth weights ↓ the number of eggs nests and primordial follicles ↑ the number of developing follicles ↑ levels of FSH, LH, estradiol, testosterone and progesterone in piglet at birth

[1]Quanities are based on a 200kg sow

[2]↑ = increased, ↓ = decreased, IgG = immunoglobulin G, GH = growth hormone, IGF-1 = insulin-like growth factor 1, FSH = follicle-stimulating hormone, LH = luteinising hormone, mTOR = mammalian target of rapamycin

1.6. Concluding remarks

The hyper-prolific sow now commonly produces over 14 piglets per litter with the top 10 % of sows producing over 17 piglets per litter. However, pre-weaning mortality levels in Britain are a growing concern for commercial industry (AHDB, 2019c). Reduced birth weights, increased anoxia and reduced colostrum consumption have been associated with pre-weaning mortality (Alonso-Spilsbury et al., 2005, Canario et al., 2007, Devillers et al., 2011). Recent research has focused on sow nutrition as a way to improve piglet viability at birth and performance to weaning. The transition period from gestation to lactation appears to be a critical time point for the sow in terms of both foetal growth and colostrum production (Hansen et al., 2012, Theil, 2015). Focusing on improving sow nutrition across this period is essential.

HMB has been found to have anti-catabolic (Ostaszewski et al., 2000), anabolic (Eley et al., 2007), lipolytic (Wilson et al., 2008, Flummer and Theil, 2012) and immunostimulatory effects (Siwicki et al., 2003). Research suggests that the use of HMB as a supplement enhances performance, and a few studies have provided promising results with regard to the effect of supplementing HMB to sows in gestation on sow and litter performance. However, the replication is poor, the results are inconsistent and the dosage and duration of administration ambiguous.

1.7. Aims of this research

The aims of this research are to determine the effects of supplementing HMB to sow diets during the transition period on litter and piglet performance to weaning and on colostrum production. In addition, this work will attempt to determine the optimum dose and duration of feeding supplemental HMB to sows during the transition period.

Specific objectives:

- To determine whether there is a dose-dependent effect of supplementing HMB to sow diets during the transition period on piglet birth weight, viability, performance to weaning and on colostrum production.
- To determine whether there is an effect of the duration of feeding supplemental HMB to sows during the transition period on piglet birth weight, viability, performance to weaning and on colostrum production.

- To determine how to optimise any beneficial effects HMB may have on sow and litter performance through additional nutritional supplements to the sow during the transition period and throughout lactation.

2.

General Methods

2.1. General methods overview and ethics statement

This chapter has been included to prevent the repeat of similar methodology across experiments. All experimental protocols received ethical approval from the University of Leeds Animal Welfare and Review Body. Veterinary advice was sought if there were any problems. All experiments complied with the Animal (Scientific Procedures) Act 1986, as revised by the Directive 2010/63/EU. Pig housing and husbandry practices were in line with the Council Directive 2008/120/EC standards and the Welfare of Farmed Animals (England) Regulations 2007. The principles of the '3Rs', as set out by the Directive 2010/63/EU, were applied throughout all experimental design processes. The number of replicates required per experiment was determined using Equation 3 as stated in Section 1.5 (Morris, 1999).

2.2. Animal Husbandry

All experiments were conducted at the University of Leeds farm, Spen Farm. Spen Farm consists of an indoor and an outdoor pig production system. Unless stated otherwise, each experiment was conducted on the indoor production system. All experiments on the indoor production system used cross-bred gilts/sows sourced from JSR [(Large White × Landrace) × JSR Geneconverter 900]. Any experiment on the outdoor production system used gilts/sows sourced from Rattlerow [(Large White × Landrace × Duroc) × Tendershire].

2.2.1. Standard Spen Farm practice

2.2.1.1. Indoor production system

Unless stated otherwise the pigs used in each experiment were treated according to standard Spen Farm practice before and after the experiment. Spen Farm's indoor production system consisted of a 200 sow herd and worked on a three week batch farrowing system. Pigs were reared on site from birth until slaughter. Until September 2017 (for experiments 1 and 2), from service until ~ d 109 of gestation, sows were housed in groups of ~ eight, in straw yards and floor fed. As of September 2017 (for experiment 3), from service until ~ d 109 of gestation, sows were housed on straw at the adjacent Wise Warren Farm as one dynamic herd and fed from electronic sow feeders (ESFs; Nedap Electronic Sow Feeder,

Nedap Livestock Management, The Netherlands). Sows were housed individually in conventional farrowing crates in the farrowing house (~ d 109) in rooms containing between 6 and 10 individual farrowing pens measuring 1.4 m × 3.0 m. Each pen had a creep area at the front with a heat lamp. Sows were randomly allocated a room and a pen based on their treatment for the purpose of all experiments. Sows were expected to farrow on d 115 of gestation. Farrowing was not induced for any of the experiments. Sows remained in farrowing crates with their piglets for ~ four weeks. Whilst in the farrowing house piglets were fed by the sow, had free access to water and would normally have received a commercial creep feed. However, for the purpose of all experiments, no creep feed was offered to the piglets in order to determine the effect of maternal milk production on piglet growth. Piglets received the following management treatment: tagged on the left ear for identification, teeth clipped, tails docked and an intra-muscular injection of iron (200 mg) as gleptoferron within 24 hours of birth, an oral shot of Baycox (0.4 ml) for the prevention of coccidiosis on d 4, and an intra-muscular injection of Suvaxyn PCV Mhyo (2 ml) at weaning, for the prevention of Porcine Circovirus and pneumonia caused by Mycoplasma hyopneumoniae. At weaning sows were returned to the main herd and served again four days later. Piglets were weaned at ~ 28 d and were moved into a flat deck weaner-grower facility.

2.2.1.2. Outdoor production system

The outdoor production system consisted of a 220 sow unit which also worked on a three week batch farrowing system. Piglets were reared on site from birth until weaning. Sows were kept in group outdoor paddocks with access to straw bedded arcs and fed from ESFs from service until ~ d 108 of gestation. On d 108 of gestation, sows were moved to individual farrowing paddocks measuring 15 m × 15 m with access to a straw bedded arc with a creep fender attached to the front to provide a protective run for piglets. Each paddock also had an individual feeding stall which the sow could be locked away in. Sows were randomly allocated a farrowing paddock for the purpose of any experiment. Farrowing was not induced for any experiments. When on trial, piglets were tagged on the left ear for identification. Fenders were removed from the arcs on ~ d 14 of lactation. Piglets were weaned at ~ 26 d and given an intra-muscular injection of Suvaxyn PCV Mhyo (2 ml). At weaning piglets were sold. Sows were returned to the main outdoor herd and served again four days later.

2.2.2. Feed and water

Gestation and lactation diets were formulated and manufactured by Associated British Nutrition (ABN, Peterborough UK), the industry sponsors of these trials and a subsidiary of Associated British Agriculture (AB Agri, Peterborough, UK). All diets were pelleted to 6 mm. The diets for experiments 1 and 2 (described in Chapters 3 and 4, respectively) were produced in bulk and silos were filled up weekly. The diets for experiment 3 (described in Chapter 5) were produced in bulk, but bagged. Representative samples of diets were collected weekly and stored at - 20 °C pending analysis. Details of the diet specifications are provided in the relevant chapters. Throughout each experiment sows had free access to water. In each experiment, sows were fed a restricted allocation of feed during gestation once daily. From the start of each trial until movement to farrowing accommodation, sows were brought out of their straw yards and fed a pre-weighed amount of feed in individual feeding stalls. Once in the farrowing house, they were fed in their farrowing crates. Sows were fed their gestation diets from the start of the trial until they farrowed. Any feed refusals were weighed back. Sows were fed a pre-weighed amount of feed based on their body weight and parity on entry to the trial. Body weight was used to calculate the sows' energy requirement for maintenance using the equation for body maintenance and then they were given an additional amount for growth based on their parity (NRC, 2012):

Equation 4

$$Maintenance\ MJNE/Day: \frac{BW^{0.75} \times 0.415}{0.96} \times 0.71$$

Where BW = body weight (kg)

Parity 1: + 10 MJNE/day

Parity 2 and above: + 6.5 MJNE/day

Sows were allocated their lactation diets from farrowing until weaning by farm staff. Feed allowance in lactation was increased daily to appetite. Each sow had an individual bag of feed in front of her pen which was weighed back and topped up twice weekly in order to calculate feed intake throughout lactation.

2.3. HMB

The HMB used in each of the experiments came as HMB calcium anhydrous (sourced from Direct Food Ingredients, Macclesfield, UK); a white crystalline powder with a minimum of 99 % purity. It will be referred to throughout this thesis as HMB. In experiments 1 and 2 the same batch of HMB was used and it was provided to the sows as a top-dressing onto standard diet. When provided as a top-dressing the HMB was dissolved in 10 ml apple squash and 40 ml water to encourage intake. The control groups received the apple squash and water as a top-dressing onto the same commercial diet but without the HMB. In experiment 3 a different batch of HMB was used and it was incorporated directly into the relevant diet during manufacture.

2.4. Measurements

Sows were weighed and back-fat measurements were taken on entry to the trial, movement to the farrowing house and at weaning. Back-fat measurements were taken at the P2 position using a digital back-fat indicator (Renco Lean-Meter, Renco Corporation, Minneapolis, MN, USA for experiments 1 and 2 and BFM-1 Backfat Meter, SonopTek, Beijing, China for experiment 3) which was placed on the back of the sow on the last rib, 6 - 7 cm from the side of the backbone (Oliviero et al., 2009). Oil was used as a lubricant between the probe and the sow's skin. Back-fat measurements were taken on the sow's left side each time. Sows were given body condition scores using the following scoring system: 1 = emaciated: shoulders, individual ribs, hips and backbone are visually apparent; 2 = thin: shoulders, ribs, hips and backbone are quite easily felt when pressure is applied with the palm of the hand; 3 = acceptable/optimal: shoulders, ribs, hips and backbone can only be felt when pressure is applied; 4 = fat: shoulders, ribs, hips and backbone cannot be felt even when pressure is applied; 5 = grossly fat: fat deposits are clearly visible (AHDB, 2017b).

Cameras (D-Link DCS-4603) were placed on the back ceiling of the farrowing house such that one camera covered the backs of two farrowing pens. Cameras were connected to a switch board (NETGEAR PROSAFE SMART SWITCH) and an NVR (D-LINK NVR, DNR-20-20-04P). Cameras were set to record throughout the farrowing period and switched off once both sows the camera was covering had farrowed.

Piglets were weighed and tagged within 24 hours of birth, at an average d 7 and at weaning. Litter sizes (numbers alive, dead and mummified) were recorded. Any piglets that died during the trial were recorded and weighed. Fostering of piglets between sows was completed between 24 and 48 hours post-partum to allow for adequate colostrum intake from the maternal sow. Fostering was kept within treatment. To do this numbers were placed above each sow to detail which treatment group each sow was in. Piglets were then able to be fostered between sows of the same number. All fostering of piglets was recorded. Fostered piglets were included in performance analysis of the litter they were transferred onto. Foster sows which were used were excluded from the analysis.

2.4.1. Colostrum samples

Colostrum samples were collected as close to the start of farrowing as possible (preferably before the birth of the third piglet). Approximately 15 ml was collected manually from a combination of functional teats, aliquoted out and then frozen at - 20 °C until analysis.

2.4.2. Estimation of colostrum intake and yield

Individual colostrum intake by piglets was estimated by piglet weight gain between birth and 24 hours using Equation 2 (as described in Section 1.3.3.1) (Theil et al., 2014b). The duration of colostrum suckling (D) was taken as time between first and second weighing. Colostrum yield from the onset of farrowing until 24 hours post-partum was calculated as the sum of the intakes by each piglet in the litter plus the weight of sample that was taken for analysis. Piglets that died prior to 24 hours were not included in this calculation.

2.5. Laboratory analysis

2.5.1. Colostrum preparation for immunoglobulin analysis

Colostrum samples were thawed in the fridge overnight at 4 °C. Once thawed, samples were vortexed for 15 secs to ensure a homogenous mixture. 500 µl of colostrum was transferred to an eppendorf and centrifuged at 3,000 g for 10 mins to separate the fat from skim colostrum, using a standard bench top micro-centrifuge. 200 µl of skimmed colostrum was removed from the centrifuged sample and transferred to another eppendorf. This was then vortexed for 5 secs, aliquoted out into three eppendorfs and re-frozen at - 20 °C until IgA, IgG and

IgM analysis. Skimmed colostrum was analysed within one week of being centrifuged.

2.5.1.1. Immunoglobulin analysis

Total IgA, total IgG and total IgM were measured in all skimmed colostrum samples by enzyme-linked immunosorbent assay (ELISA). Immunoglobulin concentrations were analysed using commercial kits (IgA [E100-102]; IgG [E100-104]; IgM [E100-117] ELISA Quantitation Kit, Bethyl Laboratories, Montogomery, TX).

Wash buffer, coating buffer, blocking buffer and sample diluent were prepared by re-combining the packets with deionised (DI) water to the volume described on the packet, and the enzyme substrate TMB and stop solution came as a ready to use solution (E101 and E115; Bethyl Laboratories, Montogomery, TX). All components were used at room temperature.

Skimmed colostrum aliquots were thawed in the fridge on the morning of analysis. Samples, standards and HRP conjugate solution were diluted on the day of analysis. Each sample and standard was run in duplicate. Affinity purified coating antibodies for IgA, IgG or IgM, were diluted in coating buffer (0.05 M Sodium Carbonate-Bicarbonate, pH 9.6) and 100 µl of diluted coating antibody was added to each well of a 96 well plate. The plate was left for one hour at room temperature and then washed five times by aspiration with Tris-buffered saline (TBS) (50 mM Tris, 0.14 M NaCl, 0.05 % Tween 20, pH 8.0). After washing, 200 µl of blocking buffer (50 mM Tris, 0.14 M NaCl, 1 % BSA, pH 8.0) was added to each well and the plate was incubated at room temperature for 30 mins. The plate was then washed five times and blotted. Colostrum samples were diluted (1:200,000 for IgA, 1:500,000 for IgG and 1:25,000 for IgM). Standard dilutions from 0 to 1000 ng/ml for IgA and IgM and from 0 to 500 ng/ml for IgG were prepared. 100 µl of standard or diluted sample was added to each well and incubated for one hour at room temperature. The plate was washed five times and blotted. 100 µl of diluted horseradish peroxidase (HRP) conjugated goat anti-pig IgA, IgG or IgM detection antibody was added to each well and incubated for one hour at room temperature. The HRP was diluted as follows: 1:75,000 for IgA and IgG and 1:50,000 for IgM. The plate was washed five times and blotted. 100 µl of enzyme substrate 3,3', 5,5'- Tetramethylbenzidine (TMB), was added to each well. The plate was left to develop in a dark room at room temperature for a set time (IgA: 12 mins; IgG: 9

mins; IgM: 14 mins). A dark blue colour was formed, the intensity of which was directly proportional to the amount of IgA, IgG or IgM present in the skimmed colostrum sample. The reaction was stopped after the designated time period by the addition of 100 µl of stop solution (0.18 M H_2SO_4) to each well. The colour changed from a dark blue to a dark yellow again the intensity of the yellow was directly proportional to the amount of immunoglobulin present in the sample.

The absorbance was measured at 450 nm using a SPECTAmax™ 340 (Molecular Devices, California, USA) within 15 mins of the stop solution being added. The concentrations of IgA, IgG and IgM were calculated using the average absorbance value against the standards (minus the blank from each absorbance). Colostrum absorbance data were put into GraphPad PRISM (version 8, GraphPad Software, California, USA). Absorbance values were $\log_{10}$ transformed and analysed by a 4-parameter curve. Data were then log transformed back and multiplied by the dilution factor to get the concentrations of IgA, IgG and IgM. Intra and inter-assay CVs were calculated to determine variance between plates.

2.5.2. Feed analysis

Dietary samples were sent for Sciantec Analytical Services Ltd (Cawood, UK) for crude protein, crude fibre, total fat and amino acid analysis. Crude fibre was analysed using the Ankom 220 Analyser. Crude protein was analysed by the DUMAS method. Oil A was determined by direct solvent extraction. Amino acid composition as analysed via ion exchange chromatography. See Appendix A.1 for further details.

2.6. CCTV footage

CCTV footage was used to work out the farrowing duration of each sow defined as the time of birth of the first piglet until the time of birth of the last piglet (van Rens and van der Lende, 2004, van Dijk et al., 2005) and was also used to work out between birth intervals for each piglet and average time to suckle for each piglet. When no personnel were present at a farrowing the video footage was used to determine whether any piglets found dead were still born or whether they had survived parturition and died afterwards.

3.

The effects of supplementing sows with β-hydroxy β-methyl butyrate in late gestation on piglet performance and colostrum production are dose-dependent

3.1. Abstract

HMB supplementation to sow diets in gestation has been shown to improve piglet and litter pre-weaning performance, however the optimum inclusion level is unclear. This study aimed to determine whether there were dose-dependent effects of HMB supplementation of sow diets in gestation on colostrum production, piglet birth weight and growth to weaning. A total of 140 (Large White × Landrace) multiparous sows were randomly assigned to one of four treatments on d 100 of gestation. The treatments included a control diet supplemented with HMB at doses of: 0 (CT + 0), 5 (CT + 5), 15 (CT + 15) or 45 (CT + 45) mg/kg of the sow's body weight. Sows were fed their treatment diets from d 100 of gestation until parturition and the study ended at weaning when the piglets were ~ 28 d old.

The total number of piglets born, born alive and born dead were not affected by maternal dietary treatments. HMB supplementation to sows increased total live born litter weight and average piglet 24 hour weight in a quadratic manner (*P*=0.002 and *P*=0.004, respectively) compared with the CT + 0 treatment. HMB supplementation to sows improved piglet week one ADG in a linear fashion (*P*=0.047) and increased average piglet week one weight in a quadratic manner (*P*=0.021) compared with the CT + 0 treatment. There were no differences in average wean weights between maternal dietary treatments. HMB supplementation to sows reduced the 24 hour mortality percentage in a quadratic manner (*P*=0.045) and this remained a trend at weaning (*P*=0.055).

There was a quadratic increase in colostrum yield (*P*=0.004) and in colostrum intake of piglets (*P*=0.005) in response to HMB supplementation. Additionally, HMB supplementation increased the IgG concentration of sow colostrum in a linear fashion (*P*=0.002); however, there were no effects of HMB supplementation on IgA or IgM concentrations.

In conclusion, this study demonstrated that supplementation of HMB to sows in late gestation improved piglet 24 hour weight in a quadratic dose-dependent manner. In addition, HMB supplementation to sows improved the quality of colostrum produced linearly and the quantity of colostrum produced in a quadratic dose-response manner, and this is reflected in the increased early performance of piglets and the tendency for a reduction in percentage mortality by weaning.

3.2. Introduction

Pre-weaning mortality of piglets is an ongoing issue for commercial pig units, with mortality rates in British herds averaging ~ 12 % (AHDB, 2019c). The drive for hyper-prolific sows has increased the average number of piglets born per litter from 12.5 pigs per litter in 2010, to 14.6 pigs per litter in 2018 (AHDB, 2019c). Whilst it is economically more beneficial to produce more piglets per sow, it has come with negative side effects such as: increased still births, increased low birth weights and increased variation in within litter birth weight (Canario et al., 2007), increased anoxia (Alonso-Spilsbury et al., 2005) and reduced individual colostrum consumption (Devillers et al., 2011).

The transition period between gestation and lactation is a critical period for the sow and her litter as colostrum is synthesised (Schneider, 1991, Hansen et al., 2012), nutrients are relocated from the blood to the mammary tissue and milk production begins (Hansen et al., 2012). In addition, approximately one third of foetal weight gain occurs in the last 10 days of gestation (Noblet et al., 1985, Theil, 2015). Therefore, the diet the sow receives over this period is of key importance for parturition and the piglets' subsequent performance (Theil, 2015).

β-hydroxy β-methyl butyrate (HMB), a metabolite of leucine, has been studied in recent years in both animal and human research. It has been shown to enhance protein synthesis through the activation of the mTOR pathway and attenuate protein degradation via the down regulation of the ubiquitin-proteasome pathway (Smith et al., 2004, Eley et al., 2007). HMB has also been shown to have lipolytic and immunostimulatory effects (Krakowski et al., 2002, Wilson et al., 2008). Positive effects of HMB supplementation have been found in many species including: fish, mice and rats, broilers and pigs (Szcześniak et al., 2015). Moreover, several studies have investigated the impact of HMB supplementation to sow diets in late gestation on sow and litter productivity. HMB has been found to improve piglet birth weight (Tatara et al., 2007, Tatara et al., 2012, Blicharski

et al., 2017), colostrum yield (Flummer and Theil, 2012) and colostrum IgG concentration (Krakowski et al., 2002). However, the results are inconsistent. The doses used in sow studies have been found vary from 10 mg/kg BW daily (Nissen et al., 1994) to 200 mg/kg BW daily (Blicharski et al., 2017). Establishing the optimum dose of HMB supplementation may explain some of the discrepancies.

3.2.1. Hypothesis

HMB supplementation to sow diets for the last 15 days of gestation will improve colostrum production and piglet performance to weaning in a dose-dependent manner.

3.2.2. Study aims

- To determine the effect of HMB supplementation to sows at different doses on immunoglobulin concentrations (IgA, IgG and IgM) in sow colostrum.
- To determine the effect of HMB supplementation to sows at different doses on the yield of sow colostrum produced.
- To determine whether there is a dose-dependent effect of HMB supplementation to sows on piglet birth weight and total litter weight.
- To determine whether there is a dose-dependent effect of HMB supplementation to sows on piglet growth rate from birth to weaning.
- To determine the effect of HMB supplementation to sows at different doses on pre-weaning mortality.

3.3. Materials and Methods

3.3.1. Experimental design and dietary treatments

This experiment was a dose-response design with four treatments. Sows were fed experimental diets from d 100 of gestation until parturition. After parturition sows were fed a commercial lactation diet. All sows received the same standard gestation diet and HMB was provided as a top-dressing to this diet at doses of 0, 5, 15 or 45 mg/kg BW dissolved in apple squash (Section 2.3). Sows were fed an individual pre-weighed quantity of feed based on their body weight on entry to the trial (d 100 BW ± SEM = 270.7 ± 4.41 kg) using Equation 4 (as described in Section 2.2.2) (NRC, 2012).They were also fed individual quantities of HMB based on their body weight on d 100 of gestation.

The treatments included: (CT + 0) the control diet top-dressed with 0 mg HMB per kg of sow BW, (CT + 5) the control diet top-dressed with 5 mg HMB per kg of

sow BW, (CT + 15) the control diet top-dressed with 15 mg HMB per kg of sow BW and (CT + 45) the control diet top-dressed with 45 mg HMB per kg of sow BW. The doses of HMB were selected based on levels previously tested in the literature; they were selected to cover a wide range of doses in order to obtain a more accurate estimation of the pattern of response. The standard gestation diet was formulated to contain 9.23 MJ NE/kg and 3.9 g/kg SID lysine and meet all nutrient requirements of the gestating sow (NRC, 2012).

In lactation all sows were fed the same commercial lactation diet (9.99 MJ NE/kg and 8.2 g/kg SID lysine) formulated to meet all nutrient requirements of the lactating sow (NRC, 2012). Feed allowance in lactation was increased by ~ 0.5 kg/d to appetite. The diet compositions and calculated nutrient levels for the control gestation (prior to treatment with HMB) and for the lactation diet are presented in Table 3.1.

Table 3.1. Composition and calculated nutrient specifications for the gestation and lactation diets (%, as-fed basis)

Diet	Gestation diet[1]	Lactation diet
Ingredient		
Barley	15.00	-
Wheat	44.67	47.78
Wheat feed	30.00	13.17
Soyabean meal	1.50	7.39
Maize meal	-	10.00
Bakery meal	-	4.35
Rapeseed extract	-	4.00
Sunflower meal	-	2.72
Vitamin-mineral premix[2]	0.25	0.25
L-Lysine liquid	0.10	0.57
DL-Methionine	-	0.04
Threonine	-	0.14
L-Tryptophan	-	0.00
Choline chloride	0.03	0.03
Yeast	-	0.10
Limestone	1.35	0.92
Dicalcium phosphate	0.20	0.77
Salt	0.50	0.26
Sodium bicarbonate	-	0.03
Soya oil	-	1.71
Vegetable fat	1.40	0.75
Glucose syrup + Raffinate	5.00	5.00
Calculated nutrient composition		
Net energy (MJ/kg)	9.23	9.99
Crude protein (%)	11.64	15.30
Crude fibre (%)	4.00	4.00
SID Lysine (%)	0.39	0.82
SID Methionine + Cystine (%)	0.37	0.50
SID Threonine (%)	0.31	0.57
SID Tryptophan (%)	0.12	0.16
SID Leucine	0.64	0.87
Calcium (%)	0.69	0.79
Total Phosphorus (%)	0.43	0.56
Digestible Phosphorus (%)	0.24	0.33

[1]β-hydroxy β-methyl butyrate was added as a top-dressing to the gestation diet at doses of 0, 5, 15 or 45 mg/kg BW to create the CT + 0, CT + 5, CT + 15 and CT + 45 treatments respectively

[2]Vitamin and trace mineral premix provided per kg of the diet: 10,000 IU vitamin A, 1850 IU vitamin D_3, 50 IU vitamin E, 4 mg vitamin K, 1.5 mg thiamine (B_1), 4 mg riboflavin (B_2), 3.5 mg pyridoxine (B_6), 15 ug vitamin B_{12}, 12 mg pantothenic acid, 20 mg nicotinic acid, 200 µg biotin, 2 mg folic acid, 15 mg copper, 1 mg iodine, 80 mg iron, 50 mg manganese, 0.25 mg selenium, 100 mg zinc, 100 mg oxy-nil dry and 150 mg phytase.

3.3.2. Animals and Management

One hundred and forty mixed parity sows (Large White × Landrace [JSR Genepacker 90, JSR, UK]) were used across six consecutive batches (22 to 27 sows per batch) and followed for one parity. On d 100 of gestation sows were allocated to one of the four dietary treatments, primarily on the basis of parity (range 1:8) and then matched for previous litter history, d 100 body weight and back-fat thickness. Sows were housed throughout gestation and lactation according to standard Spen Farm practice (as described in Section 2.2.1.1) but were moved to farrowing accommodation on d 112 of gestation. Sows were fed as described in Section 2.2.2.

Piglets received the management treatment as stated in Section 2.2.1.1. Cross fostering commenced as stated in Section 2.4. Foster sows were introduced when the number of piglets was still too high post fostering. One foster sow was introduced in batch one and two foster sows were introduced in batch six. Across the whole trial 2,102 piglets were born to the 140 sows and of these 117 were stillborn.

3.3.3. Measurements

Measurements including sow weights, back-fat thickness, feed intake, litter sizes and piglet weights were recorded as described in Section 2.4. Cameras were placed in the farrowing house as described in Section 2.4. Colostrum samples were collected as described in Section 2.4.1, before the birth of the third piglet. Piglets averaged 7.8 ± 0.29 d and 27.9 ± 0.29 d (average age ± SEM) at week one and at weaning, respectively.

3.3.4. Farrowings attended

Sixty five litters had fully supervised farrowings and these were used to study performance within the first 24 hours of farrowing. At farrowing each piglet was given a vitality score based on its behaviour in the first 15 secs after birth using the following scoring system devised by Baxter et al. (2008) 0 = no movement and no breathing after 15 secs; 1 = no movement after 15 secs but the piglet is breathing or attempting to breath; 2 = piglet shows some movement within 15 secs, breathing or attempting to breath; 3 = good movement, good breathing, piglet attempts to stand within 15 secs. When the piglet's umbilical cord had broken, piglets were weighed, their temperatures taken using a tympanic ear thermometer (Tesco Digital Ear Thermometer, Tesco, UK) and they were tagged

on the left ear for subsequent identification. Piglets were placed back at the point they were picked up from. Piglet temperatures were taken again 24 hours after the start of farrowing and were re-weighed.

3.3.5. Laboratory analysis

Colostrum samples were analysed as described in Sections 2.5.1 and 2.5.1.1. Samples from the same batch were analysed on the same plate. The intra- and inter-assay CVs were: 3.0 % and 9.5 % for IgA, 2.9 % and 9.2 % for IgG, and 3.4 % and 10.9 % for IgM.

3.3.6. Calculations and statistical analysis

Data were analysed using SPSS Statistics (version 21.0, SPSS Inc., Chicago IL, USA). All data (including performance, colostrum and piglet birth data) were analysed using polynomial contrasts for unequally spaced increments to test for linear and quadratic responses to HMB. Treatment means were compared using the following single degree-of-freedom orthogonal contrasts: CT + 0 vs CT + 5, CT + 15, CT + 45; CT + 5 vs CT + 15, CT + 45; CT + 15 vs CT + 45. Data were first tested for homogeneity of variance and normality using the Levene's test and the Kolmogorov-Smirnov test, respectively. Data displaying heteroscedasticity or non-normal data were $\log_{10}$ transformed prior to statistical analysis. Transformed data were back transformed for inclusion in the respective tables. If after log transformation the data still did not conform to homogeneity or normality, then a Generalised Linear Model were used on the untransformed data. Farrowing duration, birth interval, vitality score, time to suckle, CV for wean weights and IgA concentration in colostrum required $\log_{10}$ transformation. A generalised linear model was used to analyse the number of piglets born dead per litter.

The sow/litter was the experimental unit for all analyses. Birth data were based on the sow's genetic litter and post 24 hours data were based the suckling litter (post-fostering). Foster sows were not included in the analysis. The statistical model included treatment, parity and batch as fixed factors. Total number of pigs born was used as a covariate for average birth weight and total litter birth weight. Litter size post-fostering and age were used as covariates, when significant, for analysis of piglet performance to weaning. Mortality was calculated as the percentage of piglets that died, out of the total number of piglets that were born alive in a litter and was based on the sow's genetic litter. Total number of piglets born was used as a covariate for mortality. Birth intervals, vitality scores and time

to suckle were averaged out for each sow, thus the sow was the experimental unit for analysis. Colostrum intake by piglets was determined using Equation 2 (as described in Section 1.3.3.1) and yield was determined by the sum of intakes by piglets in that litter (Section 2.4.2) (Theil et al., 2014b). Total born was used as a covariate for vitality and colostrum analysis. Parity has been found to influence levels of immunoglobulins in sow colostrum (Cabrera et al., 2012), therefore when it was significant it was included as a covariate instead of a fixed factor for immunoglobulin analysis as by Leonard et al. (2010). Significance was reported at $P<0.05$ and as trends if $P<0.1$. Data are expressed as least-square means with their pooled standard error of the mean (SEM).

3.4. Results

Overall, the litters performed well throughout the experiment. Across the whole trial 10 litters were excluded from post birth analysis (3 from CT + 0, 4 from CT + 5 and 3 from CT + 15) due to sow sickness or the requirement to use them as foster sows. Performance data up to 24 hours represents a total of 140 litters split across the treatments as follows: CT + 0, $n = 34$; CT + 5, $n = 38$; CT + 15, $n = 35$; CT + 45, $n = 33$. Post birth analysis represents a total of 130 litters split across the treatments as follows: CT + 0, $n = 31$; CT + 5, $n = 34$; CT + 15, $n = 32$; CT + 45, $n = 33$. The results presented are mean ± SEM.

3.4.1. HMB intake

The daily intake of HMB for sows in the CT + 5 treatment averaged 1.21 ± 0.040 g/d and ranged from 0.85 to 1.75 g/d. The total intake of HMB for sows fed CT + 5 averaged 17.39 ± 0.590 g and ranged from 12.53 to 25.32 g. The daily intake of HMB for sows in the CT + 15 treatment averaged 3.64 ± 0.102 g/d and ranged from 2.50 to 4.79 g/d. The total intake of HMB for sows in the CT + 15 treatment averaged 52.81 ± 1.58 g and ranged from 32.49 to 70.43 g. The daily intake of HMB for sows in the CT + 45 treatment averaged 10.81 ± 0.288 g/d and ranged from 7.79 to 14.09 g/d. The total intake of HMB for sows fed CT + 45 averaged 153.48 ± 4.614 g and ranged from 103.84 to 205.56 g.

3.4.2. Sow characteristics

Sow characteristics are presented in Table 3.2. The average sow parity was 3.3 ± 0.34. Sow weights and back-fat measurements were similar on entry to the trial and averaged 271.4 ± 3.97 kg and 15.5 ± 0.69 mm, respectively across all

treatments. There was a tendency for a linear dose-response relationship between gestation length and treatment (*P*=0.092). On average the gestation length of sows in the HMB treatments was 0.58 d shorter than that of sows receiving the CT + 0 treatment (*P*=0.036). Sow average daily feed intake (ADFI) throughout lactation was similar across treatments (*P*=0.604), however, there was a trend for a quadratic dose-response relationship between total feed intake (FI) and HMB supplementation (*P*=0.078). Sows in the CT + 15 and CT + 45 treatments had a 2.7 % higher total FI in lactation compared with sows in the CT + 5 treatment (*P*=0.068). Sow weight and back-fat changes from d 100 to d 112 of gestation were similar across treatments and averaged + 10.1 ± 1.14 kg and + 0.4 ± 0.71 mm, respectively. Sow weight and back-fat changes from d 112 of gestation to weaning were similar across treatments and averaged - 45.1 ± 3.70 kg and - 0.3 ± 0.93 mm, respectively.

3.4.3. Litter performance

Litter performance is presented in Table 3.3. Total numbers of piglets born were similar across treatments (*P*=0.426) and averaged 15.1 ± 0.71 piglets/litter. Numbers of piglets born alive and born dead were not affected by treatment (*P*=0.551 and *P*=0.682 respectively). HMB supplementation increased total born litter weight in a quadratic manner (*P*=0.002); supplementing the standard gestation diet with HMB at a dose of 15 mg/kg BW increased total born litter weight by 8.4 % compared with the CT + 0 treatment. Total born litter weight was 8.4 % heavier in the CT + 15 treatment compared with the CT + 45 treatment (23.2 vs 21.4 kg for CT + 15 vs CT + 45, respectively; *P*=0.005). HMB supplementation also increased total live born litter weight in a quadratic fashion (*P*<0.001); HMB supplementation increased total live born weight by 5.2, 12.0 and 2.1 %, in treatments CT + 5, CT + 15 and CT + 45, respectively compared with CT + 0. The quadratic relationship between litter weight and HMB supplementation was also apparent at week one (*P*=0.021), with litter weights being 1.8, 7.8 and 1.4 % higher in treatments CT + 5, CT + 15 and CT + 45, respectively compared with CT + 0. There was a tendency for sows in the CT + 15 treatment to have a heavier litter weight at week one compared with sows in the CT + 45 group (30.5 vs 28.7 kg for CT + 15 vs CT + 45, respectively; *P*=0.075). By weaning the relationship between HMB supplementation and litter weight remained a quadratic trend (*P*=0.056).

The numbers of piglets on each sow at week one and at weaning were similar across treatments. HMB supplementation reduced percentage 24 hour mortality in a quadratic manner (*P*=0.045); percentage 24 hour mortality was 41.3 % lower in the CT + 15 treatment than the CT + 0 treatment. The quadratic relationship between percentage mortality and HMB supplementation remained a trend at weaning (*P*=0.055).

Table 3.2. The effects of β-hydroxy β-methyl butyrate on sow characteristics when supplemented to sow diets at doses of 0, 5, 15 or 45 mg/kg body weight for ~ 15 days prior to parturition[1]

HMB mg/kg BW[2]	Parity	Gestation length (d)	Sow d100 weight (kg)	Weight change (kg)		Sow d100 back-fat (mm)	Back-fat change (mm)		Lactation FI[2] (kg)	
				d100 to d112	d112 to wean		d100 to d112	d112 to wean	Total FI[3]	ADFI[3]
0	3.3	114.4	272.7	+ 10.3	- 44.2	14.9	+ 1.0	- 0.4	156.9	5.9
5	3.4	113.9	271.4	+ 10.0	- 45.6	15.5	+ 0.2	- 0.3	156.9	5.9
15	3.3	113.8	270.8	+ 9.8	- 46.7	15.9	- 0.1	+ 0.2	162.5	6.0
45	3.1	113.6	270.7	+ 10.1	- 43.7	15.5	+ 0.5	- 0.7	160.0	5.9
SEM	0.34	0.30	3.97	1.14	3.70	0.69	0.71	0.93	2.45	0.11
				P-value						
Overall	0.957	0.184	0.968	0.987	0.883	0.682	0.840	0.853	0.139	0.604
Linear	0.628	**0.092**	0.707	0.967	0.744	0.637	0.780	0.639	0.236	0.969
Quadratic	0.800	0.234	0.752	0.728	0.467	0.266	0.212	0.480	**0.078**	0.253
				Orthogonal contrasts						
0 vs 5 + 15 + 45	0.967	**0.036**	0.638	0.756	0.738	0.280	0.233	0.915	0.223	0.741
5 vs 15 + 45	0.738	0.532	0.852	0.955	0.898	0.739	0.970	0.991	**0.068**	0.444
15 vs 45	0.650	0.709	0.979	0.840	0.471	0.623	0.517	0.381	0.373	0.279

[1]Data represents a total of 140 litters split across the four treatments as follows: 0 *n* = 34; 5 *n* = 38; 15 *n* = 35; 45 *n* = 33
[2] HMB = β-hydroxy β-methyl butyrate, BW = body weight, FI = feed intake, ADFI = average daily feed intake
[3]Corrected for litter size

Table 3.3. The effects of β-hydroxy β-methyl butyrate on litter performance to weaning when supplemented to sow diets at doses of 0, 5, 15 or 45 mg/kg body weight for ~ 15 days prior to parturition[1]

Variable	HMB mg/kg BW[2]				SEM	*P*-value			*Orthogonal contrasts*		
	0	5	15	45		Overall	Linear	Quadratic	0 vs 5 + 15 + 45	5 vs 15 + 45	15 vs 45
Numbers of piglets											
Total born	14.9	15.1	14.9	15.6	0.71	0.426	0.120	0.863	0.201	0.612	0.327
Alive[3]	14.1	14.2	14.0	13.8	0.23	0.551	0.163	0.888	0.611	0.283	0.380
Dead[3]	0.9	0.9	1.0	1.2	0.23	0.551	0.163	0.888	0.611	0.283	0.380
Week 1	11.4	11.6	11.7	11.4	0.29	0.844	0.911	0.390	0.535	0.920	0.517
Wean	10.9	11.3	11.4	11.1	0.33	0.478	0.849	0.159	0.173	0.869	0.445
Litter weights (kg)											
Total born[3]	21.4	21.7	23.2	21.4	0.56	**0.012**	0.680	**0.002**	0.176	0.286	**0.005**
Total live born[3]	19.1	20.1	21.4	19.5	0.61	**0.005**	0.871	**<0.001**	**0.029**	0.510	**0.006**
Week 1[4,5]	28.3	28.8	30.5	28.7	0.86	0.127	0.855	**0.021**	0.197	0.339	**0.075**
Wean[4,5]	79.1	80.1	82.7	79.0	1.83	0.261	0.705	**0.056**	0.403	0.689	**0.076**
Litter gains (kg)											
Birth to week 1[4,5]	9.3	9.7	10.7	10.2	0.71	0.369	0.318	0.152	0.220	0.244	0.568
Week 1 to wean[4]	50.5	51.4	52.3	50.2	1.56	0.631	0.644	0.225	0.568	0.921	0.238
Birth to weaning[4]	60.0	61.5	63.4	61.1	1.78	0.447	0.827	0.107	0.248	0.669	0.276
24 hour mortality[3] (%)	7.5	8.4	4.4	8.2	1.48	0.990	0.766	**0.045**	0.733	0.169	**0.038**
Week 1 mortality[3] (%)	15.4	16.6	10.8	15.6	2.47	0.165	0.969	**0.068**	0.630	0.155	**0.089**
Wean mortality[3] (%)	19.3	18.6	13.7	17.6	2.51	0.220	0.632	**0.055**	0.267	0.229	0.180

[1]Data represents a total 140 litters split across the four treatments as follows: 0 *n* = 34; 5 *n* = 38; 15 *n* = 35; 45 *n* = 33
[2]HMB = β-hydroxy β-methyl butyrate, BW = body weight
[3]Corrected for total born
[4]Corrected for litter size
[5]Corrected for age

3.4.4. Litter characteristics at farrowing and colostrum production

Litter characteristics at farrowing and colostrum intake and yield are presented in Table 3.4. HMB supplementation did not impact farrowing duration (*P*=0.423), birth interval (*P*=0.483), vitality score (*P*=0.315), average time to suckle (*P*=0.346) or 24 hour temperature of piglets (*P*=0.993). HMB supplementation increased the average live birth weight of piglets (*P*=0.049) and 24 hour weight of piglets (*P*=0.010) in a quadratic manner. Piglets gained an average of 76 ± 15.9 g from birth to 24 hours. HMB supplementation increased piglet 24 hour weight gain in a quadratic fashion (*P*=0.019).

Colostrum intake of piglets alive at 24 hours after birth averaged 409 ± 19.9 g/piglet, and the average individual piglet intake of each litter ranged from 246 to 806 g/piglet per litter. HMB supplementation increased average piglet colostrum intake in a quadratic fashion (*P*=0.005); piglet colostrum intake was 10.2, 22.3 and 13.5 % higher in treatments CT + 5, CT + 15 and CT + 45, respectively than the CT + 0 treatment. There were no differences in colostrum intake between the CT + 5, CT + 15 and CT + 45 groups.

Total yield of colostrum averaged 5.4 ± 0.29 kg/sow and ranged from 3.1 to 8.4 kg. HMB supplementation increased total yield of colostrum in a quadratic manner (*P*=0.004); sows receiving CT + 15 had a 28.5 % higher colostrum yield compared with sows receiving CT + 0. Sows in the CT + 15 treatment produced more colostrum than sows receiving the CT + 45 treatment (5.9 vs 5.6 kg for CT + 15 vs CT + 45, respectively; *P*=0.039).

3.4.5. Piglet growth performance

Piglet growth performance from 24 hours to weaning is presented in Table 3.5. Piglet weight variations (CVs) are presented in Table 3.6. HMB supplementation increased average piglet weight at 24 hours (total), and average piglet live weight at 24 hours in a quadratic manner (*P*=0.002 and *P*=0.004, respectively). HMB supplementation increased average piglet live weight at 24 hours by 3.0, 8.4 and 2.9 % compared with the CT + 0 treatment, for treatments CT + 5, CT +15 and CT + 45, respectively. There was a tendency for piglets from sows receiving CT + 15 to be 5.3 % heavier at 24 hours than piglets from sows receiving CT + 45 (*P*=0.062). HMB supplementation reduced the CV of average piglet weights at 24 hours (total) in a quadratic manner (*P*=0.022). In addition, HMB reduced the percentage of piglets born weighing ≤ 1.1 kg in a quadratic fashion (*P*=0.008)

when considering the total number of piglets born (including stillborn piglets). Sows in the CT + 15 treatment had the lowest percentage of piglets weighing ≤ 1.1 kg compared with the CT + 0 treatment (11.8 vs 18.5 % for CT + 15 vs CT + 0, respectively). Of all the live born piglets, HMB also reduced the percentage of piglets weighing ≤ 1.1 kg in a quadratic manner (*P*=0.005). However, sows receiving CT + 45 group had the highest number of piglets weighing ≤ 1.1 kg.

HMB supplementation increased piglet week one ADG in a linear fashion (*P*=0.047); piglets from sows in the CT + 45 treatment had a 13.5 % higher ADG than piglets from sows in the CT + 0 treatment. HMB increased average piglet week one weight in a quadratic manner (*P*=0.021); there was a tendency for piglets from sows fed CT + 15 to be heavier than piglets from sows fed CT + 45 at week one (2.67 vs 2.52 kg for CT + 15 vs CT + 45, respectively; *P*=0.093). Piglets from sows supplemented with HMB were numerically heavier at weaning than piglets from sows receiving CT + 0, however this was not significant (*P*=0.391). HMB reduced the CV of piglet weights at weaning in a quadratic manner (*P*=0.026); piglets from sows in the CT + 15 treatment had the lowest within litter variation in pig weight at weaning within a litter compared with those fed CT + 0 (18.91 vs 22.67 % for CT + 15 vs CT + 0, respectively).

Table 3.4. The effect of β-hydroxy β-methyl butyrate on litter characteristics at farrowing when supplemented to sow diets at doses of 0, 5, 15 or 45 mg/kg body weight for ~ 15 days prior to parturition[1]

HMB mg/kg BW[2]	Farrowing duration (mins)[3,4]	Birth interval (mins)[3,4]	Vitality score[3,4]	Time to suckle (mins)[3,4]	Birth weight (kg)[4,5]	24 hour weight (kg)[4,5]	24 hour gain (g)[4,5]	24 hour temp (°C)	CI (g)[2,4,5]	CY[2,4,5] (kg)
0	259.13	20.31	1.75	19.26	1.38	1.44	29.87	36.99	366.83	4.60
5	267.24	21.36	1.71	17.05	1.35	1.46	81.71	36.99	404.14	5.40
15	234.04	20.24	1.78	19.25	1.46	1.58	95.01	36.99	448.76	5.91
45	277.86	22.08	1.66	19.36	1.30	1.43	98.16	37.04	416.20	5.56
SEM	28.538	3.015	0.061	1.802	0.046	0.047	15.940	0.160	19.907	0.291
					P-Value					
Overall	0.423	0.483	0.315	0.346	**0.063**	0.061	**0.005**	0.993	**0.024**	**0.009**
Linear	0.775	0.606	0.177	0.540	0.168	0.636	**0.011**	0.772	0.147	**0.064**
Quadratic	0.121	0.138	0.414	0.971	**0.049**	**0.010**	**0.019**	0.951	**0.005**	**0.004**
					Orthogonal contrasts					
0 vs 5 + 15 + 45	0.593	0.532	0.496	0.535	0.841	0.301	**0.001**	0.897	**0.010**	**0.002**
5 vs 15 + 45	0.579	0.959	0.902	**0.090**	0.490	0.400	0.380	0.886	0.181	0.228
15 vs 45	0.139	0.154	**0.079**	0.960	**<0.001**	**0.016**	0.876	0.819	0.201	**0.039**

[1]Data represents a total of 102 litters split across the four treatments as follows: 0 *n* = 24; 5 *n* = 27; 15 *n* = 26; 45 *n* = 25
[2]HMB = β-hydroxy β-methyl butyrate, BW = body weight, CI = colostrum intake, CY = colostrum yield
[3]Non-normal data, $\log_{10}$ transformed
[4]Corrected for total number of pigs born
[5]Smaller sample size: 0 *n* = 15; 5 *n* = 18; 15 *n* = 16; 45 *n* = 16

Table 3.5. The effect of β-hydroxy β-methyl butyrate on piglet growth performance from 24 hours until weaning when supplemented to sow diets at doses of 0, 5, 15 or 45 mg/kg body weight for ~ 15 days prior to parturition[1]

HMB mg/kg BW[2]	Average piglet weights (kg)					Piglet ADG[2] (g)	
	24 hour (total)[3]	24 hour (alive)[3]	24 hour (post fostering)[3]	Week one[4]	Wean[4]	Week one	Week one to wean
0	1.45	1.48	1.47	2.47	7.19	126	236
5	1.50	1.52	1.50	2.51	7.23	133	236
15	1.58	1.60	1.59	2.67	7.46	138	239
45	1.47	1.52	1.50	2.52	7.17	143	232
SEM	0.037	0.038	0.039	0.075	0.168	7.0	6.6
			P-value				
Overall	**0.017**	**0.034**	**0.042**	0.121	0.391	0.193	0.823
Linear	0.885	0.586	0.646	0.712	0.761	**0.047**	0.537
Quadratic	**0.002**	**0.004**	**0.006**	**0.021**	0.107	0.397	0.501
			Orthogonal contrasts				
0 vs 5 + 15 + 45	**0.067**	**0.042**	**0.089**	0.175	0.551	0.071	0.976
5 vs 15 + 45	0.459	0.228	0.199	0.268	0.605	0.307	0.966
15 vs 45	**0.012**	**0.062**	**0.053**	**0.093**	0.124	0.492	0.343

[1]Data represents a total of 140 litters split across the four treatments as follows: 0 *n* = 34; 5 *n* = 38; 15 *n* = 35; 45 *n* = 33
[2]HMB = β-hydroxy β-methyl butyrate, BW = body weight, ADG = average daily gain
[3]Corrected for total born
[4]Corrected for age

Table 3.6. The effect of β-hydroxy β-methyl butyrate on the variation of piglet weights when supplemented to sow diets at doses of 0, 5, 15 or 45 mg/kg body weight for ~ 15 days prior to parturition[1]

HMB mg/kg BW[2]	Percentage of litter ≤ 1.1 kg at birth (%)[3]		Coefficient of Variation (%)				
	Of total litter	Of live born piglets	24 hour (total)[3]	24 hour (alive)[3]	24 hour (post fostering)[3]	Week one	Wean[4]
0	18.46	17.75	22.03	22.52	20.70	21.67	22.67
5	17.46	17.14	21.83	22.06	20.85	20.30	22.12
15	11.83	10.28	18.94	21.22	18.83	18.81	18.91
45	21.43	19.17	21.54	21.55	20.64	20.54	22.72
SEM	2.826	2.768	1.263	1.478	1.417	1.456	1.556
			P-Value				
Overall	**0.025**	**0.022**	0.105	0.870	0.529	0.422	0.117
Linear	0.188	0.464	0.825	0.611	0.982	0.743	0.617
Quadratic	**0.008**	**0.005**	**0.022**	0.503	0.196	0.101	**0.026**
			Orthogonal contrast				
0 vs 5 + 15 + 45	0.551	0.383	0.280	0.503	0.652	0.202	0.516
5 vs 15 + 45	0.753	0.358	0.193	0.626	0.404	0.659	0.421
15 vs 45	**0.003**	**0.005**	**0.069**	0.842	0.256	0.307	**0.032**

[1]Data represents a total of 140 litters split across the four treatments as follows: 0 *n* = 34; 5 *n* = 38; 15 *n* = 35; 45 *n* = 33
[2]HMB = β-hydroxy β-methyl butyrate, BW = body weight
[3]Corrected for total born
[4]Non-normal data, $\log_{10}$ transformed

3.4.6. Immunoglobulin concentrations

Colostrum IgA, IgG and IgM concentrations are presented in Table 3.7. Colostrum IgG concentration averaged 67.1 ± 6.58 mg/ml and ranged from 11.1 to 185.3 mg/ml. HMB supplementation increased colostrum IgG concentration in a linear manner (*P*=0.001). Colostrum IgG concentration of sows in treatments CT + 5, CT + 15 and CT + 45 were 6.4, 20.3 and 43.7 % higher respectively than that of sows fed CT + 0. Sow in the CT + 5 treatment had a reduced IgG concentration compared with those in the CT + 15 and CT + 45 treatments (60.8 vs 68.7 and 82.0 mg/ml for CT + 5 vs CT + 15 and CT + 45, respectively; *P*=0.05). Colostrum IgA concentration averaged 15.6 ± 3.00 mg/ml and ranged from 1.6 to 93.7 mg/ml. HMB supplementation did not affect colostrum IgA concentration (*P*=0.937). Colostrum IgM concentration averaged 3.9 ± 0.24 mg/ml and ranged from 1.1 to 8.2 mg/ml. There was no effect of HMB supplementation on colostrum IgM concentration (*P*=0.542).

Table 3.7. The effects of β-hydroxy β-methyl butyrate on sow colostrum IgA, IgG and IgM concentrations (mg/ml), when supplemented to sow diets at doses of 0, 5, 15 or 45 mg/kg body weight for ~ 15 days prior to parturition

HMB mg/kg BW[1]	Colostrum immunoglobulin concentration (mg/ml)		
	IgA[2,5,6]	IgG[3]	IgM[4,6]
0	15.0	57.1	3.8
5	14.3	60.8	3.7
15	16.5	68.7	4.2
45	16.5	82.0	3.8
SEM	3.00	6.58	0.24
	P-Value		
Overall	0.937	**0.012**	0.542
Linear	0.863	**0.001**	0.975
Quadratic	0.873	0.680	0.237
	Orthogonal contrasts		
0 vs 5 + 15 + 45	0.846	**0.039**	0.832
5 vs 15 + 45	0.550	**0.050**	0.368
15 vs 45	0.838	0.102	0.252

[1]HMB = β-hydroxy β-methyl butyrate, BW = body weight
[2]0 *n*=34; 5 *n*=22; 15 *n*=26; 45 *n*=33
[3]0 *n*=33; 5 *n*=25; 15 *n*=29; 45 *n*=29
[4]0 *n*=34; 5 *n*=24; 15 *n*=31; 45 *n*=31
[5]Non-normal data, $\log_{10}$ transformed
[6]Corrected for parity

3.5. Discussion

3.5.1. The effects of HMB on sow characteristics and litter performance

HMB supplementation at all doses was effective in increasing total live born litter weight compared with the control diet. There were no differences in total number of piglets born or total number of live born piglets between the treatment groups, therefore the observed litter weight differences were attributed towards the heavier weights of the individual piglets. This may be due to the anti-catabolic effects of HMB (Blicharski et al., 2017). HMB has been found to reduce protein degradation (Ostaszewski et al., 2000), as well as stimulate protein synthesis (Smith et al., 2005) leading to enhanced skeletal muscle growth of the foetuses and increased litter weight.

One of the main aims of this study was to determine whether there was a dose-dependent response to HMB supplementation on litter weight. It was hypothesised that increasing the dose of HMB supplementation would further improve litter weights. This study found that HMB supplementation increased total live born litter weight in a quadratic manner with the most beneficial dose being 15 mg/kg BW. Supplementing HMB at doses of 5 and 45 mg/kg BW both increased total live born litter weight compared with the control (by 5.2 and 2.1 % respectively), however this increase was lower than that of sows receiving the 15 mg/kg dose where the total live born litter weight was 12.0 % heavier than the control.

Sows in the 45 mg/kg group may have had lower total live born litter weight compared with sows in the 15 mg/kg group due to the tendency for a linear decrease in gestation length with increased dose of HMB supplementation. Sows receiving the 45 mg/kg dose numerically had the shortest gestation length and thus the foetuses had the least time to develop (Rydhmer et al., 2008). It has been suggested that circulating cytokines may be involved in the onset of labour (Steinborn et al., 1995, Arntzen et al., 1998). HMB has been shown to influence non-specific cell mediated and humoral immunity (Peterson et al., 1999b, Krakowski et al., 2002, Siwicki et al., 2003). HMB was found to directly influence lymphocyte blastogenesis in *in vitro* studies with sheep lymphocytes (Nissen and Abumrad, 1997) as well as increase T and B lymphocyte activities in *in vivo* studies in fish (Siwicki et al., 2003). T lymphocytes activate B lymphocytes via the

release of interleukins, a type of cytokine that drives the differentiation of B lymphocytes into plasma cells (Murphy, 2012). Plasma cells generate immunoglobulins and in the current study HMB was found to increase the concentration of IgG in sow colostrum in a linear fashion. Therefore, HMB may have increased the levels of circulating cytokines released, through the increased activation of T cells, leading to the early onset of labour. Increasing the dose of HMB may have augmented this effect leading to a slight reduction in gestation length. This may have resulted in reduced litter weights for sows in the 45 mg/kg group compared with sows receiving the 15 mg/kg dose.

In addition, as mentioned in Section 1.4.2.1 HMB supplementation has been found to decrease plasma levels of glutamine (Holecek et al., 2009) and glutamine deficiency decreases protein synthesis in skeletal muscles (Holecek and Sispera, 2014). Therefore, it is possible that highest dose of HMB supplementation may have interfered with the metabolism of other amino acids such as glutamine which are required for protein synthesis. This may have reduced the litter weights of sows receiving the 45 mg/kg dose compared with those receiving the 15 mg/kg dose.

Supplementing sows with HMB increased litter weight at week one in a quadratic fashion with a tendency for a quadratic increase in litter weight at weaning. This effect is most likely due to the increase in total born litter weight caused by HMB supplementation. In addition, HMB supplementation increased colostrum yield and colostrum intake in a quadratic fashion. Colostrum intake influences piglet pre-weaning growth (Declerck et al., 2016) therefore would have also increased litter weights.

In the current study there were no differences in sow ADFI between treatments in lactation. However, there was a trend for sows fed the higher doses of HMB (15 or 45 mg/kg BW) to have increased total FI compared with sows fed the 5 mg/kg dose. As there was a linear tendency for HMB to reduce gestation length, sows fed the higher doses of HMB were fed their lactation diets for ~ 0.3 d longer. Across all treatments sow ADFI in lactation was 5.9 kg, therefore sows fed the higher doses of HMB would have received ~ 1.8 kg more of their lactation diet compared with sows fed the 5 mg/kg dose due to farrowing earlier. When this was accounted for the tendency for sows fed the 15 and 45 mg/kg doses of HMB to have higher total FI than sows fed the 5 mg/kg dose was no longer apparent.

Flummer and Theil (2012) reported lower mortality amongst piglets in the colostrum period from sows supplemented with HMB during late gestation. The current study found a quadratic relationship between HMB supplementation and 24 hour mortality. Sows fed the 15 mg/kg dose had the lowest percentage 24 hour mortality compared with all other groups. Piglet birth weight, colostrum intake and colostrum yield were all increased by HMB supplementation in this trial. A negative association between piglet birth weight and colostrum intake, with pre-weaning mortality has been reported in multiple studies (Farmer and Quesnel, 2009, Declerck et al., 2016). Piglets receiving a higher quantity of colostrum will receive more energy for growth and thermoregulation (Devillers et al., 2011) reducing their chances of mortality. Additionally, HMB supplementation increased the concentration of IgG in sow colostrum. New born piglets are dependent on immunoglobulins absorbed from colostrum for protection against disease (Rooke and Bland, 2002). Therefore, the reduced mortality level may be an indirect effect of HMB supplementation through its direct effects on birth weight and colostrum production.

3.5.2. The effects of HMB supplementation on litter characteristics at farrowing, colostrum intake and yield

It was hypothesised that there would be a dose-dependent response of piglet birth weight to HMB supplementation, a higher dose of HMB would have a more pronounced positive effect on piglet birth weight. HMB supplementation improved piglet birth weight in a quadratic manner. Positive effects of HMB on average piglet birth weight have previously been reported (Tatara et al., 2007, Wan et al., 2015, Blicharski et al., 2017) and this effect is thought to be due to its involvement in the regulation of skeletal muscle turnover (Szcześniak et al., 2015). The lack of effect of the 45 mg/kg dose may be due to the slightly shorter gestation length observed with this treatment resulting in the foetuses having less time to develop and thus a lower birth weight (Rydhmer et al., 2008). Alternatively, it may be due to high doses of HMB interfering with the metabolism of other amino acids required for protein synthesis (Holecek et al., 2009).

The reported effects of HMB supplementation to sows on average piglet birth weight are inconsistent. However, the dosage used across studies has varied vastly and this current study demonstrated a dose-dependent effect of HMB supplementation on average piglet birth weight, which may explain some of the

discrepancies between earlier studies. Tatara et al. (2007) found average piglet birth weight was increased by 24 % when sows were supplemented with HMB at a dose of 50 mg/kg BW in the last two weeks of gestation. This dose was similar to the 45 mg/kg dose used in the current study, which did not increase birth weight. Average piglet birth weight in the control group of the current study was higher than the average piglet weight in the control group of the Tatara et al. (2007) study. The effects of HMB may not be as noticeable if the piglets are heavier without supplementation as they may be close to their genetic potential. The Tatara et al. (2007) study also used Polish Landrace sows whereas the present study used Large White × Landrace sows, perhaps the difference in genetics may explain the discrepancies in studies. In addition, it should be noted that the Tatara et al. (2007) study only used six sows per treatment which is quite low replication for performance parameters.

Flummer et al. (2012) found no effect of supplementing sow diets with HMB at a dose of 15 mg/kg BW on piglet birth weight. This study only administered HMB for the last 10 d of gestation compared with the 15 day duration used in the present study, suggesting there may be an influence of duration of supplementation on birth weight. However, it should also be noted that this study only used five sows in total with only two sows in the HMB treatment group.

Few published studies have looked at the effects of HMB supplementation on colostrum intake and yield, and to our knowledge none have looked at the effects of dose of HMB supplementation. Flummer and Theil (2012) found colostrum yield, as measured per piglet, was 18 % higher than the control group when sows were supplemented with 2.5 g HMB for 7 d pre-partum. In the current study, there was a tendency for piglets from sows supplemented with HMB at all doses to have a higher colostrum intake than piglets from the control sows. HMB at all doses also successfully increased colostrum yield. HMB is thought to have a lipolytic effect causing fat mobilisation and elevating plasma levels of non-esterified fatty acids (NEFA) which may be used as energy for the production of colostrum (Wilson et al., 2008, Theil et al., 2014a).

The range of colostrum yield produced by sows in the present study was slightly higher than that reported by Devillers et al. (2007) but in line with the values reported by Hasan et al. (2019). The study by Devillers et al. (2007) was performed over 10 years ago and so it may be that colostrum yield has increased

as sows have become more prolific and as nutrition has improved. In addition, the study by Devillers et al. (2007) used the equation devised by Devillers et al. (2004) to estimate colostrum intake whereas the current study used the equation by Theil et al. (2014b). As mentioned in Section 1.3.3.1, the equation by Devillers et al. (2004) is thought to underestimate colostrum intake as it is based on bottle fed piglets as opposed to sow reared piglets (Theil et al., 2014b). It was hypothesised that there would be a dose-dependent response of HMB supplementation to sows on colostrum yield and intake by piglets. It was thought that a higher dose of HMB would further augment its lipolytic effects (Wilson et al., 2008). This study found that colostrum yield and intake both increased in a quadratic manner in relation to increased dose of HMB supplementation. Average colostrum intake was numerically 7.8 % higher for piglets from sows fed the 15 mg/kg dose compared with piglets from sows fed the 45 mg/kg dose, but this was not significant. Again, this could be an indirect result of HMB supplementation. Average piglet birth weight from sows fed the 15 mg/kg dose was higher than those fed the 45 mg/kg dose; larger piglets have been found to gain more from suckling than smaller piglets as they are better at stimulating the sow's teat, therefore can drain more colostrum from them (King et al., 1997). Colostrum intake has been shown to increase by 26 - 37 g per 100 g increase in body weight (Le Dividich et al., 2004 and Devillers et al., 2005 as cited by Le Dividich et al., 2005). This may have resulted in an increased colostrum intake from piglets in the 15 mg/kg treatment.

Colostrum yield for sows fed the 5, 15 and 45 mg/kg doses was increased by 18, 28 and 21 %, respectively compared with the control. The sows receiving the 5 mg/kg dose consumed ~ 17.4 g HMB over the experimental period. This quantity is almost the exact amount that sows in the Flummer and Theil (2012) study received. They fed sows with HMB at a dose of 2.5 g/d for only 7 d which equates to 17.5 g HMB. They found that colostrum yield per piglet was increased to the same degree as the 5 mg/kg dose in the current study, by 18 %. Colostrum yield is highly variable and has been found to range from 1.5 to 9.2 kg (Devillers et al., 2007, Quesnel, 2011, Hasan et al., 2019). Colostrum yield is influenced by many factors such as parity, sow weight and back-fat changes (Devillers et al., 2007, Decaluwe et al., 2013), therefore caution should be taken when making comparisons between studies.

The higher colostrum intake and colostrum yield observed in the HMB treatment groups are reflected in the piglet 24 hour weight gain. Piglets from sows supplemented with HMB had an increased weight gain compared to the control. The average colostrum intake of piglets from the HMB supplemented treatments was 423 g/piglet, whereas in the control group it was only 367 g/piglet. Theil et al. (2014b) found that 312 g of colostrum is needed to maintain body weight, therefore the increased weight gain of the treatment groups compared to the control group is due to their increased colostrum intake. Piglets from sows fed the 45 mg/kg dose had the highest 24 hour weight gain compared with the piglets in the control group. Although piglets from sows fed the 45 mg/kg dose did not consume as much colostrum as piglets from sows fed the 15 mg/kg dose, piglets from sows fed the 45 mg/kg dose were lighter at birth and colostrum intake has been found to be more beneficial in piglets with low versus high birth weights (Declerck et al., 2016). Le Dividich et al. (2005) discussed that the 24 hour weight gain of piglets that survived to weaning averaged 70 g/kg body weight. The average 24 hour weight gain of piglets in the HMB treatments in the current study were all over 70 g/piglet, whereas in the control group the average 24 hour weight gain was less than 30 g/piglet. This may have resulted in the tendency for the reduced mortality at weaning in the HMB treatments compared with the control.

3.5.3. The effects of HMB supplementation on piglet growth performance

HMB supplementation improved average piglet 24 hour weight. In this study, HMB supplementation at all doses increased colostrum yield and intake and thus piglet 24 hour weight gain compared with the control. HMB supplementation increased average piglet 24 hour weight in a quadratic manner; piglets from sows receiving the 15 mg/kg dose had the highest average 24 hour weights. This may be because sows fed the 15 mg/kg dose produced a higher yield of colostrum and piglets from those sows consumed more colostrum.

HMB reduced the variation in average 24 hour weights of piglets within a litter, for piglets from sows receiving the 5 mg/kg and 15 mg/kg, as displayed by the reduced CVs. However, the variation in average piglet 24 hour weights was highest in piglets from sows fed the 45 mg/kg dose. In addition, HMB appeared to reduce the percentage of piglets weighing ≤ 1.1 kg for sows fed the 5 mg/kg and 15 mg/kg dose, however the percentage of piglets weighing ≤ 1.1 kg was highest for sows fed the 45 mg/kg dose. It is well established that weight variation

within a litter can effect piglet survival and weight gain (Milligan et al., 2002b). Smaller litter mates suffer due to direct competition from heavier litter mates for access to functional and productive teats (English et al., 1977; English and Morrison et al., 1984 as cited by Milligan et al., 2002b). High birth weight variation of litters is associated with increased levels of mortality (Milligan et al., 2002b). Impaired growth and development of the mammalian foetus during pregnancy can result in IUGR and birth weights are often used as a criteria to detect IUGR on farms (Wu et al., 2006). Approximately 15 - 20 % of piglets have a birth weight below 1.1 kg; the survival and postnatal growth of these piglets is restricted (Wu et al., 2006). The effect HMB had on protein synthesis and skeletal muscle turnover would have also increased the growth of small piglets and may even have had a larger impact on them, resulting in less piglets born under 1.1 kg. Wan et al. (2016) found that supplementing normal body weight (BW 2.51 kg) and IUGR (BW 1.85 kg) piglets with a diet containing 0.08 % (as-fed) HMB from d 7 until d 28 post-partum increased the expression of mTOR in the IUGR piglets but not the piglets of normal body weight. Sows fed the 45 mg/kg dose had a shorter gestation length than sows in the other groups, therefore the foetuses had less time to develop (Rydhmer et al., 2008). Additionally, although litter size was used as a covariate in the model, sows fed the 45 mg/kg dose had the highest litter sizes. Combined these factors may have resulted in an increased number of small piglets as well as an increased variation of weights in litters from sows fed the 45 mg/kg dose.

Average piglet week one weight was improved by HMB supplementation in a quadratic manner. Piglets from sows fed the 15 mg/kg dose remained heavier at week one compared with piglets from other treatments. Birth weight is a major factor determining future growth (Gondret et al., 2005); the heavier week one weights observed in piglets from sows receiving the 15 mg/kg dose of the current study may be due to their higher birth weights. In the first week of lactation piglets from all the HMB treatment groups had a higher ADG compared with piglets in the control group. Piglet week one ADG increased with higher maternal supplementation of HMB in a linear fashion, however the ADG of piglets from sows receiving the 45 mg/kg dose was not significantly higher than sows receiving the 15 mg/kg dose. In agreement, a study by Wan et al. (2015) found piglets from sows supplemented with 4 g/d HMB from day 35 of gestation until parturition had a 19 % higher week one ADG compared with the control group. In

the present study HMB increased colostrum yield and intake; colostrum intake is related to piglet pre-weaning growth (Edwards, 2002, Devillers et al., 2007), therefore the increase in week one ADG is most likely a result of the piglets receiving more colostrum. Nissen et al. (1994) found supplementing sow diets with HMB in gestation increased the fat in colostrum by 41 %. Colostrum fat was not measured in the current study, however if levels of fat were increased by HMB supplementation, then piglets would have received more energy for growth which may have also contributed to the increased week one ADG.

Although HMB increased average birth weight and average week one weight, it did not improve week one to wean ADG, therefore although there was a numeric difference in overall HMB treatment on average weaning weight, this was not significant. It was predicted by Devillers et al. (2004) that piglets need to consume 250 g of colostrum in order to achieve a good body weight gain. The average colostrum intake from all treatments, including the control, was over 250 g/piglet suggesting all piglets had adequate colostrum intake for growth, which may explain why there was no significant difference in average piglet weight at weaning. The variation in piglet weaning weights within a litter were reduced with HMB supplementation for litters from sows fed the 5 and 15mg/kg doses. Litters with more variable weights at birth have been found to have more variable weights at weaning (Milligan et al., 2002b). Sows fed HMB at a dose of 5 or 15 mg/kg had the lowest variation in piglet weights at birth which is likely why they showed reduced variation in weights at weaning.

3.5.4. The effects of HMB supplementation on colostrum immunoglobulin concentrations

Several studies have suggested that HMB is an immunostimulant (Krakowski et al., 2002, Siwicki et al., 2003, Flummer et al., 2012) but the mechanism behind this is still unclear. A study in rainbow trout demonstrated that lysosomal activity and total plasma IgG concentration was enhanced by HMB supplementation, and this was further improved with increased dose of HMB (Siwicki et al., 2003). Flummer et al. (2012) found piglets from sows supplemented with HMB had heavier spleens on d 28; this suggests improved immune function which is in agreement with the immunostimulatory effect. Krakowski et al. (2002) found that the IgG concentration of sow colostrum was increased by 27 % when

supplemented with HMB at a dose of 15 mg/kg BW for three weeks, from six weeks prior to parturition.

In agreement with Krakowski et al. (2002) and in line with the immunostimulatory effects of HMB, HMB successfully increased the level of IgG in sow colostrum at all doses in the current study. As hypothesised, increasing the dose of supplementation of HMB to sows increased the concentration of IgG in sow colostrum in a linear fashion. The average concentration of IgG in colostrum in the current study were in line with those reported in other studies, however the range was slightly larger (Hurley, 2015). As previously mentioned, HMB has been found to increase T and B lymphocyte activity in fish (Siwicki et al., 2003) and T lymphocytes aid in the differentiation of B lymphocytes into plasma cells (Murphy, 2012). It is possible that this increase in IgG release from plasma cells may be a result of increased T cell activation by HMB and that this was further augmented with increased dose of HMB. Colostrum IgG is derived from sow serum (Bourne and Curtis, 1973); this suggests that HMB supplementation resulted in an increase in IgG release from plasma cells in serum, which were then transferred to mammary secretions during colostrum formation (Salmon et al., 2009). The IgG concentration of sow serum was not measured in the current study.

Concentrations of IgA and IgM were not impacted by HMB supplementation. Average concentrations of IgA and IgM in colostrum were similar to those reported in the literature, however in regard to the concentration of IgA, the range was slightly larger than previously found (Hurley, 2015). The majority of colostrum IgA is synthesised in the mammary gland and only 40 % is derived from sow serum (Bourne and Curtis, 1973). If HMB increased the level of IgA in sow serum this may not have been reflected in colostrum. The lack of effect of HMB on IgA and IgM may also be due to the lower levels of these immunoglobulins in comparison to IgG in early colostrum.

3.6. Conclusions

In conclusion, supplementing sow diets with HMB for the last 15 days of gestation had positive effects on piglet birth weight, pre-weaning growth and colostrum production. HMB supplementation increased piglet birth weight in a quadratic fashion and this is most likely a result of HMB stimulating protein synthesis. HMB supplementation also increased colostrum yield and colostrum intake in a quadratic fashion which may be due its lipolytic effects which would have resulted

in more energy for the production of colostrum. The concentration of IgG in colostrum was enhanced in a linear fashion up until the highest dose tested, which is most likely due to the activation of T cells by HMB. Combined, these factors resulted in piglets from sows supplemented with HMB having an increased week one performance and reduced mortality to weaning. The optimum dose from these results in terms of both piglet performance and colostrum production was 15 mg/kg BW. However, the optimum duration of time which sows should be supplemented with HMB for is still unknown. More research is needed in order to confirm this.

4.

Increasing the duration of β-hydroxy β-methyl butyrate supplementation to sows in gestation increases colostrum immunoglobulin concentrations

4.1. Abstract

HMB supplementation to sow diets has previously been shown to have beneficial effects on litter performance. The effects have been shown to be dose-dependent, with the optimum dose being 15 mg/kg of the sow's body weight. Therefore, this study aimed to determine whether there was an optimum duration of HMB supplementation to sow diets on colostrum quality, piglet birth weight and piglet performance to weaning. A total of 127 (Large White × Landrace) multiparous sows were randomly assigned to one of four treatments on d 93 of gestation. The treatments were feeding a HMB supplemented diet for 0 (CT + 0), 6 (CT + 6), 15 (CT + 15) or 22 (CT + 22) days prior to their parturition date at a dose of 15 mg/kg of the sow's body weight. The study ended at weaning when the piglets were ~ 27 d old.

Total litter weight and average piglet 24 hour weight were similar across treatments and there were no differences in piglet performance to weaning between the treatments. There was a linear tendency for an increase in colostrum concentration of IgG and a linear increase in colostrum concentration of IgM, in response to increased duration of HMB supplementation (P=0.074 and P=0.042, respectively). Additionally, there was a quadratic increase in the concentration of IgA in colostrum in response to increased duration of HMB supplementation (P=0.024), with no benefit of supplementation beyond 15 d.

In conclusion, this study found that HMB had a positive effect on colostrum IgG concentration and demonstrated that there was an effect of the duration of supplementing HMB. In addition, this study found that there were positive effects of increasing the duration of feeding HMB to sows on colostrum concentrations of IgA and IgM.

4.2. Introduction

β-hydroxy β-methyl butyrate (HMB), a metabolite of leucine, has been shown to have beneficial effects in animal studies. Previous research has shown that HMB has had positive effects on protein metabolism, cholesterol synthesis and lipolysis in multiple species including: fish, broilers, mice, and pigs (Ostaszewski et al., 2000, Smith et al., 2005, Wilson et al., 2008, Szcześniak et al., 2015). Experiment 1 (described in Chapter 3) demonstrated that HMB had beneficial dose-dependent effects on sow and litter performance when provided to the sow for ~ 15 days prior to parturition. HMB increased colostrum yield, colostrum intake, colostrum IgG concentration and piglet birth weight. Experiment 1 identified the optimum dosage of HMB supplementation to be 15 mg/kg BW.

However, the optimum duration of supplementation with HMB is unknown. The limited available previous literature has inconsistences around the duration that HMB has been supplemented to sow diets. The durations ranged from 3 d prior to parturition (Nissen et al., 1994) to 80 d prior to parturition (Wan et al., 2015) and the results are conflicting with regard to piglet birth weight and performance. Flummer et al. (2012) found no effect of supplementing HMB to sows at a dose of 15 mg/kg BW for 10 days prior to parturition. Wan et al. (2015) found that litter weights at birth were increased when HMB was supplemented to sows at a dose of 4000 mg/d from day 35 of gestation until parturition. The duration of feeding HMB may influence piglet and litter birth weights.

4.2.1. Hypothesis

Increasing the duration of feeding HMB to sows in gestation will improve sow, litter and piglet performance from birth to weaning.

4.2.2. Aims

- To determine the effect of duration of HMB supplementation to sow gestation diets on immunoglobulin concentrations (IgA, IgG and IgM) in sow colostrum.
- To determine the effect of HMB supplementation to sow gestation diets for different time periods on average piglet 24 hour and litter weights.
- To determine the effect of duration of HMB supplementation to sow gestation diets on piglet growth rates and mortality.

4.3. Materials and methods

4.3.1. Experimental design and dietary treatments

This experiment examined the effects of feeding HMB for 0, 6, 15 or 22 days prior to parturition. After parturition all sows were fed the same commercial lactation diet. All treatment groups received the same standard gestation diet and HMB was provided as a top-dressing to this diet for 0, 6, 15 or 22 days prior to parturition at a dose of 15 mg/kg BW dissolved in apple squash (as described in Section 2.3). The 15 mg/kg BW dose was selected based on the results of experiment 1. All sows received the same quantity of apple squash and water from 22 days prior to parturition. Sows were fed individual quantities of feed based on their body weight on entry to the trial (d 93 BW $\pm$ SEM = 235.5 $\pm$ 4.36 kg) using Equation 4 (as described in Section 2.2.2) (NRC, 2012). They were also fed individual quantities of HMB based on their body weight on d 93 of gestation.

The treatments included: (CT + 0) the control diet top-dressed with HMB for zero days, (CT + 6) the control diet top-dressed with HMB from d 109 of gestation, (CT + 15) the control diet top-dressed with HMB from d 100 of gestation and (CT + 22) the control diet top-dressed with HMB from d 93 of gestation. These time points for supplementation were selected for the following reasons: d 100 of gestation matched the time period used in experiment 1 and this would allow for comparisons between studies, d 93 was selected as it is approximately when lactogenesis is thought to begin (Quesnel et al., 2009), and d 109 is when sows are moved to the farrowing house, therefore it was selected from a practical point of view. The control diet was formulated to contain 9.23 MJ NE/kg and 3.9 g/kg SID lysine and meet all nutrient requirements for the gestating sow (NRC, 2012).

In lactation, all sows were fed the same commercial lactation diet which was formulated to contain 9.99 MJ NE/kg and 8.2 g/kg SID lysine and meet all nutrient requirements for the lactating sow (NRC, 2012). Feed allowance in lactation commenced at ~ 3.0 kg/d and was increased by ~ 0.5 kg/d to appetite split across two daily meals. The diet compositions and calculated nutrient levels for the control gestation and for the lactation diet are presented in Table 4.1.

Table 4.1. Composition, calculated and analysed nutrient specifications for the gestation and lactation diets (%, as-fed basis)

Diet	Gestation diet[1]	Lactation diet
Ingredient		
Barley	15.00	-
Wheat	44.67	47.78
Wheat feed	30.00	13.17
Soyabean meal	1.50	7.39
Maize meal	-	10
Bakery meal	-	4.35
Rapeseed extract	-	4
Sunflower meal	-	2.72
Vitamin-mineral premix[2]	0.25	0.25
L-Lysine liquid	0.1	0.57
DL-Methionine	-	0.04
Threonine	-	0.14
L-Tryptophan	-	0
Choline chloride	0.03	0.03
Yeast	-	0.1
Limestone	1.35	0.92
Dicalcium phosphate	0.2	0.77
Salt	0.5	0.26
Sodium bicarbonate	-	0.03
Soya oil	-	1.71
Vegetable fat	1.4	0.75
Glucose syrup + Raffinate	5	5
Calculated nutrient composition		
Net energy (MJ/kg)	9.23	9.99
Crude protein (%)	11.64	15.3
Crude fibre (%)	4	4
SID Lysine (%)	0.39	0.82
SID Methionine + Cystine (%)	0.37	0.5
SID Threonine (%)	0.31	0.57
SID Tryptophan (%)	0.12	0.16
SID Leucine	0.64	0.87
Calcium (%)	0.69	0.79
Total Phosphorus (%)	0.43	0.56
Digestible Phosphorus (%)	0.24	0.33
Analysed nutrient composition		
Dry matter (%)	86.35	86.7
Crude protein (%)	12.15	15.2
Crude fibre (%)	3.55	4.20
Fat (%)	3.61	5.39
Lysine (%)	0.47	0.85
Leucine (%)	0.73	0.97

[1]β-hydroxy β-methyl butyrate was added as a top-dressing to the gestation diet at a dose of 15 mg/kg BW and fed from d 109, d 115 or d 93 to create the CT + 6, CT + 15 and CT + 22 treatments, respectively.

[2]Vitamin and trace mineral premix provided per kg of the diet: 10,000 IU vitamin A, 1850 IU vitamin D_3, 50 IU vitamin E, 4 mg vitamin K, 1.5 mg thiamine (B_1), 4 mg riboflavin (B_2), 3.5 mg pyridoxine (B_6), 15 ug vitamin B_{12}, 12 mg pantothenic acid, 20 mg nicotinic acid, 200 µg biotin, 2 mg folic acid, 15 mg copper, 1 mg iodine, 80 mg iron, 50 mg manganese, 0.25 mg selenium, 100 mg zinc, 100 mg oxy-nil dry and 150 mg phytase.

4.3.2. Animals and management

One hundred and twenty seven mixed parity sows (Large White × Landrace [JSR Genepacker 90, JSR, UK]) were used across six consecutive batches (17 to 28 sows per batch) and followed for one parity. On d 93 of gestation sows were allocated to one of four dietary treatments, primarily on the basis of parity (range 1:9) and then matched for previous litter history, d 93 body weight and back-fat thickness. Sows were housed throughout gestation and lactation according to standard Spen Farm practice as described in Section 2.2.1.1. Sows were fed as described in Section 2.2.2.

Piglets received the management treatment as stated in Section 2.2.1.1. Cross fostering commenced as stated in Section 2.4. Foster sows were introduced when the number of piglets was still too high post fostering. Four foster sows were introduced across the trial; two in batch one, one in batch five and one in batch six. Across the whole trial 1,909 piglets were born to the 127 sows and of these 122 were stillborn.

4.3.3. Measurements

Measurements including sow weights, back-fat, feed intake, litter sizes and piglet weights were recorded as described in Section 2.4. Cameras were placed in the farrowing house (as described in Section 2.4). Cameras were used to give piglets a score based on their behaviour in the first 15 secs after birth using the following scoring system adapted from Baxter et al. (2008): 0 = no visible signs of movement within 15 secs; 1 = some signs of movement within 15 secs e.g, gasping or breathing; 2 = piglet shows movement within 15 secs but does not attempt to stand; 3 = piglet shows good movement and attempts to stand within 15 secs. Colostrum samples were collected (as described in Section 2.4.1) as close to the start of parturition as possible. The time of sample collection in relation to the start of parturition was noted based on the video recordings. Piglets averaged 6.9 ± 0.28 d and 26.8 ± 0.29 d (average age ± SEM) at week one and at weaning respectively.

4.3.4. Laboratory analysis

Dietary samples were collected weekly throughout the experiment. Subsamples of these were collected and mixed thoroughly and sent to Sciantec Analytical Services Ltd (Cawood, UK) for crude protein, crude fibre, total fat and amino acid

analysis. See section 2.5.2 for details. This was done for both the gestation and lactation diets.

Colostrum samples were analysed as described in Sections 2.5.1 and 2.5.1.1. Samples from the same batch were analysed on the same plate. The intra- and inter-assay CVs were 3.2 % and 13.0 % for IgA, 2.8% and 10.5 % for IgG, and 4.2 % and 21.1 % for IgM.

4.3.5. Calculations and statistical analysis

Data were analysed using SPSS Statistics (version 21.0, SPSS Inc., Chicago IL, USA). All data (including performance, colostrum and piglet birth data) were analysed using polynomial contrasts for unequally spaced increments to test for linear and quadratic responses to HMB. Treatment means were compared using the following single degree-of-freedom orthogonal contrasts: CT + 0 vs CT + 6, CT + 15 and CT + 22; CT + 6 vs CT + 15 and CT + 22 and CT + 15 vs CT + 22. Data were first tested for homogeneity of variance and normality using the Levene's test and the Kolmogorov-Smirnov test, respectively. Data displaying heteroscedasticity or non-normal data were $\log_{10}$ transformed prior to statistical analysis. Transformed data were back transformed for inclusion in the respective tables. Birth interval and colostrum concentrations of IgA and IgM required $\log_{10}$ transformation.

The sow/litter was the experimental unit for all analyses. Birth data were based on the sow's genetic litter and post 24 hours data were based on the suckling litter (post-fostering). Foster sows were not included in analysis. The statistical model included treatment, parity and batch as fixed factors. Due to the low number of sows above parity six (n=7), parity six and above were grouped together. Total number of pigs born was used as a covariate for average 24 hour and total litter 24 hour weights. Litter size post-fostering and age were used as covariates, when significant, for analysis of performance to weaning. Mortality was calculated as the percentage of piglets that died, out of the total number of piglets that were born alive in a litter, and was based on the sow's genetic litter. Total number of piglets born alive was used as a covariate for mortality. Birth intervals, vitality scores and time to suckle were averaged for each sow, thus the sow was the experimental unit for analysis. Total born was used as a covariate for vitality analysis. When parity was significant it was used as a covariate instead of a fixed factor for immunoglobulin analysis as in experiment 1. Significance was

reported at $P<0.05$ and as trends if $P<0.1$. Data are expressed as least-square means with their pooled standard error of the mean (SEM).

4.4. Results

Overall, the litters performed well throughout the experiment. Across the whole trial six litters were excluded from post 24 hour performance analysis (one from CT + 22, two from CT + 15 and three from CT + 6). They were excluded as the sows were A further four litters were excluded from weaning weight analysis as they weaned early due to the requirement for foster sows (two from CT + 0, one from CT + 6 and one from CT + 15). Performance data up to 24 hours represents the mean of 127 litters split across the treatments as follows: CT + 0, $n = 33$; CT + 6, $n = 32$; CT + 15, $n = 32$; CT + 22, $n = 30$. All the values presented are mean ± SEM.

4.4.1. Dietary analysis

The analysed nutrient content of the gestation and lactation experimental diets are presented in Table 4.1. The levels of CP, CF and fat were similar for both sub-samples of gestation diet and for both sub-samples of lactation diet analysed and they were all in line with the formulated the values.

4.4.2. HMB intake

The daily intake of HMB for sows in the HMB treatment groups averaged 3.35 ± 0.061 g/d and ranged from 2.27 to 5.04 g/d. The total intake of sows in the CT + 6 treatment averaged 23.49 ± 1.338 g and ranged from 14.31 to 30.24 g. The total intake of sows in the CT + 15 treatment averaged 50.82 ± 1.767 g and ranged from 33.98 to 79.44 g. The total intake of sows in the CT + 22 treatment averaged 73.88 ± 2.477 g and ranged from 62.60 to 111.96 g.

4.4.3. Sow performance

Sow characteristics are presented in Table 4.2. The average sow parity was 3.0 ± 0.34. Sow weights and back-fat thickness measurements were similar on entry to the trial and averaged 235.3 ± 4.39 kg and 12.1 ± 0.78 mm respectively, across all treatments. There was a tendency for a quadratic dose response relationship between gestation length and treatment ($P=0.090$); sows in the CT + 6 treatment had the longest (116.0 d) and sows in the CT + 22 treatment had the shortest gestation length (115.2 d). On average the gestation length of sows receiving the CT + 22 treatment was 0.60 d shorter than that of sows receiving the CT + 15

treatment (*P*=0.054). Total sow FI across lactation was similar across all treatments (*P*=0.814); however, there was a negative dose-response relationship between ADFI throughout lactation and duration of supplementation with HMB (*P*=0.028). Sows in the CT + 22 and CT + 15 treatments had a 4.5 % lower ADFI compared with sows in the CT + 6 treatment. Sow weight and back-fat changes from entry to the trial (d 93) to d 109 were similar across treatments and averaged + 11.9 ± 0.94 kg and - 0.8 ± 0.93 mm respectively. Sow back-fat changes from d 109 to weaning were similar across treatments and averaged + 0.9 ± 0.90 mm. Sows in the CT + 0 treatment lost more weight from d 109 to weaning than sows on the other treatments (-31.2 vs - 25.3 kg for CT + 0 vs CT + 6, CT + 15, CT + 22, respectively; *P*=0.023).

Table 4.2. The effects of β-hydroxy β-methyl butyrate on sow characteristics when supplemented to sow diets at a dose of 15 mg/kg body weight for 0, 6, 15 or 22 days prior to parturition[1]

Days on HMB[2]	Parity	Gestation length (d)	Sow d 93 weight (kg)	Weight changes (kg)		Sow d 93 back-fat (mm)	Back-fat changes (mm)		Lactation FI[2] (kg)	
				D 100 to d 109	D 109 to wean		D 100 to d 109	D109 to wean	Total FI	ADFI
0	2.9	115.8	239.3	+ 11.3	- 31.2	12.3	- 0.6	+ 0.9	197.5	7.7
6	2.9	116.0	234.2	+ 12.4	- 24.7	12.1	- 1.0	+ 1.1	195.3	7.8
15	3.0	115.9	233.6	+ 11.8	- 26.4	11.9	- 1.0	+ 0.1	193.5	7.5
22	3.0	115.2	234.2	+ 12.0	- 24.8	12.1	- 0.5	+ 1.5	195.2	7.4
SEM	0.34	0.24	4.39	0.94	2.32	0.78	0.93	0.90	3.06	0.12
					P-value					
Overall	0.994	0.128	0.768	0.845	0.136	0.984	0.972	0.710	0.814	0.163
Linear	0.796	**0.091**	0.665	0.464	0.178	0.867	0.655	0.869	0.508	**0.028**
Quadratic	0.907	**0.090**	0.418	0.880	0.320	0.733	0.959	0.480	0.500	0.663
					Orthogonal contrasts					
0 vs 6 + 15 + 22	0.804	0.713	0.296	0.445	0.023	0.719	0.816	0.989	0.397	0.212
6 vs 15 + 22	0.882	0.171	0.862	0.661	0.755	0.924	0.678	0.776	0.794	**0.078**
15 vs 22	0.971	**0.054**	0.962	0.869	0.604	0.889	0.956	0.259	0.699	0.484

[1]Data represents a total of 127 litters split across the four treatments as follows: 0 *n* = 33; 6 *n* = 32, 15 *n* = 32, 22 *n* = 30
[2]HMB = β-hydroxy β-methyl butyrate, FI = feed intake, ADFI = average daily feed intake

4.4.4. Litter performance

Litter performance is presented in Table 4.3. The total number of piglets born were similar across treatments (*P*=0.936) and averaged 15.0 ± 0.60 piglets/sow. However, HMB increased the number of piglets born alive and reduced the number of piglets born dead in a linear fashion (*P*=0.028 and *P*=0.028 respectively). HMB increased the number of live born piglets by 1.5, 4.4 and 4.4 % for treatments CT + 6, CT + 15 and CT + 22, respectively, compared to the CT + 0 treatment. HMB reduced the number of born dead piglets by 15.4, 38.5 and 46.1 % in treatments CT + 6, CT + 15 and CT + 22, respectively compared with the CT + 0 treatment.

Total born litter weight and total live born litter weight were similar across treatments (*P*=0.226 and *P*=0.688, respectively) and averaged 21.8 ± 0.43 kg and 20.7 ± 0.47 kg respectively. There was a linear tendency for litters from sows in the CT + 15 or CT + 22 treatments to be lighter than those in the CT + 0 or CT + 6 treatments by week one (31.8 and 31.3 vs 33.1 and 33.2 kg for CT + 15 and CT + 22 vs CT + 0 and CT + 6, respectively; *P*=0.069). Litter weights were similar across all treatments at weaning (*P*=0.465).

HMB had no effect on percentage 24 hour mortality (*P*=0.524). By week one there was a linear tendency for mortality to be higher in the HMB treatment groups (*P*=0.079), however this was not apparent by weaning (*P*=0.467). Mortality to weaning in the CT + 0 treatment for this trial was very low for the farm (10.9 %).

4.4.5. Litter characteristics at farrowing

Litter characteristics at farrowing are presented in Table 4.4. Farrowing duration from the birth of the first piglet to the birth of the last piglet averaged 247.5 ± 29.26 mins and ranged from 27.4 to 703.5 mins. Farrowing duration from the birth of the first piglet until the expulsion of the placenta averaged 318.0 ± 32.2 mins and ranged from 76.2 to 766.1 mins. Farrowing duration from birth of the first piglet, until birth of the last piglet was similar across treatments (*P*=0.587); however sows in the CT + 22 treatment numerically had the shortest farrowing duration compared with the sows in the CT + 0 treatment (225.4 vs 278.0 mins for CT + 22 vs CT + 0, respectively). HMB shortened farrowing duration when measuring from the birth of the first piglet until the expulsion of the placenta (*P*=0.045). The farrow duration of sows receiving the CT + 22, CT + 15, and CT + 6 treatments were 85.2, 57.9 and 24.6 mins shorter than the CT + 0 treatment. Average piglet

time to udder was similar across treatments (*P*=0.185); however, piglets from sows receiving the CT + 6 treatment had the shortest time to udder compared with the CT + 0 treatment (10.8 vs 15.8 mins for CT + 6 vs CT + 0, respectively).

Table 4.3. The effects of β-hydroxy β-methyl butyrate on litter performance when supplemented to sow diets at a dose of 15 mg/kg body weight for 0, 6, 15 or 22 days prior to parturition[1]

Variable	Days on HMB[2]				SEM	*P*-value			*Orthogonal contrasts*		
	0	6	15	22		Overall	Linear	Quadratic	0 vs 6 + 15 + 22	6 vs 15 + 22	15 vs 22
Numbers of piglets											
Total born	15.0	14.7	15.2	15.0	0.60	0.936	0.848	0.942	0.912	0.567	0.796
Alive[3]	13.7	13.9	14.3	14.3	0.22	0.157	**0.028**	0.637	**0.061**	0.187	0.854
Dead[3]	1.3	1.1	0.8	0.7	0.22	0.157	**0.028**	0.637	**0.061**	0.187	0.854
Week 1	12.3	11.8	12.3	11.4	0.27	**0.014**	**0.031**	0.376	**0.053**	0.980	0.008
Wean	12.1	11.5	12.1	11.3	0.28	**0.061**	0.163	0.575	0.131	0.572	0.028
Litter weights (kg)											
Total born[3]	21.9	22.4	21.5	21.2	0.43	0.226	0.104	0.415	0.706	**0.044**	0.668
Total live born[3]	20.5	21.1	20.5	20.5	0.47	0.688	0.722	0.476	0.635	0.267	0.944
Week 1[4,5]	33.1	33.2	31.8	31.3	0.83	0.301	**0.069**	0.743	0.301	0.114	0.652
Wean[4,5]	85.4	86.4	83.2	83.4	1.70	0.465	0.194	0.864	0.575	0.137	0.947
Litter gains (kg)											
Birth to week 1[4,5]	13.5	13.3	12.6	12.1	0.66	0.374	**0.080**	0.797	0.241	0.225	0.523
Week 1 to wean[4]	52.5	53.2	51.8	52.0	1.39	0.883	0.595	0.854	0.933	0.425	0.944
Birth to weaning[4,5]	65.8	66.6	64.2	64.3	1.61	0.650	0.296	0.880	0.655	0.233	0.972
24 hour mortality[3] (%)	3.4	5.7	4.8	5.2	1.21	0.524	0.438	0.431	0.167	0.631	0.806
Week 1 mortality[3] (%)	9.2	11.3	11.7	13.5	1.67	0.317	**0.079**	0.927	0.115	0.534	0.420
Wean mortality[3] (%)	10.9	13.3	14.5	14.2	1.89	0.467	0.351	0.221	0.133	0.641	0.897

[1]Data represents a total of 127 litters split across the four treatments as follows: 0 *n* = 33; 6 *n* = 32, 15 *n* = 32, 22 *n* = 30
[2]HMB = β-hydroxy β-methyl butyrate
[3]Corrected for total born
[4]Corrected for litter size
[5]Corrected for age
[6]Corrected for number born live

Table 4.4. The effects of β-hydroxy β-methyl butyrate on litter characteristics at farrowing when supplemented to sow diets at a dose of 15 mg/kg body weight for 0, 6, 15 or 22 days prior to parturition[1]

Days on HMB[2]	Farrowing duration (mins)[3,4]	Farrowing duration to placenta (mins)[4]	Birth interval (mins)[5]	Vitality score[4]	Time to udder (mins)
0	277.99	359.94	19.88	1.61	15.81
6	250.46	335.35	18.12	1.49	10.79
15	236.14	302.03	16.93	1.42	16.16
22	225.39	274.70	16.38	1.45	14.32
SEM	29.263	32.157	2.050	0.064	1.999
		P-value			
Overall	0.587	0.252	0.157	0.191	0.185
Linear	0.183	**0.045**	0.350	**0.059**	0.795
Quadratic	0.761	0.988	0.929	0.222	0.488
		Orthogonal contrasts			
0 vs 6 + 15 + 22	0.222	0.130	0.483	**0.038**	0.360
6 vs 15 + 22	0.574	0.218	0.545	0.490	**0.063**
15 vs 22	0.793	0.545	0.895	0.779	0.511

[1]Data represents a total of 81 litters split across the four treatments as follows: 0 *n* = 21; 6 *n* = 21, 15 *n* = 17, 22 *n* = 22
[2]HMB = β-hydroxy β-methyl butyrate
[3]Farrowing duration from the birth of the first piglet to the birth of the last piglet
[4]Corrected for total litter size
[5]Non-normal data, $\log_{10}$ transformed

4.4.6. Piglet performance

Piglet growth performance to weaning is presented in Table 4.5. Average piglet 24 hour weight was similar across treatments (*P*=0.312). There was a linear tendency for HMB to affect average piglet 24 hour live weight (*P*=0.099). Piglets from sows receiving the CT + 6 treatment were on average 8.5 % heavier 24 hour live weights than piglets from sows receiving the CT + 15 and CT + 22 treatments.

Week one ADG was similar across treatments (*P*=0.478). Piglets from sows receiving the CT + 6 treatment were numerically heaviest at week one, however this was not significant (2.8 vs 2.7, 2.6 and 2.6 kg [CT + 6 vs CT + 0, CT + 15, and CT + 22, respectively]; *P*=0.342). Week one to wean ADG was also similar across treatments (*P*=0.574). Piglets from sows fed the CT + 6 treatment were numerically heaviest at weaning, however this was not significant (7.4 vs 7.2, 7.0 and 7.1 kg [for groups CT + 6 vs CT + 0, CT + 15 and CT + 22, respectively]; *P*=0.269).

Table 4.5. The effect of β-hydroxy β-methyl butyrate on piglet performance from 24 hours to weaning when supplemented to sow diets at a dose of 15 mg/kg body weight for 0, 6, 15 or 22 days prior to parturition[1]

Days on HMB[2]	Average piglet weights (kg)					Piglet ADG[2] (kg)	
	24 hour (alive and dead)[3]	24 hour (alive)[3]	24 hour (post fostering)[3]	Week one[4,5]	Wean[4,5]	Week one[4,5]	Week one to wean[4]
0	1.48	1.51	1.51	2.73	7.24	0.175	0.226
6	1.52	1.56	1.56	2.77	7.41	0.178	0.231
15	1.46	1.47	1.47	2.64	7.04	0.167	0.221
22	1.45	1.47	1.48	2.62	7.14	0.167	0.223
SEM	0.030	0.031	0.031	0.068	0.141	0.006	0.007
			P-value				
Overall	0.312	0.133	0.206	0.342	0.269	0.478	0.574
Linear	0.212	**0.099**	0.202	0.117	0.235	0.175	0.403
Quadratic	0.498	0.561	0.599	0.676	0.881	0.821	0.801
			Orthogonal contrasts				
0 vs 6 + 15 + 22	0.920	0.735	0.938	0.513	0.804	0.522	0.932
6 vs 15 + 22	**0.060**	**0.015**	**0.035**	**0.089**	**0.061**	0.150	0.168
15 vs 22	0.926	0.941	0.809	0.822	0.599	0.930	0.796

[1]Data represents a total of 127 litters split across the four treatments as follows: 0 *n* = 33; 6 *n* = 32, 15 *n* = 32, 22 *n* = 30
[2]HMB = β-hydroxy β-methyl butyrate, ADG = average daily gain
[3]Corrected for total born
[4]Corrected for litter size
[5]Corrected for age

4.4.7. Immunoglobulin concentration

Colostrum immunoglobulin concentrations are presented in Table 4.6. Colostrum IgG concentration averaged 60.6 ± 5.33 mg/ml and ranged from 15.2 to 132.8 mg/ml. When looking at all the samples taken within 24 hours of the start of parturition there was a linear tendency for HMB to increase the concentration of IgG colostrum (*P*=0.085); sows in the CT + 22 treatment numerically had the highest colostrum concentration of IgG compared with the CT + 0 treatment (67.8 vs 56.7 mg/ml for CT + 22 vs CT + 0, respectively). When looking at all the samples taken within eight hours of the start of parturition there was a linear tendency for HMB to increase the concentration of IgG (*P*=0.074). Sows receiving the CT + 6, CT + 15 and CT + 22 treatments had a 0.1, 10.4 and 20.3 % higher level of IgG in colostrum respectively, compared with sows receiving the CT + 0 treatment. Of the colostrum samples taken within one hour of the start of parturition, HMB increased colostrum IgG concentration in a linear fashion (*P*=0.009). Sows receiving the CT + 15 and CT + 22 treatments had 9.0 and 25.5 % higher levels of IgG, respectively compared with the CT + 0 treatment.

Colostrum IgA concentration averaged 12.0 ± 1.03 mg/ml and ranged from 2.0 to 38.3 mg/ml. When looking at all the samples taken within 24 hours of farrowing HMB did not impact the concentration of IgA (*P*=0.280); however, the concentration of IgA was numerically higher in all the HMB supplemented groups compared with the CT + 0 treatment (12.5 vs 10.3 mg/ml for CT + 6, CT + 15 and CT + 22 vs CT + 0, respectively). When looking at the samples taken within eight hours of farrowing HMB increased the concentration of IgA in colostrum in a quadratic manner (*P*=0.024). The concentration of IgA was 20.9, 42.9 and 13.0 % higher in treatments CT + 6, CT + 15 and CT + 22, respectively compared with CT + 0. Finally, when looking at all the colostrum samples taken within one hour of the start of parturition HMB increased the concentration of IgA in a quadratic fashion (*P*=0.015). Colostrum IgA concentration was 32.2, 36.1 and 11.3 % higher for sows in treatments CT + 6, CT + 15 and CT + 22, respectively.

Colostrum IgM concentration averaged 3.6 ± 0.27 mg/ml and ranged from 1.4 to 9.5 mg/ml. Of all the samples taken within 24 hours of the start of parturition HMB did not impact colostrum IgM concentration (*P*=0.229). However, the concentration of IgM was numerically higher in all three HMB treatments

compared with the CT + 0 treatment (3.7 vs 3.3 mg/ml for CT + 6, CT + 15 and CT + 22 vs CT + 0, respectively). When looking at the samples taken within eight hours of the start of parturition HMB increased the concentration of IgM in a linear manner compared with the control group (*P*=0.042). Colostrum IgM concentration was 5.4, 14.8 and 24.8 % higher for sows in treatments CT + 6, CT + 15 and CT + 22, respectively compared with the CT + 0 treatment. Of the samples taken within one hour of the start of parturition there was a linear tendency for HMB to increase the colostrum concentration of IgM (*P*=0.077). Colostrum IgM concentration was 13.2, 15.2 and 32.7 % higher in treatments CT + 6, CT + 15 and CT + 22, respectively compared with the CT + 0 treatment.

Table 4.6. The effect of β-hydroxy β-methyl butyrate supplementation to sow diets at a dose of 15 mg/kg body weight for 0, 6, 15 and 22 days, on colostrum IgA, IgG and IgM concentrations, in samples taken within 24 hours, eight hours and one hour of the start of parturition

Immunoglobulin concentrations (mg/ml)	Days on HMB[1]				SEM	*P-value*			*Orthogonal contrasts*		
	0	6	15	22		Overall	Linear	Quadratic	0 vs 6 + 15 + 22	6 vs 15 + 22	15 vs 22
Within 24 hours of parturition[2]											
IgA[5,6]	10.3	12.3	13.4	11.9	1.03	0.280	0.148	0.168	**0.054**	0.978	0.802
IgG	56.7	58.6	59.2	67.8	5.33	0.235	**0.085**	0.468	0.321	0.387	0.117
IgM[5,6]	3.2	3.7	3.6	4.0	0.27	0.229	0.116	0.827	0.108	0.938	0.190
Within 8 hours of parturition[3]											
IgA[5,6]	10.6	12.8	15.2	12.0	1.29	**0.072**	**0.085**	**0.024**	0.026	0.681	0.155
IgG	61.8	62.4	68.2	74.3	6.07	0.287	**0.074**	0.682	0.358	0.167	0.387
IgM[5,6]	3.5	3.7	4.0	4.4	0.34	0.231	**0.042**	0.871	0.131	0.265	0.447
Within 1 hour of parturition[4]											
IgA[5,6]	10.9	14.9	14.9	12.1	1.58	**0.073**	0.242	**0.015**	**0.017**	0.381	0.295
IgG	66.4	57.0	72.4	83.3	6.29	**0.017**	**0.009**	0.141	0.537	**0.006**	0.150
IgM[5,6]	3.5	4.0	4.0	4.6	0.47	0.228	**0.077**	0.869	0.107	0.769	0.244

[1]HMB = β-hydroxy β-methyl butyrate
[2]IgA: 0 *n*=19; 6 *n*=21; 15 *n*=30; 22 *n*=28. IgG: 0 *n*=19; 6 *n*=21; 15 *n*=30; 22 *n*=28. IgM: 0 *n*=19; 6 *n*=21; 15 *n*=30; 22 *n*=28
[3]IgA: 0 *n*=11; 6 *n*=18; 15 *n*=19; 22 *n*=20.IgG: 0 *n*=11; 6 *n*=18; 15 *n*=19; 22 *n*=20. IgM: 0 *n*=11; 6 *n*=18; 15 *n*=19; 22 *n*=20
[4]IgA: 0 *n*=9; 6 *n*=11; 15 *n*=13; 22 *n*=15. IgG: 0 *n*=9; 6 *n*=11; 15 *n*=13; 22 *n*=15. IgM: 0 *n*=9; 6 *n*=11; 15 *n*=13; 22 *n*=15
[5]Non-normal data, $\log_{10}$ transformed
[6]Corrected for parity

4.5. Discussion

Before the results are discussed in detail it should be mentioned that the sows and litters performed very well throughout this experiment. The overall ADFI of all the sows throughout lactation was 28.3 % higher than sows in experiment 1. In addition, sows in the current study lost 40.5 % less weight from movement to the farrowing house to weaning than the sows in experiment 1. Sows in the current study gained an average of 0.9 mm back-fat throughout lactation whereas sows in experiment 1 lost 0.3 mm of back-fat throughout lactation; back-fat loss throughout lactation can reduce the number of piglets born in the next litter (Whittemore and Kyriazakis, 2006). Overall percentage mortality to weaning in the current experiment was 13.2 % and only 10.9 % in the control group compared with 17.8 % overall and 19.3 % in the control group of experiment 1.

Whilst the overall total born litter weight, average piglet 24 hour weight and number of live born piglets were in line with experiment 1, sows in the present study weaned on average 0.6 piglets/litter more than sows in experiment 1 with similar average weaning weights to piglets from sows in experiment 1. Therefore, sows in the current study had 5.5 % higher total litter weaning weights than sows in experiment 1. Overall, this suggests that sows in the current study out-performed sows in experiment 1.

4.5.1. The effects of HMB on sow characteristics and litter performance

One of the main aims of this study was to determine whether there was an effect of the duration of supplementation of HMB to sow diets in late gestation on litter performance from 24 hours to weaning. It was thought that HMB supplementation would increase litter weight through its ability to reduce protein degradation (Ostaszewski et al., 2000) and enhance protein synthesis (Smith et al., 2005), and that increasing the duration of supplementation would further augment this effect. In this study HMB supplementation to sows for any period failed to improve total born litter and total live born litter weight when compared with the control group. These results are in disagreement with the experiment 1, which found HMB supplementation to sow diets from d 100 of gestation improved total live born litter weight at all doses examined (5, 15 and 45 mg/kg BW).

The lack of effect of HMB on litter weights may be due to the tendency for a quadratic decrease in gestation length with increased length of HMB

supplementation. Sows supplemented with HMB for 22 days had the shortest, whilst sows supplemented with HMB for 6 days had the longest gestation lengths. A tendency for a slight reduction in gestation length with increased dose of HMB supplementation was noted in the previous study. As previously stated in Section 3.5.1, HMB could have increased levels of circulating cytokines resulting in a slightly reduced gestation length as it has been suggested that circulating cytokines may be involved in the onset of labour (Steinborn et al., 1995, Arntzen et al., 1998). Levels of circulating cytokines were not measured in this study, however the immunostimulatory effect of HMB has been demonstrated in both the current and previous study through its influence on immunoglobulin concentrations in sow colostrum.

Sows supplemented with HMB for 22 days had the shortest gestation length therefore the foetuses had the least time to develop. Sows supplemented with HMB for 6 days had the longest gestation length and so the foetuses had the longest time to develop (Rydhmer et al., 2008). This may be reflected in the litter weights; sows supplemented for 22 days numerically had the lightest litter weights, whereas sows supplemented for 6 days numerically had the heaviest litter weights.

There were no differences in total number of piglets born between the treatment groups. However, supplementation with HMB increased the total number of live born piglets and reduced the number of born dead piglets in a linear fashion. A retrospective power analysis was performed on the number of piglets born dead using Equation 3 as described in Section 1.5. A standardised difference of 33 % (the average reduction in still births across the HMB treatments) and a CV of 135 % (CV for the number born dead was calculated from data in this trial). This suggested that 134 sows would be needed per treatment to detect a significant result. This study used a total of 96 sows in the HMB treatment groups therefore more sows are needed to make this result more reliable. However, it warrants further investigation.

Wan et al. (2015) also found that supplementing sow diets with HMB at a dose of 4 g/d from d 35 of gestation until parturition decreased the number of still born piglets (2.7 vs 9.1 % still born in the control). Whilst the reason for the decreased number of still born piglets is unknown, the author suggested that it may be because HMB increases the level of IGF-1 in piglets (Tatara et al., 2007, Tatara

et al., 2012). IGF-1 has been shown to promote foetal growth (Hellström et al., 2016), therefore it is possible that piglet survival was also enhanced.

In the current study increasing the duration of feeding HMB reduced sow ADFI in lactation in a linear fashion. In addition, there was a tendency for a linear increase in litter weight gain at week one with a shorter duration of feeding HMB. Sows supplemented with HMB for 6 days had the highest week one litter weight gain and sows supplemented with HMB for 22 days had the lowest week one weight gain. A higher ADFI in lactation is directly related to increased litter weights (Eissen et al., 2003, Craig et al., 2017). Increasing the duration of feeding HMB resulted in sows consuming less feed in lactation therefore they produced less milk and had a lower litter weight gain. The number of piglets suckling a sow is also a key contributor of milk production (Toner et al., 1996). Although litter size was accounted for when analysing litter weights, sows supplemented with HMB for 22 days had the fewest piglets suckling at week one compared with the other groups which may also be why they had the lightest litters and lowest litter weight gain at week one and why they consumed the least amount of feed. In addition, piglets from sows supplemented with HMB for 6 days were numerically heavier at birth therefore these piglets would have consumed more milk so the sow would have required more feed to meet the demand for milk production.

HMB supplementation had no effect on percentage 24 hour mortality. There was a linear tendency towards increased mortality at week one in the groups supplemented with HMB for longer which may be a result of the higher numbers of piglets born alive in these groups. However, this tendency was not apparent by weaning. These results are in disagreement with the previous study which found HMB reduced 24 hour mortality when supplemented to sows at a dose of 15 mg/kg BW for ~ 15 days prior to parturition with a tendency for a reduction in mortality to weaning, compared with the control. It also disagrees with a study by Flummer et al. (2012) which found HMB supplementation to sows in late gestation reduced piglet mortality during the colostrum period. The discrepancies between the findings of the current study and those of the previous study may be due to the lack of effect of HMB on piglet birth weight as it is well established that birth weights are associated with piglet survival (Milligan et al., 2002b, Quiniou et al., 2002, Baxter et al., 2008, Panzardi et al., 2013). However, overall mortality in the control group of this current study was unusually low (10.9 %) for the farm and

lower than commercial figures reported at the time (~ 12 %) (AHDB, 2019c) which suggests that the control sows were performing above average standards.

4.5.2. The effects of HMB on characteristics at farrowing

There was a linear tendency for HMB to reduce farrowing duration from the time of birth of the first piglet to the expulsion of the placenta. Increased farrowing durations are associated with an increased number of still born piglets (van Dijk et al., 2005). However, it is unclear whether still born piglets cause prolonged farrowing durations or whether prolonged farrowing durations cause still births (van Dijk et al., 2005). Still born piglets can cause obstructions during delivery which can increase farrowing duration (van Dijk et al., 2005). In addition, piglets from sows with longer farrowing durations are subjected to more uterine contractions and are at higher risk of cord rupture or damage to the placenta which can result in deaths due to asphyxia (Herpin et al., 1996, Alonso-Spilsbury et al., 2005). Therefore, HMB may have reduced still births resulting in a reduction in farrowing duration or HMB may have reduced farrowing duration leading to a lower number of still born piglets.

Fahmy and Friend (1981) found that farrowing duration decreased with decreased gestation length. In the current study there was a tendency for HMB to reduce gestation length so it is possible that this could also be the reason for the observed reduction in farrowing duration. However, studies by van Rens and van der Lende (2004) and van Dijk et al. (2005) found the opposite; increased gestation length resulted in a reduced farrowing duration.

Higher levels of back-fat thickness also increase farrowing duration (Oliviero et al., 2010). Higher fat levels affect lipid-soluble steroids such as the progesterone: oestrogen ratio; this affects oxytocin receptor activation which is needed for parturition and therefore farrowing duration may be delayed or weakened (McCracken et al., 1999, Russell et al., 2003, Oliviero et al., 2010). Although there were no significant effects of HMB on sow back-fat thickness on entry to the farrowing house, sows supplemented with HMB for 6, 15 and 22 days numerically had less back-fat compared with the sows supplemented with HMB for 0 days on entry to the farrowing house. The lower level of back-fat in sows in the HMB group may have reduced farrowing durations.

Prolonged farrowing durations can increase the amount of uterine contractions piglets are exposed to which can cause asphyxia (Alonso-Spilsbury et al., 2005). Piglets which experience intra-partum asphyxia are likely to have reduced vitality at birth (Herpin et al., 1996). Although sows supplemented with HMB had shorter farrowing durations, piglets from sows supplemented with HMB showed no improvement in vitality in terms of vitality score or time to udder. Although control sows had longer farrowing durations their piglets had higher vitality scores, therefore the effect of reduced farrowing duration on piglets' time to udder was negated. Piglets from control sows may have had higher vitality scores due to the longer gestation length and therefore were more developed at birth.

4.5.3. The effects of HMB on piglet growth performance

It was hypothesised that HMB would increase average piglet 24 hour weight due to its role in skeletal muscle turnover (Szcześniak et al., 2015) and colostrum production (Flummer and Theil, 2012). It was thought that supplementation for a longer duration of time would further improve this. However, HMB supplementation did not improve average piglet 24 hour weight compared with the control group when supplemented to sow diets for any duration of time in this trial.

Although there were no significant differences in average 24 hour weight between the treatments, piglets from sows supplemented with HMB for 6 days numerically had the heaviest 24 hour weight, and piglets from sows supplemented with HMB for 22 days, numerically had the lowest 24 hour weight. As discussed in Section 4.5.1, this may be due the tendency for a slightly longer gestation length in sows supplemented with HMB for 6 days and a slightly shorter gestation length in sows supplemented with HMB for 22 days, resulting in the foetuses having different amounts of time to develop (Rydhmer et al., 2008).

Average week one weight and week one ADG were not affected by HMB supplementation. This again, is in disagreement with experiment 1 which found week one weight and week one ADG were increased by HMB supplementation. The lack of effect on week one weight in the current study is most likely due to the lack of effect on piglet 24 hour weight. Average week one weight and week one ADG in the control group of the current study were 10.5 % and 39.2 % higher, respectively than those in the control group of experiment 1. This suggests that piglets in the control group of the current study were performing above those in

experiment 1. The lactation ADFI of sows in the control group of the current study was 30.5 % higher than of those in the control group of experiment 1. This may be due to season; the current study was conducted across February to June, whereas experiment 1 was performed from June to October. Therefore, sows in experiment 1 may have experienced hotter temperatures. Hot temperatures can cause reduced FI (Eissen et al., 2000). Higher ADFI of sows results in increased litter weights as the sows have increased energy for milk production (Eissen et al., 2003, Craig et al., 2017). In addition, sows in the current study lost 40.5 % less weight than sows in experiment 1. As sows in the present study had a higher ADFI they were receiving more energy for milk production and body maintenance.

Average piglet weaning weight was not affected by HMB supplementation which agrees with the previous study. Birth weights are positively associated with weaning weights (Declerck et al., 2016); in the current study 24 hour weights were similar across all groups as were week one weights and week one to wean ADG so it is not surprising that weaning weights were similar.

4.5.4. The effects of HMB on colostrum immunoglobulin concentrations

A focal point of the current study was to determine whether there was an effect of duration of feeding HMB to sows on immunoglobulin concentrations in colostrum. With regard to IgG concentration, when looking at samples taken within 24 hours or eight hours of farrowing there was a tendency for HMB to increase the concentration of IgG in colostrum in a linear fashion. When looking at the samples taken within 1 hour of farrowing, HMB significantly increased the concentration of IgG in colostrum in a linear manner up until the highest duration of supplementation. This is consistent with the findings of experiment 1. As previously mentioned, HMB has been found to increase T and B lymphocyte activities (Siwicki et al., 2003). Therefore, it is possible that the increase in IgG concentration in colostrum was a result of increased T cell activation by HMB, which was further augmented when HMB was supplemented for a longer duration of time.

Supplementing HMB to sows for only 6 days prior to parturition had no effect on colostrum IgG level. Colostrum is largely synthesised prior to parturition (Quesnel et al., 2015). β-lactoglobulin is the first component of colostrum and is produced from around d 80 of gestation (Dodd et al., 1994). β-casein mRNA, a major milk protein, first appears in mammary tissue around d 90 (Lee et al., 1993).

Lactogenesis is therefore said to start at around d 90 of gestation (Quesnel et al., 2012). Perhaps supplementing HMB for only 6 days prior to parturition was not long enough to impact IgG concentration. Supplementing sow diets with HMB for 22 days prior to parturition had the largest impact on colostrum IgG concentration. This equates to supplementation from d 93 of gestation which, as stated above, is around when lactogenesis starts (Quesnel et al., 2012).

Supplementation with HMB successfully increased colostrum concentrations of IgA and IgM in a quadratic and linear fashion, respectively, when looking at the samples taken within eight and one hour of parturition. Whilst experiment 1 found no significant effect of HMB supplementation on IgA and IgM concentrations, both immunoglobulins were numerically higher than the control when HMB was supplemented at a dose of 15 mg/kg BW for ~ 15 days. As with IgG, the increase in IgA and IgM maybe a result of T cell activation by HMB, resulting in increased release of IgA and IgM from their respective plasma cells. As previously mentioned, only 40 % of colostrum IgA is derived from sow serum, the rest is synthesised by plasma cells in the mammary tissue, whereas approximately 80 % of colostrum IgM is derived from sow serum (Bourne and Curtis, 1973, Hurley and Theil, 2011). This suggests that HMB could have stimulated an increase in activity of plasma cells in sow serum, resulting in a higher production of IgA and IgM in serum, which were then transferred to mammary secretions. Alternatively, HMB may have stimulated an increase in plasma cell activity in the mammary tissue itself, which resulted in higher production of IgA and IgM in mammary tissue.

However, when looking at all the colostrum samples taken within 24 hours of farrowing, although there were numeric increases in IgA and IgM concentrations with HMB treatment, it was not significant. Colostrum concentrations of IgA and IgM show a decline throughout the first 24 hours post-partum (Hurley, 2015) therefore there may be too much variation in sampling time to look at samples taken within 24 hours.

Colostrum immunoglobulins provide the piglet with passive immunity and so can increase the piglets' chances of survival (Theil et al., 2014a) therefore, it is fairly surprising there was no reduction in piglet mortality in the groups supplemented with HMB. Piglet plasma concentration of IgG at 24 hours is positively correlated with colostrum intake (Devillers et al., 2011). However, Devillers et al. (2011)

found that piglet plasma concentration of IgG reached a plateau when piglets consumed more than 200 - 250 g of colostrum, which is likely a result of gut closure (Quesnel et al., 2012). It is possible that the average colostrum intake of piglets in this study was over 200 g, therefore the additional benefits of higher immunoglobulin concentrations in colostrum were not reflected in mortality levels.

4.6. Conclusions

In conclusion, this study suggested that HMB supplementation increased the numbers of piglets born alive and reduced the number of piglets born dead in a linear fashion. HMB supplementation increased the level of IgG in colostrum in a linear fashion up until the highest duration of time it was supplemented for. In addition, this study found that HMB supplementation increased the colostrum concentration of IgA in a quadratic manner and the concentration of IgM linearly. Whilst there were no effects of HMB supplementation on piglet performance, the sows and litters performed well in the current study without supplementation and mortality levels were exceptionally low. The optimum duration of HMB supplementation based on the results of this experiment was 15 days prior to parturition as it enhanced all concentrations of immunoglobulins, the number of piglets born alive was maximised and there were no negative effects on performance. However, more research is needed in order to ensure the piglets can benefit from the improved quality of the sows' colostrum.

5.

Supplementing β-hydroxy β-methyl butyrate and lignocellulose fibre to sows in gestation improves piglet performance to weaning with no additional benefits of supplementing glutamine to sows in lactation

5.1. Abstract

A study was conducted to determine the effects of supplementing sows with HMB and lignocellulose fibre in gestation and glutamine in lactation on colostrum production, the parturition process and on piglet performance to weaning. This study was run on both the indoor and outdoor pig production systems in order to achieve a high number of replication. A total of 170 (Large White × Landrace) multiparous sows on the indoor production system and 134 (Large White × Landrace × Duroc) multiparous sows on the outdoor production system were used in this study and followed from d 100 of gestation until weaning. The treatments were arranged as a 2 × 2 × 2 factorial design. The first factor (Factor 1) was two levels of HMB (0 or 0.15 %, as-fed) fed from d 100 of gestation until parturition (~ d 115). The second factor (Factor 2) was two levels of lignocellulose fibre (0 or 1 %, as-fed) fed in combination with Factor 1 from d 110 of gestation until parturition (~ d 115). Following parturition gestation treatments ceased and the third factor (Factor 3) which was one of two lactation diets with two levels of L-glutamine (0 or 1 %, as-fed) was introduced and fed until weaning (~ d 27 of lactation).

On the indoor production system lignocellulose fibre reduced sow gestation length compared with the control (114.6 vs 115.1 d for 1 vs 0 %, respectively; *P*=0.026). HMB supplementation increased colostrum yield compared with the control (6.6 vs 5.6 kg for 0.15 vs 0 %, respectively; *P*=0.009) and there was a tendency for HMB to increase the colostrum intake of piglets (*P*=0.083). There was also a tendency for HMB supplementation to increase the colostrum concentration of IgM (*P*=0.094). On the outdoor production system there was a tendency for HMB to reduce gestation length compared with the control (115.7 vs 116.1 d for 0.15 vs 0 %, respectively; *P*=0.087).

When the indoor and outdoor production system data were combined and analysed as a 2 × 2 × 2 × 2 model, supplementing sows with HMB increased average week one weight compared with the control (2.66 vs 2.55 kg for 0.15 vs 0 %, respectively; *P*=0.032) and this remained a trend at weaning (*P*=0.076). In addition, supplementing sows with lignocellulose fibre increased average weaning weight compared with the control (7.70 vs 7.47 kg for 1 vs 0 %, respectively; *P*=0.042). There were no effects of glutamine supplementation on average piglet week one or weaning weights.

In conclusion, this study demonstrated that HMB had beneficial effects on colostrum production in terms of colostrum yield, concentration of IgM and intake by piglets, when incorporated into a commercial sow diet. These positive effects were also reflected in improved piglet weights at week one and at weaning. Lignocellulose fibre reduced gestation length indoors with no negative effect on average birth weight which suggests lignocellulose fibre may have improved foetal development in gestation. In addition, when the data from both systems were combined supplementing sows with lignocellulose fibre increased average piglet weaning weight.

5.2. Introduction

The leucine metabolite, β-hydroxy β-methyl butyrate (HMB), has been found to influence protein metabolism by inhibiting proteolysis through the down regulation of the ubiquitin-proteasome pathway (Smith et al., 2005) and by promoting protein synthesis through the activation of the mTOR pathway (Eley et al., 2007). HMB has also been found to have lipolytic and immunostimulatory effects (Krakowski et al., 2002, Wilson et al., 2008, Szcześniak et al., 2015). Experiment 1 (described in Chapter 3) supplemented sow diets with HMB for ~ 15 days prior to parturition at a dose of 0, 5, 15 or 45 mg/kg BW and found positive quadratic dose-dependent effects on piglet birth weight and growth with the optimum dosage identified as 15 mg/kg BW. This experiment also found that HMB supplementation increased colostrum yield and colostrum intake in a quadratic manner with the optimum dosage again identified as 15 mg/kg BW. Experiment 2 (described in Chapter 4) supplemented HMB to sow diets for 0, 6, 15 or 22 d prior to parturition at a dose of 15 mg/kg BW and demonstrated that HMB increased colostrum concentrations of IgA and IgM. Both experiments 1 and 2 found that HMB supplementation increased the IgG concentration in sow

colostrum linearly up until the highest dose and duration tested. Combined, the results from experiments 1 and 2 found that the optimum dose and duration of supplementing sows with HMB was 15 mg/kg BW for 15 days prior to parturition. Including HMB in a sow diet would provide an easy, practical method of HMB administration on farm. Determining the effect of HMB on piglet performance when incorporated into a commercial diet is therefore of key importance.

However, HMB supplementation did not increase piglet weaning weights. The parturition process itself is critical as it can result in mortality due to asphyxia; those piglets which suffer asphyxia but survive have been shown to have reduced colostrum intake and reduced future performance (Alonso-Spilsbury et al., 2005). Therefore, sow nutrition across the transition period from gestation to lactation is important for piglet survival through parturition, as well as for piglet birth weight and colostrum production and thus the piglets' overall future performance (Douglas et al., 2013, Theil, 2015). Additionally, the diet the sow receives in lactation is crucial for milk production (Quesnel et al., 2015) and maximising piglet weaning weights. Reducing the time taken for the piglet to access the colostrum initially and improving the quality of sows' milk throughout lactation may be ways of ensuring that piglets capitalise on the beneficial effects HMB has on colostrum production.

Lignocellulose fibres are produced from wood and over recent years have been used as a high quality dietary fibre source in animal nutrition (Kroismayr, 2008). Some lignocellulose fibres only contain non-fermentable fibre fractions however, eubiotic lignocellulose fibres contain both non-fermentable and fermentable fractions. The non-fermentable fibres can affect the rate of passage of digesta and shift fermentable parts of the diet back from the caecum to the colon. The fermentable parts of the diet can then be fermented by colonic microflora (Kroismayr, 2008). Prolonged farrowing durations have been found to increase the level of asphyxia in pigs (Alonso-Spilsbury et al., 2005) and constipation in sows has been associated with prolonged farrowing durations (Oliviero et al., 2010). Recent research found that supplementing sow diets with 1 % Opticell eubiotic lignocellulose fibre for one week prior to parturition reduced sow farrowing durations by 40 mins (Enckevort, 2013). This may be through the effect lignocellulose has on the passage of digesta. If eubiotic lignocellulose fibre can speed up the parturition process there may be fewer piglets which suffer from

asphyxia, therefore more piglets will have a shorter latency to suckle. Supplementing eubiotic lignocellulose fibre in combination with HMB may have an additive effect on piglet performance to weaning as piglets may receive colostrum with a higher concentration of immunoglobulins faster.

Glutamine is the most abundant free amino acid in the body (Manso et al., 2012). It is primarily synthesised in the skeletal muscle, lungs, adipose tissue and liver, through the action of glutamine synthetase, from glutamate and ammonia (Watford, 2015). Glutamine is a precursor for many metabolic pathways required for growth and cell division (Watford, 2015). It has many physiological functions some of which include: protein synthesis and degradation, hormone secretion, intestinal integrity, nutrient metabolism, cell growth and differentiation, gene expression, anti-oxidant defence and immune system modulation (Wu et al., 2011). Glutamine is a conditionally essential amino acid as under normal physiological conditions the body can synthesise it in the required concentrations. However, in neonates and under various catabolic conditions such as lactation, the demand for glutamine in various tissues increases and so the rate of glutamine utilisation exceeds the rate of synthesis (Wu, 2009, Watford, 2015).

Glutamine is abundant in many physiological fluids; it is one of the most copious amino acids present in milk (Manso et al., 2012). In pigs, the concentration in milk has been found to increase from 0.1 to 4.0 mM (7 times higher than maternal plasma concentration) between d 1 and d 28 of lactation (Wu and Knabe, 1994). The high level found in milk is associated with the neonatal piglet's rapid growth and cell division requirements, particularly in the small intestine and gut-associated lymphoid tissue (Manso et al., 2012). The uptake of glutamine by the mammary gland may not be adequate for the synthesis of milk proteins (Li et al., 2009); on d 10 of lactation the mammary gland takes up 16 g of glutamine/d from the blood stream (Trottier et al., 1997) but secretes 36 g glutamine/d in milk (Haynes et al., 2009, Wu et al., 2011). Therefore, additional glutamine must be synthesised by the mammary gland (Wu et al., 2011). Catabolism of BCAAs plays an important role in glutamine synthesis by the lactating mammary gland however, this reduces the efficiency of the utilisation of dietary amino acids (Li et al., 2009, Wu et al., 2011) and can result in a loss of lean body mass in the sow (Manso et al., 2012).

In addition, HMB supplementation has been found to decrease plasma levels of glutamine (Holecek et al., 2009). Supplementing sow diets with glutamine in lactation may make up for any decrease in glutamine plasma level caused by HMB supplementation. In addition, providing supplemental glutamine to the sow in lactation may be a way to help provide the extra glutamine required for milk production, sparing BCAAs for metabolic utilisation and aiding in the maintenance of lean body mass (Wu et al., 2011, Manso et al., 2012). Supplementing sow diets during lactation with glutamine has previously been shown to increase the concentration of glutamine in sow milk (Kitt et al., 2004, Wu et al., 2011, Manso et al., 2012, Santos de Aquino et al., 2014) which would provide additional glutamine for the neonatal piglets to use for growth. Providing sows with supplemental glutamine may be a way to allow the piglets to reach their maximum growth potential throughout the pre-weaning period.

5.2.1. Hypothesis

Supplementing sows with HMB and lignocellulose fibre in gestation and with glutamine in lactation will improve sow, litter and piglet performance from birth to weaning.

5.2.2. Aims

- To determine whether incorporating HMB into a commercial sow feed and feeding it to sows in gestation will increase immunoglobulin concentrations (IgA, IgG, IgM) in colostrum.
- To determine whether feeding sows a commercial sow diet containing HMB in gestation will increase the yield of colostrum produced.
- To determine the effect of feeding sows a commercial sow diet containing HMB in gestation on litter and piglet birth weights and thus overall litter and piglet performance to weaning.
- To determine the effect of feeding sows lignocellulose fibre over the transition period on farrowing duration.
- To determine the effect of supplementing sow diets with lignocellulose fibre over the transition period on piglet viability and growth to weaning.
- To determine the effect of supplementing sow diets with both HMB and lignocellulose fibre on piglet performance to weaning.
- To determine whether supplementing sows with glutamine in lactation improves piglet performance.

- To determine the effect of feeding sows supplemental glutamine in lactation on sow weight loss throughout lactation.
- To determine the effect of supplementing sows with HMB and lignocellulose in gestation and with glutamine in lactation on piglet performance to weaning.

5.3. Materials and methods

5.3.1. Experimental design and dietary treatments

The experimental design is presented in Figure 5.1. Treatments were arranged as a 2 × 2 × 2 factorial design with two levels of HMB (0 [CT] or 0.15 %, as-fed [CT + HMB]; Factor 1) and two levels of lignocellulose fibre (0 [NLF] or 1 %, as-fed [LF]; Factor 2) in the gestation phase, followed by two levels of L-glutamine (0 [Lact] or 1 %, as-fed [Lact + Gln]; Factor 3) in the lactation phase. Factor 1 was offered as standard gestation diets, with the two supplementary levels of HMB from d 100 of gestation until parturition (~ d 115). Factor 2 (lignocellulose) was introduced to the diets of half of the sows from d 110 of gestation until parturition (~ d 115). Following parturition gestation treatments ceased and all sows went onto one of two lactation diets (differing in supplementary levels of L-glutamine) until weaning (~ d 27 of lactation). The trial was run in parallel on both the indoor and outdoor production systems and both the indoor and outdoor herds received feed from the same batch.

The concentration of 0.15 % HMB was selected based on the dose of 15 mg/kg BW which was used in experiment 1 and experiment 2. It was selected as the minimum dietary concentration required for every sow to receive a minimum dose of 15 mg/kg BW, if they were fed to body maintenance using Equation 4 (as described in Section 2.2.2). The range of sow weights from experiments 1 and 2 were used to determine this. The concentration of 1 % eubiotic lignocellulose fibre (lignocellulose fibre; Opticell®, sourced from Agromed, Austria) was selected based on a preliminary in house study conducted on the university farm. The level of L-glutamine (glutamine; sourced from DSM Nutritional Products) used in the study was based on a study by Wu et al. (2011) which found supplementing gilt diets with 1 % L-glutamine throughout lactation resulted in an ~ 7 % increase in piglet weaning weights and a reduction in pre-weaning mortality of ~ 78.5 % compared to the control.

In gestation sows were fed individual quantities of feed based on their parity and body weight on d 100 of gestation. Sows were fed a pre-weighed amount of feed using Equation 4 (as described in Section 2.2.2) (NRC, 2012). In lactation, sow feed allowance was increased by ~ 0.5 kg/d to appetite. The diet compositions and calculated nutrient levels of the test diets during gestation are presented in Table 5.1 and during lactation are presented in Table 5.2. The gestation and lactation diets were formulated to meet all nutrient requirements of the gestating and lactating sow respectively (NRC, 2012).

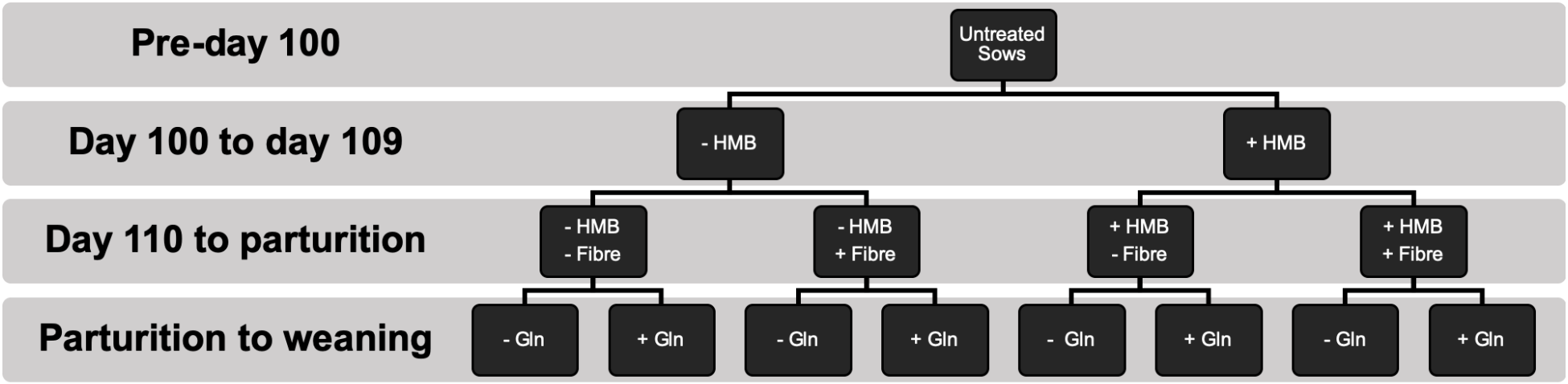

Figure 5.1. The 2 × 2 × 2 experimental design for Experiment 3

HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine

On d 100 of gestation sows were split into two groups and fed a standard gestation diet with or without 0.15 % (as-fed) supplemental HMB (+ HMB, - HMB; Factor 1). On d 110 of gestation the second factor was introduced, with half the sows in each group going on to receive 1 % (as-fed) supplemental lignocellulose (+ Fibre, - Fibre; Factor 2) in combination with their previous HMB treatment. After parturition gestation treatments ceased and sows were fed a standard lactation diet with or without 1 % (as-fed) supplemental L-glutamine (+ Gln, - Gln; Factor 3). This experimental design was run in parallel on both the indoor and outdoor production systems.

Table 5.1. Composition and nutrient specifications of the gestation diets (%, as-fed basis)

Supplemental HMB[1] (%, as-fed)	0		0.15	
Supplemental lignocellulose (%, as-fed)	0	1	0	1
Ingredients				
Barley	30.00	30.00	30.00	30.00
Wheat	25.30	26.70	25.19	26.59
Wheat feed	39.73	37.11	39.69	37.07
Rapeseed extract	1.30	1.30	1.30	1.30
Vitamin-mineral premix[2]	0.25	0.25	0.25	0.25
L-Lysine liquid	0.03	0.03	0.03	0.03
Choline Chloride	0.04	0.04	0.04	0.04
Limestone	1.00	1.00	1.00	1.00
Salt	0.28	0.28	0.28	0.28
Sodium Bicarbonate	0.35	0.38	0.35	0.38
Lignocellulose	0.00	1.00	0.00	1.00
Soya oil	0.23	0.42	0.23	0.42
Vegetable fat	0.50	0.50	0.50	0.50
Molaferm std[3]	1.00	1.00	1.00	1.00
HMB	0.00	0.00	0.15	0.15
Calculated nutrient composition				
Net energy (MJ/kg)	9.08	9.07	9.07	9.06
Crude protein (%)	12.29	12.03	12.27	12.01
Crude fibre (%)	5.13	5.57	5.12	5.57
NDF (%)	19.16	19.37	19.14	19.35
SID Lysine (%)	0.39	0.39	0.39	0.39
SID Methionine + Cystine (%)	0.40	0.39	0.40	0.39
SID Threonine (%)	0.34	0.33	0.34	0.33
SID Tryptophan (%)	0.14	0.14	0.14	0.14
SID Leucine (%)	0.67	0.65	0.67	0.64
SID Glutamic acid (%)	2.70	2.70	2.70	2.70
Calcium (%)	0.53	0.52	0.53	0.52
Total Phosphorus (%)	0.46	0.44	0.46	0.44
Digestible Phosphorus (%)	0.23	0.22	0.23	0.22

[1]HMB = β-hydroxy β-methyl butyrate

[2]Vitamin and trace mineral premix provided per kg of the diet: 10,000 IU vitamin A, 1850 IU vitamin D_3, 50 IU vitamin E, 4 mg vitamin K, 1.5 mg thiamine (B_1), 4 mg riboflavin (B_2), 3.5 mg pyridoxine (B_6), 15 µg vitamin B_{12}, 12 mg pantothenic acid, 20 mg nicotinic acid, 200 µg biotin, 2 mg folic acid, 15 mg copper, 1 mg iodine, 80 mg iron, 50 mg manganese, 0.25 mg selenium, 100 mg zinc, 100 mg oxy-nil dry and 150 mg phytase.

[3]Liquid blend of molasses used as a binding agent

Table 5.2. Composition and nutrient specifications of the lactation diets (%, as-fed basis)

Supplemental L-Glutamine (%, as-fed)	0	1
Ingredients		
Barley	30.00	30.00
Wheat	34.55	33.81
Wheat feed	15.00	15.00
Soyabean meal	10.90	10.90
Rapeseed extract	2.50	2.50
Vitamin-mineral premix[1]	0.25	0.25
L-Lysine Liquid	0.53	0.53
DL-Methionine	0.05	0.05
Choline Chloride	0.04	0.04
Sow additive pack	0.10	0.10
Limestone	0.88	0.88
Dicalcium Phosphate	0.90	0.90
Salt	0.31	0.31
Soya oil	1.83	1.60
Vegetable fat	0.79	0.75
Molaferm Std[2]	1.00	1.00
L-Glutamine	0.00	1.00
Calculated nutrient composition		
Net energy (MJ/kg)	9.88	9.84
Crude protein (%)	15.39	16.51
Crude fibre (%)	4.06	4.04
SID Lysine (%)	0.84	0.83
SID Methionine + Cystine (%)	0.52	0.52
SID Threonine (%)	0.58	0.58
SID Tryptophan (%)	0.17	0.17
SID Leucine (%)	0.90	0.89
SID Glutamic acid (%)	3.19	4.17
SID Glutamine (%)	2.94	3.91
Calcium (%)	0.79	0.79
Total Phosphorus (%)	0.53	0.53
Digestible Phosphorus (%)	0.33	0.33

[1]Vitamin and trace mineral premix provided per kg of the diet: 10,000 IU vitamin A, 1850 IU vitamin D_3, 50 IU vitamin E, 4 mg vitamin K, 1.5 mg thiamine (B_1), 4 mg riboflavin (B_2), 3.5 mg pyridoxine (B_6), 15 µg vitamin B_{12}, 12 mg pantothenic acid, 20 mg nicotinic acid, 200 µg biotin, 2 mg folic acid, 15 mg copper, 1 mg iodine, 80 mg iron, 50 mg manganese, 0.25 mg selenium, 100 mg zinc, 100 mg oxy-nil dry and 150 mg phytase.
[2]Liquid blend of molasses used as a binding agent

5.3.2. Indoor production system

5.3.2.1. Animals and Management

One hundred and seventy mixed parity sows (Large White × Landrace [JSR 9T, JSR, UK]) were used across seven consecutive batches (18 to 28 sows per batch) and followed for one parity. On d 100 of gestation sows were allocated to one of eight dietary treatments, primarily on the basis of parity (range 1:7), and then matched for previous litter history, d 100 body weight and back-fat thickness. Sows were housed throughout gestation and lactation according to standard Spen Farm practice as described in Section 2.2.1.1. Sows were fed as described in Section 2.2.2.

Piglets received the management treatment as stated in Section 2.2.1.1. Cross fostering commenced as stated in Section 2.4. Foster sows were introduced when the number of piglets was still too high post fostering. Seven foster sows were used across the seven batches; one in each of batches one, two, three, four and six and two in batch seven. Across the whole trial 2,666 piglets were born to the 170 sows and of these 124 were still born.

5.3.2.2. Measurements

Measurements including sow weights, back-fat, feed intake, litter sizes and piglet weights were recorded as described in Section 2.4. Cameras were placed in the farrowing house (as described in Section 2.4). Video footage was used to give piglets a score based on their behaviour in the first 15 secs after birth using the following scoring system adapted from Baxter et al. (2008): 0 = no visible signs of movement within 15 secs; 1 = some signs of movement within 15 secs e.g, gasping or breathing; 2 = piglet shows movement within 15 secs but does not attempt to stand; 3 = piglet shows good movement and attempts to stand within 15 secs. Daily faecal scores were taken for each sow from d 113 of gestation until parturition using the following numeric scoring system described by Oliviero et al. (2009): 0: no faeces, 1: dry and pellet shaped, 2: between dry and normal, 3: normal and soft, but firm and well formed, 4: between normal and wet, still formed but not firm, 5: very wet faeces, unformed and liquid. Colostrum samples were collected before the birth of the third piglet (as described in Section 2.4.1). Piglets averaged 6.8 ± 0.18 d and 27.7 ± 0.19 d (average age ± SEM) at week one and at weaning respectively.

5.3.2.3. Farrowings attended

Forty-four litters had fully supervised farrowings and were used to work out colostrum yield and intake. At farrowing, when the umbilical cord broke, piglets were picked up, weighed and placed back at the point they were picked up from. Piglets were re-weighed 24 hours after the start of farrowing and their temperatures taken using a tympanic ear thermometer (Tesco Digital Ear Thermometer, Tesco, UK). A further 14 litters were weighed within four hours of the start of farrowing and re-weighed 24 hours later in order to have a higher number of litters to use for average 24 hour weight gain.

5.3.3. Outdoor production system

5.3.3.1. Animals and Management

One hundred and thirty-four mixed parity sows (Large White × Landrace × Duroc) were used across seven consecutive batches (9 to 24 sows per batch). On d 100 of gestation sows were allocated to one of eight dietary treatments, primarily on the basis of parity (range 1:6), and then matched for previous litter history, d 100 body weight and back-fat thickness. Sows were housed according to standard Spen Farm practise for the outdoor production system (Section 2.2.1.2). Sows were fed a pre-determined amount of feed using Equation 4 (described in Section 2.2.2) from ESFs from d 100 until d 109 of gestation. On d 109, when sows were in their farrowing paddocks until parturition, sows were fed the same pre-determined amount of feed in their individual feeding stalls. Due to a technical issue with the ESFs sows in batches five and seven were brought into the farrowing paddocks early (on d 100 of gestation) and fed their pre-determined amount of feed in their individual feeding stalls. After parturition sows were fed their experimental lactation diets.

Piglets received the management treatment as stated in Section 2.2.1.2. Cross fostering to even up litter sizes commenced from 24 until 72 hours post-partum. Fostering was kept within treatment and all fostering was recorded. No foster sows were used outdoors. Across the whole trial 1,707 piglets were born to the 134 sows and of these 18 were still born.

5.3.3.2. Measurements

Measurements including sow weights, back-fat, condition score and litter sizes were recorded as described in Section 2.4. Sow feed intake was measured throughout gestation and lactation. Farrowing date for all sows was recorded. Piglets were weighed within 24 hours of farrowing when possible. If a sow would not come out the arc, piglets were weighed as close to the farrowing date as possible and the date they were tagged on was recorded. When sows farrowed on the weekend piglets were tagged the following Monday. Piglets were then weighed again on average week one (week one age ± SEM = 5.8 ± 0.19 d) and at weaning (wean age ± SEM = 25.8 ± 0.19 d).

5.3.4. Laboratory analysis

Subsamples of dietary samples (which had been collected weekly throughout the experiment) were collected, mixed thoroughly and sent to Sciantec Analytical Services Ltd (Cawood, UK) for crude protein, crude fibre, fat and AA analysis. See Appendix A.1 for details. Further subsamples of dietary samples collected throughout the experiment from the gestation diets were ground to a fine powder (10 -15 g per sample) using a coffee grinder and sent to Heartland Assays (Iowa, USA) for HMB analysis. HMB was analysed via gas chromatography-mass spectrometry.

Colostrum samples were analysed as described in Sections 2.5.1 and 2.5.1.1. Samples from the same batch were analysed on the same plate. The intra- and inter- assay CVs were: 2.7 % and 11.6% for IgA, 2.2 % and 10.5 % for IgG and 3.0 % and 8.4 % for IgM.

5.3.5. Calculations and statistical analysis

Indoor and outdoor data were analysed as separate models. All data were analysed using the General Linear Model (GLM) procedure of SPSS Statistics (version 21.0, SPSS Inc., Chicago IL, USA) with the sow as the experimental unit. Data were first tested for homogeneity of variance and normality using the Levene's test and the Kolmogorov-Smirnov test, respectively. Data displaying heteroscedasticity or non-normal data were $\log_{10}$ transformed prior to statistical analysis. Transformed data were back transformed for inclusion in the respective tables. Farrowing duration, birth intervals, time to udder and colostrum IgA concentration required $\log_{10}$ transformation.

Birth data were based on the sow's genetic litter and post 24 hours data were based on the suckling litter (post-fostering). Foster sows were not included in analysis. The statistical model for birth data (gestation length, numbers of piglets born, average birth weight and litter weights, farrowing characteristics, colostrum analysis) included the effect of HMB and lignocellulose fibre and the associated HMB × lignocellulose interaction, with batch and parity as fixed factors. Non-significant interactions were removed from the model and the main effects were analysed separately. Total number of piglets born was used as a covariate for average piglet birth weight and total litter weight. As it was not always possible to tag all the litters outdoors within 24 hours of parturition the age at which the litter was tagged (in relation to when the sow farrowed) was included as a factor when analysing the outdoor data. Birth intervals, vitality scores and time to suckle were averaged out for each sow, thus the sow was the experimental unit for analysis. Colostrum intake was determined using Equation 2 (as described in Section 1.3.3.1) and yield was determined as the sum of the intakes by piglets in that litter (as described in Section 2.4.2) (Theil et al., 2014b). As piglets were re-weighed at approximately 24 hours old the duration of colostrum suckling (D) was included as 1440 mins as by Lavery (2018). Total born was used as a covariate for vitality and colostrum analysis. Parity was used as a covariate instead of a fixed factor for immunoglobulin analysis when significant. Faecal scores were averaged out for each sow, scores of zero were not included, but instead recorded as the sow experiencing constipation. The number of sows in each treatment group that experienced some level of constipation were analysed using a Pearson Chi Squared test.

The statistical model for post 24 hours data (week one weights, wean weights, ADG and mortality) included the effect of Factor 1, Factor 2 and Factor 3 with the associated Factor 1 × Factor 2, Factor 1 × Factor 3, Factor 2 × Factor 3 and Factor 1 × Factor 2 × Factor 3 interactions, with batch and parity as fixed factors. Non-significant interactions were removed from the model and the main effects were analysed separately. Litter size post-fostering and age were used as covariates when significant for analysis of performance to weaning. Mortality was calculated as the percentage of piglets that died out of the total number of piglets that were born alive in a litter and was based on the sow's genetic litter. Total number of piglets born was used as a covariate for mortality.

The indoor and outdoor data were also collated and run as combined (2 × 2 × 2 × 2) model using the same methods as above with the additional inclusion of production system (indoor or outdoor) as a fixed factor and the associated interactions. Batch could not be included in the combined model as it confounded the effect of production system. Total number of piglets born was used as a covariate for average piglet 24 hour weight however, it was not included as a covariate for total litter weight as outdoor sows had smaller litters than indoor sows it interfered with the results. In addition, litter size post-fostering and age were used as covariates when significant for piglet analysis of performance to weaning but were not included for litter performance to weaning as again litters were smaller outdoors and therefore they interfered with analysis.

Significance was reported at $P<0.05$ and as trends if $P<0.1$. Data are expressed as least-square means with their pooled standard error of the mean (SEM).

5.4. Results

5.4.1. Dietary analysis

The analysed nutrient content of the gestation and lactation experimental diets are presented in Table 5.3. With regard to the gestation diets, the crude protein levels were in line with the formulated values and with each other. The crude fibre levels were slightly lower than formulated in all the gestation diets; however, the levels in the two diets that were supplemented with 1 % lignocellulose fibre (CT + LF and CT + HMB + LF) were still higher than the two diets that were not supplemented with lignocellulose fibre (CT + NLF and CT + HMB + NLF). The levels of HMB in the CT + HMB + NLF and the CT + HMB + LF diets were in line with the formulated values. Trace amounts of HMB were found in the CT + LF diet which is most likely due to the diet going down the production line after a diet which contained HMB. Overall, the analysed nutrient levels in the gestation diets were considered appropriate for use.

The crude protein levels in the in the Lact + Gln diet were slightly higher than that of the Lact diet. Glutamic acid concentrations were analysed and the difference between the levels in the Lact and the Lact + Gln diets was used as an indication of supplementary glutamine. The difference in levels of glutamic acid between the Lact and Lact + Gln diets was ~ 0.83 % which was considered satisfactory for use.

Table 5.3. Analysed nutrient content of experimental diets (as-fed)

Supplemental HMB[1] (%, as fed)	0		0.15		Supplemental L-glutamine (%, as fed)	
Supplemental lignocellulose fibre (%, as fed)	0	1	0	1	0	1
DM (%)	87.9	87.9	88.4	88.4	88.8	87.9
Oil A (%)	3.06	3.67	3.44	3.81	4.94	3.63
Crude protein (%)	13.2	13.7	13.3	13.1	15.3	16.7
Crude fibre (%)	4.5	5.1	4.8	5.0	4.2	4.2
Ash (%)	4.0	5.5	4.7	5.1	4.5	5.4
NaCl (%)	0.38	0.41	0.38	0.43	0.44	0.47
Lysine (%)	0.48	0.49	0.49	0.48	0.93	0.94
Leucine (%)	0.77	0.79	0.79	0.77	0.95	1.02
Glutamic acid (%)	2.76	2.75	2.85	2.72	3.18	4.01
HMB (mg/g)	0.0	0.1	1.4	1.3	-	-

[1]HMB = β-hydroxy β-methyl butyrate

5.4.2. Indoor production system

Of the initial 170 sows six were excluded from all analyses (three from batch two, one from batch 3, one from batch four and one from batch six) due to litter sizes of below 5 or above 22 piglets, leaving 164 sows for birth analysis split across the treatments as follows: 0 % HMB, *n* = 84; 0.15 % HMB, *n* = 80, 0 % lignocellulose fibre, *n* = 79; 1 % lignocellulose fibre, *n* = 85; 0 % glutamine, *n* = 80; 1 % glutamine, *n* = 84. Due to a health problem in the herd 21 % of these remaining litters were treated with electrolytes for scour: 23 % of CT + NFL, 23 % of CT + HMB + NFL, 27 % of CT + LF and 15 % of CT + HMB + LF. Six litters were removed from post birth analysis due to ill health (two from batch two, three from batch three and one from batch four), resulting in 158 litters for post 24 hours analysis split across the treatments as follows: 0 % HMB, *n* = 80; 0.15 % HMB, *n* = 78, 0 % lignocellulose fibre, *n* = 76; 1 % lignocellulose fibre, *n* = 82; 0 % glutamine, *n* = 77; 1 % glutamine, *n* = 81. A separate analysis of litter and piglet performance post 24 hours was also conducted on the litters which had not been affected by scour (115 litters) and excluded those which had (43 litters). All the values presented are mean ± SEM.

5.4.2.1. Sow characteristics

Sow characteristics are presented in Table 5.4. Sow gestation length averaged 114.9 ± 0.17 d across all treatments. Lignocellulose fibre reduced sow gestation length (*P*=0.026); sows fed lignocellulose fibre had ~ 0.5 d shorter gestation length than sows that did not receive lignocellulose fibre (114.6 vs 115.1 d for 1 vs 0 %, respectively). Sows fed HMB in gestation had a 4.4 % higher ADFI in lactation than sows that did not receive HMB (*P*=0.004). There was also a tendency for sows fed HMB in gestation to have a higher total FI in lactation than sows not fed HMB (*P*=0.059). Sows supplemented with glutamine lost more weight from d 109 to weaning compared with sows that were not supplemented with glutamine (-35.8 vs -30.0 kg for 1 vs 0 %, respectively; *P*=0.013). There was also a tendency for sows supplemented with lignocellulose fibre to lose more weight from d 109 to weaning than sows that were not supplemented with lignocellulose fibre (-35.1 vs -30.7 kg for 1 vs 0 % respectively; *P*=0.059). There was a tendency for sow d 109 to weaning weight change to be influenced by a lignocellulose fibre × glutamine interaction (*P*=0.083). Sows supplemented with both lignocellulose fibre and glutamine lost more weight than sows which did not receive lignocellulose fibre or glutamine. There was also a tendency for sow back-

fat change from d 109 to weaning to be influenced by a lignocellulose fibre × glutamine interaction (*P*=0.080). Glutamine supplementation increased the amount of back-fat lost in sows which had not been supplemented with lignocellulose fibre. However, glutamine supplementation had no effect on the back-fat loss of sows which had been supplemented with lignocellulose fibre.

5.4.2.2. Litter performance of all indoor litters

Litter performance for all of the indoor litters, including those which were affected by scour, is presented in Table 5.5. There were no treatment interactions so data presented are the main effects. Total number of piglets born were similar across treatments for both HMB (*P*=0.111) and lignocellulose fibre (*P*=0.986) and averaged 15.8 ± 0.39 piglets/litter. Total born litter weight and total live born litter weight averaged 21.8 ± 0.34 kg and 21.1 ± 0.41 kg, respectively, across all treatments. Total born litter weight and total live born litter weight were not affected by HMB (*P*=0.611 and *P*=0.854, respectively) or lignocellulose fibre (*P*=0.316 and *P*=0.505, respectively).

HMB supplementation to sows increased total litter week one weight gain by 13.8 % compared with litters from sows that were not supplemented with HMB (13.2 vs 11.6 kg for 0.15 vs 0 %, respectively; *P*=0.026). As a result of this there was a tendency for HMB to increase litter weights at week one (*P*=0.076); sows supplemented with HMB on average had 4.4 % heavier litters at week one compared with sows that did not receive HMB (32.2 vs 30.1 kg, for 0.15 vs 0 %, respectively). However, this trend was not apparent at weaning (*P*=0.886). Litter weights at week one and weaning were not impacted by lignocellulose fibre (*P*=0.474 and *P*=0.631, respectively) or by glutamine (*P*=0.467 and *P*=0.546, respectively).

As lignocellulose fibre reduced gestation length the average age of litters at week one and at weaning were increased by lignocellulose fibre (*P*=0.032 and *P*=0.019, respectively). Litters from sows supplemented with lignocellulose fibre were on average 0.5 d and 0.6 d older at week one and weaning respectively, than litters from sows which did not receive lignocellulose fibre. The age gap changed between week one and weaning as some litters were weaned early due to the need for foster sows and were therefore excluded from weaning analysis. Percentage mortality was similar across treatments and averaged 14.8 ± 1.4 % by weaning.

Table 5.4. The main effects of β-hydroxy β-methyl butyrate and lignocellulose fibre supplementation to sows in gestation and L-glutamine supplementation to sows in lactation on sow characteristics on the indoor production system[1]

Variable	HMB[2]		Fibre		Gln		SEM	*P*-value[2]		
	0 %	0.15 %	0 %	1 %	0 %	1 %		HMB	Fibre	Gln
Parity	2.6	2.6	2.6	2.6	2.6	2.6	0.17	0.930	0.856	0.815
Sow weights (kg)										
D100	232.5	235.3	-	-	-	-	1.88	0.264	-	-
D109	239.9	242.2	241.8	240.4	239.9	242.2	1.93	0.380	0.582	0.397
D100 to d109 change	+ 7.4	+ 7.0	+ 7.5	+ 6.9	-	-	0.50	0.499	0.423	-
D109 to wean change[3]	- 33.2	- 32.7	- 30.7	- 35.1	- 30.0	- 35.8	1.69	0.805	**0.059**	**0.013**
Sow back-fat (mm)										
D100	14.1	14.0	-	-	-	-	0.34	0.774	-	-
D109	14.4	14.5	14.5	14.4	14.2	14.7	0.29	0.744	0.693	0.267
D100 to d109 change	+ 0.3	+ 0.6	+ 0.3	+ 0.6	-	-	0.27	0.506	0.317	-
D109 to wean change[3]	- 3.4	- 3.6	- 3.5	- 3.5	- 3.4	- 3.6	0.25	0.476	0.836	0.680
Condition score										
Day 100	3.1	3.0	-	-	-	-	0.04	0.220	-	-
Farrow	3.1	3.1	3.1	3.1	3.1	3.2	0.03	0.675	0.603	0.513
Wean	2.9	2.9	2.9	2.9	3.0	2.9	0.06	0.878	0.896	0.113
Gestation length (d)[4]	114.8	114.9	115.1	114.6	-	-	0.17	0.746	**0.026**	-
Sow faecal scores	3.0	2.9	2.9	3.0	-	-	0.06	0.310	0.114	-
Lactation FI (kg)[2,5]										
Total FI	170.6	176.0	173.1	173.5	174.3	172.3	2.06	**0.059**	0.895	0.462
ADFI[2]	6.4	6.6	6.6	6.4	6.5	6.5	0.07	**0.004**	0.133	0.530

[1]Data represents a total of 164 litters for data to parturition and 151 litters for data to wean
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine, FI = feed intake, ADFI = average daily feed intake
[3]Lignocellulose fibre × glutamine interaction for d 109 to wean weight change and d 109 to wean back-fat change (*P*=0.083 and *P*=0.080 respectively)
[4]Corrected for total born
[5]Corrected for litter size

Table 5.5. The main effects of β-hydroxy β-methyl butyrate and lignocellulose fibre to sows in gestation and L-glutamine supplementation to sows in lactation on litter performance on the indoor production system[1]

Variable	HMB[2]		Fibre		Gln		SEM	*P*-value[2]		
	0%	0.15%	0%	1%	0%	1%		HMB	Fibre	Gln
Numbers of piglets										
Total Born	16.3	15.4	15.8	15.8	-	-	0.39	0.111	0.986	-
Alive[3]	15.3	15.1	15.2	15.1	-	-	0.11	0.273	0.683	-
Dead[3,4]	0.5	0.7	0.5	0.6	-	-	0.10	0.303	0.655	-
Week one	12.3	12.2	12.2	12.3	12.1	12.4	0.18	0.514	0.464	0.171
Wean	11.8	11.8	11.7	11.9	11.8	11.8	0.20	0.832	0.623	0.824
Litter weights (kg)										
Total born[3]	21.7	21.9	21.5	22.0	21.7	21.8	0.34	0.611	0.316	0.914
Live born[3]	21.2	21.1	21.0	21.3	21.1	21.2	0.41	0.854	0.505	0.785
Week one[5,6]	30.8	32.2	31.2	31.8	31.2	31.8	0.56	**0.076**	0.474	0.467
Wean[5,6]	88.1	88.3	87.7	88.7	87.6	88.8	1.43	0.886	0.631	0.546
Total litter gains (kg)										
Week one[5,6]	11.6	13.2	12.4	12.5	12.4	12.4	0.50	**0.026**	0.876	0.947
Week one to wean[5]	56.4	55.4	55.6	56.2	56.1	55.7	1.21	0.547	0.752	0.833
Birth to wean[5,6]	67.3	67.6	67.2	67.7	67.3	67.6	1.46	0.873	0.825	0.858
Week one age (d)	6.9	6.7	6.5	7.0	6.8	6.8	0.18	0.507	**0.032**	0.877
Wean (d)	27.8	27.6	27.4	28.0	27.8	27.7	0.19	0.387	**0.019**	0.836
24 hour mortality[3] (%)	4.7	5.1	4.5	5.3	4.8	5.0	0.82	0.678	0.493	0.891
Week one mortality[3] (%)	12.9	12.5	13.0	12.4	13.3	12.2	1.38	0.841	0.763	0.555
Wean mortality[3] (%)	14.9	14.7	15.7	13.9	14.7	14.9	1.43	0.890	0.377	0.940

[1]Data represents a total of 164 litters for data to parturition, 158 litters for data to week one and 151 litters for data to weaning
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine
[3]Corrected for total born
[4]Non-normal data, generalised linear model used
[5]Corrected for litter size
[6]Corrected for age

5.4.2.3. Litter characteristics at farrowing and colostrum production

Litter characteristics at farrowing are presented in Table 5.6. There were no treatment interactions therefore data presented are the main effects. The main effect of glutamine was included in the model analysing 24 hour weight, 24 hour weight gain, colostrum intake and colostrum yield, as the sows had received one feed on their lactation diets before these measurements were taken. However, it did not appear to have any effect on any of the parameters measured or influence any of the other treatments tested. Therefore, as the sows had only received one feed on glutamine it was removed from the model and only the effects of HMB and lignocellulose fibre are presented.

Farrowing duration from the time of birth of the first piglet until the time of birth of the last piglet averaged 226.3 ± 14.29 mins and ranged from 61.5 to 602.4 mins. There was no effect of treatment on farrowing duration from the birth of the first piglet until the birth of the last piglet (HMB, P=0.396; lignocellulose fibre, P=0.796). Piglets from sows supplemented with HMB had higher vitality scores compared with piglets from sows that were not supplemented with HMB (1.9 vs 1.7 for 0.15 vs 0 %, respectively; P<0.001).

When looking at the litters that were weighed at farrowing, there was no effect of treatment on the birth weight (HMB, P=0.514; lignocellulose fibre, P=0.235) or on the 24 hour weight (HMB, P=0.240; lignocellulose fibre, P=0.253) of piglets. However, piglets from sows supplemented with HMB had 47.8 % higher 24 hour weight gain compared with piglets from sows that did not receive HMB (106.8 vs 72.3 g for 0.15 vs 0 %, respectively; P=0.047).

Colostrum intake of piglets alive at 24 hours averaged 449 ± 18.4 g/piglet and the average intake in each litter ranged from 280 to 759 g/piglet. There was a tendency for HMB supplementation to increase colostrum intake (P=0.083); piglets from sows supplemented with HMB on average consumed 11.1 % more colostrum than piglets from sows that were not supplemented with HMB (472.7 vs 425.3 g for 0.15 vs 0 %, respectively). Total colostrum yield averaged 6.1 ± 0.26 kg/sow and ranged from 2.2 to 8.9 kg/sow. HMB supplementation increased colostrum yield by 18.2 % compared with sows that were not supplemented with HMB (6.6 vs 5.6 kg for 0.15 vs 0 %, respectively; P=0.009).

Table 5.6. The effects of β-hydroxy β-methyl butyrate and lignocellulose fibre to sows in gestation on litter characteristics at farrowing and colostrum production[1]

Variable	HMB[2]		Fibre		SEM	*P*-Value[2]	
	0%	0.15%	0%	1%		HMB	Fibre
Farrow duration (min)[3,4]	214.51	238.07	224.02	228.57	14.285	0.396	0.796
Farrow duration to placenta (min)[3]	284.05	285.82	285.36	284.51	14.712	0.930	0.966
Birth interval (mins)[3,4]	14.98	16.13	15.26	15.85	1.064	0.612	0.647
Average vitality score	1.66	1.86	1.73	1.80	0.036	**<0.001**	0.134
Time to udder (mins)[3,4]	14.87	14.10	13.79	15.18	1.164	0.450	0.338
Litters weighed within 4 hours of the start farrowing							
Birth weight[3] (kg)	1.35	1.34	1.35	1.34	0.041	0.828	0.784
24 hour weight[3] (kg)	1.42	1.43	1.43	1.42	0.045	0.953	0.841
24 hour weight gain[3] (g)	72.0	88.3	78.5	81.8	12.85	0.263	0.815
24 hour temperature (°C)	36.8	36.8	36.7	36.9	0.22	0.902	0.606
Litters weighed at farrowing							
Birth weight[3] (kg)	1.34	1.38	1.39	1.32	0.040	0.514	0.235
24 hour weight[3] (kg)	1.44	1.51	1.51	1.44	0.042	0.240	0.253
Weight gain (g)	72.3	106.8	93.7	85.5	11.72	**0.047**	0.628
Colostrum intake[3] (g)	425.3	472.7	458.3	439.7	18.40	**0.083**	0.488
Colostrum yield[3] (kg)	5.61	6.63	6.31	5.92	0.256	**0.009**	0.293

[1]Data represents a total of 108 litters for farrow durations, birth interval, vitality score and time to udder; 57 litters for litters weighed within 4 hours of farrowing; 44 litters for litters weighed at farrowing
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre
[3]Corrected for litter size
[4]Non-normal data, $\log_{10}$ transformed

5.4.2.4. Piglet performance for all piglets

Piglet growth performance from 24 hours to weaning for all piglets, including those from litters which were effected by scour, is presented in Table 5.7. There were no treatment interactions, therefore data presented are the main effects. Piglet live 24 hour weight averaged 1.42 ± 0.021 kg across all treatments. There were no significant effects of HMB (*P*=0.265) or lignocellulose fibre (*P*=0.425) on average piglet live 24 hour weight.

Piglet week one and wean weights averaged 2.56 ± 0.045 kg and 7.53 ± 0.103 kg, respectively across all treatments. There was a tendency for maternal HMB supplementation to increase average piglet week one weight by 4.6 % compared with piglets from sows which did not receive HMB (2.62 vs 2.50 kg for 0.15 vs 0 %, respectively; *P*=0.059). There was also a tendency for maternal HMB supplementation to increase average piglet weaning weight by 3.2 % compared with piglets from sows which did not receive HMB (7.65 vs 7.41 kg for 0.15 vs 0 %, respectively; *P*=0.089). Lignocellulose fibre and glutamine had no impact on average piglet week one (lignocellulose fibre, *P*=0.383; glutamine, *P*=0.521, respectively) or weaning weights (lignocellulose fibre, *P*=0.882; glutamine *P*=0.701, respectively).

Table 5.7. The main effects of β-hydroxy β-methyl butyrate and lignocellulose fibre to sows in gestation and L-glutamine supplementation to sows in lactation, on piglet performance from 24 hours to weaning on the indoor production system[1]

Variable	HMB[2]		Fibre		Gln		SEM	*P*-Value[2]		
	0 %	0.15 %	0 %	1 %	0 %	1 %		HMB	Fibre	Gln
Average weights (kg)										
24 hour (total)[3]	1.39	1.42	1.40	1.42	-	-	0.021	0.291	0.438	-
24 hour (live)[3]	1.40	1.44	1.41	1.43	-	-	0.021	0.265	0.425	-
24 hour (post foster)[4]	1.41	1.44	1.42	1.43	1.42	1.42	0.022	0.221	0.986	0.984
Week one[4,5]	2.50	2.62	2.54	2.59	2.54	2.58	0.045	**0.059**	0.383	0.521
Wean[4,5]	7.41	7.65	7.52	7.54	7.50	7.56	0.103	**0.089**	0.882	0.701
ADGs (g)[2]										
Week one[5]	165	167	166	166	163	169	4.5	0.693	0.997	0.282
Week one to wean[4]	235	240	237	238	237	237	3.8	0.327	0.931	0.952
Birth to wean[4]	219	224	222	222	221	223	3.5	0.286	0.962	0.609

[1]Data represents a total of 164 litters for data to parturition, 158 litters for data to week one and 151 litters for data to weaning
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre Gln = L=glutamine, ADG = average daily gain
[3]Corrected for total born
[4]Corrected for litter size
[5]Corrected for age

5.4.2.5. Immunoglobulin concentrations

Colostrum IgA, IgG and IgM concentrations are presented in Table 5.8. There were no HMB × lignocellulose fibre interactions and so data displayed are the main effects. Colostrum IgG concentration averaged 56.8 ± 1.77 mg/ml and ranged from 21.9 to 119.9 mg/ml. IgG concentration was not impacted by HMB (*P*=0.114) or by lignocellulose fibre (*P*=0.314). Colostrum IgA concentration averaged 10.5 ± 0.35 mg/ml and ranged from 4.8 to 19.7 mg/ml. IgA concentration was not impacted by HMB (*P*=0.878) or by lignocellulose fibre (*P*=0.917). Colostrum IgM concentration averaged 4.4 ± 0.16 mg/ml and ranged from 0.5 to 9.2 mg/ml. There was a tendency for HMB to increase IgM concentration by 8.8 % compared with the control (4.6 vs 4.2 mg/ml for 0.15 vs 0 %, respectively; *P*=0.094).

Table 5.8. The effect of β-hydroxy β-methyl butyrate and lignocellulose fibre supplementation to sows in gestation on concentrations of IgA, IgG and IgM in sow colostrum on the indoor production system[1]

Variable[2]	HMB[3]		Fibre		SEM	*P*-value[3]	
	0 %	0.15 %	0 %	1 %		HMB	Fibre
IgA[4]	10.6	10.4	10.4	10.6	0.35	0.878	0.917
IgG	58.8	54.9	55.6	58.1	1.75	0.114	0.314
IgM	4.2	4.6	4.3	4.5	0.16	**0.094**	0.379

[1]Data represents a total of 136 sows
[2]Corrected for parity
[3]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre
[4]Non-normal data, $\log_{10}$ transformed

5.4.2.6. Performance of litters which were not affected by scour

Litter performance for litters which were not affected by scour is presented in Table 5.9. There were no treatment interactions so the data presented are the main effects. Litters from sows supplemented with HMB gained more weight in week one compared with litters from sows that were not supplemented with HMB (14.4 vs 12.3 kg for 0.15 vs 0 %, respectively; *P*=0.009). There was a tendency for litters from sows supplemented with lignocellulose fibre to gain more weight from birth to weaning than litters from sows that were not supplemented with lignocellulose fibre (73.7 vs 69.8 kg for 1 vs 0 %, respectively; *P*=0.079). In addition, there was a tendency for lignocellulose fibre supplementation to increase litter weight at weaning by 4.7 % compared with litters from sows that were not supplemented with lignocellulose fibre (90.8 vs 86.7 for 1 vs 0 %, respectively; *P*=0.079). Glutamine supplementation did not influence litter weights at week one (*P*=0.619) or at weaning (*P*=0.721). Percentage mortality was similar across treatments and averaged 13.9 ± 1.65 % to weaning.

5.4.2.7. Performance of piglets from litters which were not affected by scour

Piglet performance for piglets from litters which were not affected by scour is presented in Table 5.10. There were no treatment interactions so the data presented are the main effects. Piglets from sows supplemented with HMB were 6.0 % heavier at week one compared with piglets from sows which were not supplemented with HMB (2.66 vs 2.51 kg for 0.15 vs 0 %, respectively; *P*=0.027). There was also a tendency for piglets from sows supplemented with HMB to be heavier at weaning compared with piglets from sows which did not receive HMB (7.71 vs 7.46 kg for 0.15 vs 0 %, respectively; *P*=0.099). There was a tendency for piglets from sows supplemented with lignocellulose fibre to be 4.7 % heavier than piglets from sows that were not supplemented with lignocellulose fibre at week one (2.65 vs 2.53 kg for 1 vs 0 %, respectively; *P*=0.072). This was not apparent at weaning (*P*=0.410). There was no effect of glutamine on weights at week one (*P*=0.462) or at weaning (*P*=0.460).

Table 5.9. The main effects of β-hydroxy β-methyl butyrate and lignocellulose fibre to sows in gestation and L-glutamine supplementation to sows in lactation on litter performance on the indoor production system, excluding any litters which had been affected by scour[1]

Variable	HMB[2]		Fibre		Gln		SEM	*P*-value[2]		
	0%	0.15%	0%	1%	0%	1%		HMB	Fibre	Gln
Numbers of piglets										
Post foster	13.8	13.3	13.7	13.4	13.6	13.6	0.28	0.165	0.451	0.993
Week one	12.4	12.3	12.1	12.5	12.3	12.3	0.20	0.775	0.182	0.939
Wean	11.8	11.9	11.7	12.0	11.8	11.9	0.22	0.877	0.395	0.897
Litter weights (kg)										
Total post foster	19.0	18.8	18.4	19.3	18.6	19.1	0.44	0.541	0.132	0.365
Week one[4,5]	30.8	32.3	31.0	32.1	31.3	31.8	0.70	0.117	0.271	0.619
Wean[4,5]	87.9	89.6	86.7	90.8	88.3	89.1	1.65	0.459	**0.079**	0.721
Litter gains (kg)										
Week one[4,5]	12.3	14.4	13.1	13.6	13.4	13.3	0.61	**0.009**	0.515	0.844
Week one to wean[4]	58.1	58.7	57.3	59.5	58.3	58.5	1.29	0.733	0.202	0.923
Birth to wean[4]	70.5	72.9	69.8	73.7	71.9	71.6	1.62	0.265	**0.073**	0.886
Age (d)										
Week one	6.7	6.8	6.5	7.0	6.8	6.7	0.22	0.514	**0.099**	0.528
Wean	27.6	27.8	27.4	28.0	27.8	27.6	0.23	0.640	**0.081**	0.563
Mortality (%)[3]										
Day	4.3	5.6	4.6	5.3	4.5	5.3	1.02	0.372	0.609	0.545
Week one	12.1	11.8	12.7	11.2	11.5	12.4	1.58	0.903	0.465	0.655
Wean	14.2	13.6	15.1	12.7	13.5	14.3	1.65	0.777	0.288	0.738

[1]Data represents a total of 115 litters which were not affected by scour and excludes the 43 litters which were and needed treatment with electrolytes
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine
[3]Corrected for total born
[4]Corrected for litter size
[5]Corrected for age

Table 5.10. The main effects of β-hydroxy β-methyl butyrate and lignocellulose fibre to sows in gestation and L-glutamine supplementation to sows in lactation, on piglet performance from 24 hours to weaning on the indoor production system, excluding any litters which had been affected by scour[1]

Variable	HMB[2]		Fibre		Gln		SEM	*P*-value[2]		
	0 %	0.15 %	0 %	1 %	0 %	1 %		HMB	Fibre	Gln
Average weights (kg)										
24 hour (post foster)[3]	1.40	1.46	1.41	1.44	1.42	1.43	0.025	0.104	0.389	0.806
Week one[3,4]	2.51	2.66	2.53	2.65	2.56	2.61	0.050	**0.027**	**0.072**	0.462
Wean[3,4]	7.46	7.71	7.52	7.65	7.53	7.64	0.110	**0.099**	0.410	0.460
ADGs (g)[2]										
Week one[3,4]	167	173	166	174	168	172	4.7	0.358	0.180	0.521
Week one to wean[3]	237	241	238	240	239	239	4.0	0.433	0.728	0.896
Birth to wean[3]	222	226	223	225	223	226	3.6	0.356	0.643	0.506

[1]Data represents the mean of 115 litters which were not affected by scour and excludes the 43 litters which were and needed treatment with electrolytes
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre Gln = L=glutamine, ADG = average daily gain
[3]Corrected for litter size
[4]Corrected for age

5.4.3. Outdoor production system

Overall, the litters performed well throughout the experiment. Batch seven was affected by seasonal infertility which resulted in a batch size of only nine sows. One sow was excluded from all analysis (in batch seven) due to savaging behaviour resulting in only eight sows left in batch seven and 133 sows left for overall analysis split across the treatments as follows: 0 % HMB, *n* = 64; 0.15 % HMB, *n* = 69, 0 % lignocellulose fibre, *n* = 66; 1 % lignocellulose fibre, *n* = 67; 0 % glutamine, *n* = 67; 1 % glutamine, *n* = 66. All the values presented are mean ± SEM.

5.4.3.1. Sow characteristics

Sow characteristics are presented in Table 5.11. There were no treatment interactions so data presented are the main effects. Sow weights and back-fat thickness were similar on entry to the trial and averaged 213.0 ± 2.45 kg and 11.0 ± 0.36 mm, respectively. Sow weight and back-fat changes from d 100 to movement to the farrowing paddocks (d 109) were similar across treatments. Sow gestation length averaged 115.9 ± 0.18 d, across all treatments. There was a tendency for sows fed HMB to have shorter gestation lengths (by 0.4 d) compared with sows not fed HMB (*P*=0.087). Sows fed lignocellulose fibre numerically had a 0.2 d shorter gestation length than sows not fed lignocellulose fibre, however this was not significant (*P*=0.384). Sow ADFI in lactation was not impacted by HMB (*P*=0.489), lignocellulose fibre (*P*=0.705) or glutamine (*P*=0.926). Sow weight and back-fat changes from d 109 to weaning were similar across treatments and averaged -28.2 ± 2.70 kg and -1.9 ± 0.32 mm, respectively.

5.4.3.2. Litter performance

Litter performance is presented in Table 5.12. There were no treatment interactions and so data presented are the main effects. Total number of piglets born were similar across treatments and averaged 12.5 ± 0.36 piglets/litter across all treatments. Total born litter weights and total live born litter weights averaged 20.2 ± 0.42 kg and 20.0 ± 0.43 kg respectively, across all treatments. Total born litter weight and total live born litter weight were not affected by HMB (*P*=0.598 and *P*=0.538, respectively) or lignocellulose fibre (*P*=0.699 and *P*=0.656, respectively).

Total litter weights at week one and at weaning averaged 30.2 ± 0.62 kg and 84.3 ± 1.23 kg respectively, across all treatments. There was a tendency for week one

litter weight gain to be increased by glutamine supplementation (11.0 vs 9.8 kg for 1 vs 0 %, respectively; *P*=0.072). In addition, litters from sows supplemented with HMB numerically gained 6.9 % more weight than litters from sows that were not supplemented with HMB (10.8 vs 10.1 kg for 0.15 vs 0 %, respectively; *P*=0.307). However, total week one and weaning litter weights were not affected by HMB (*P*=0.563 and *P*=0.491, respectively), lignocellulose fibre (*P*=0.844 and *P*=0.265, respectively) or glutamine (*P*=0.844 and *P*=0.858, respectively). Percentage mortality was similar across treatments and averaged 9.6 ± 1.13 % by weaning.

5.4.3.3. Piglet performance

Piglet growth performance from 24 hours to weaning is presented in Table 5.13. There was an interaction between HMB and glutamine supplementation for week one ADG which is presented in Figure 5.2. Glutamine supplementation had no effect on piglet performance to weaning in piglets from sows which had received HMB. However, glutamine improved the ADG of piglets from sows which had not received HMB. There were no other treatment interactions therefore data presented are the main effects.

Piglet 24 hour live weights averaged 1.64 ± 0.032 kg and piglet week one and wean weights averaged 2.68 ± 0.056 kg and 7.77 ± 0.118 kg, respectively across all treatments. Average week one weight was similar across treatments (HMB, *P*=0.401; lignocellulose fibre, *P*=0.618; glutamine, *P*=0.787), as was wean weight (HMB, *P*=0.349; lignocellulose fibre, *P*=0.204; glutamine, *P*=0.937). However, piglets from sows supplemented with HMB were numerically 2.3 % heavier at week one (2.71 vs 2.65 kg for 0.15 vs 0 %, respectively) and 1.9 % heavier at weaning, than piglets from sows that were not supplemented with HMB (7.74 vs 7.60 kg for 0.15 vs 0 %, respectively). Piglets from sows supplemented with lignocellulose fibre were numerically 1.4 % heavier at week one and 2.7 % heavier at weaning, compared with piglets from sows that were not supplemented with lignocellulose fibre.

Table 5.11. The main effects of β-hydroxy β-methyl butyrate and lignocellulose fibre supplementation to sows in gestation and L-glutamine supplementation to sows in lactation on sow characteristics on the outdoor production system[1]

Variable	HMB[2]		Fibre		Gln		SEM	*P*-value[2]		
	0 %	0.15 %	0 %	1 %	0 %	1 %		HMB	Fibre	Gln
Parity	3.0	2.9	2.9	3.1	2.8	3.2	0.23	0.692	0.401	0.171
Sow weights (kg)										
D100	212.5	213.5	-	-	-	-	2.44	0.745	-	-
D109	207.1	204.6	210.8	201.0	204.1	208.0	5.29	0.727	0.163	0.584
D100 to d109 change	+ 5.4	+ 4.3	+ 4.8	+ 4.9	-	-	1.41	0.526	0.922	-
D109 to wean change	- 28.0	- 28.3	- 28.9	- 27.4	- 27.6	- 28.7	2.70	0.926	0.675	0.759
Sow back-fat (mm)										
D100	11.1	11.0	-	-	-	-	0.36	0.770	-	-
D109	11.1	10.8	10.8	11.1	11.1	10.6	0.37	0.506	0.518	0.661
D100 to d109 change	+ 0.1	- 0.2	- 0.2	+ 0.1	-	-	0.24	0.447	0.245	-
D109 to wean change	- 2.1	- 1.6	- 1.8	- 1.9	- 1.9	- 1.7	0.32	0.288	0.714	0.623
Condition score										
Day 100	2.9	2.9	-	-	-	-	0.04	0.423	-	-
Farrow	3.2	3.1	3.1	3.1	3.2	3.1	0.04	0.055	0.554	0.212
Wean	2.8	2.8	2.8	2.8	2.8	2.8	0.06	0.750	0.814	0.982
Gestation length (d)[3]	116.1	115.7	116.0	115.8	-	-	0.18	**0.087**	0.384	-
Lactation FI (kg)[2]										
Total FI	183.7	184.4	183.1	185.0	184.8	183.3	0.92	0.583	0.122	0.215
ADFI[2]	7.8	7.2	7.8	7.7	7.8	7.8	0.07	0.489	0.705	0.926

[1]Data represents a total of 133 sows
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine, FI = feed intake, ADFI = average daily feed intake
[3]Corrected for total born

Table 5.12. The main effects of β-hydroxy β-methyl butyrate and lignocellulose fibre supplementation to sows in gestation and L-glutamine supplementation to sows in lactation on litter performance on the outdoor production system[1]

Variable	HMB[2]		Fibre		Gln		SEM	*P*-Value[2]		
	0 %	0.15 %	0 %	1 %	0 %	1 %		HMB	Fibre	Gln
Numbers of piglets										
Total Born	12.5	12.6	12.7	12.4	-	-	0.36	0.997	0.647	-
Alive	12.3	12.4	12.5	12.2	-	-	0.36	0.835	0.551	-
Dead[3]	0.2	0.2	0.2	0.2	-	-	0.06	0.463	0.210	-
Week one	11.1	11.4	11.5	11.0	11.2	11.3	0.32	0.359	0.261	0.261
Wean	10.9	11.2	11.3	10.8	11.0	11.1	0.29	0.461	0.180	0.861
Litter weights (kg)										
Total born[4]	20.0	20.3	20.3	20.1	-	-	0.42	0.598	0.669	-
Live born[4]	19.8	20.2	20.1	19.9	-	-	0.43	0.538	0.656	-
Week one[5,6]	29.9	30.4	30.1	30.2	29.7	30.6	0.62	0.563	0.844	0.844
Wean[5,6]	83.8	84.9	83.4	85.3	84.2	84.5	1.23	0.491	0.265	0.858
Total litter weight gains (kg)										
Week one	10.1	10.8	10.3	10.5	9.8	11.0	0.52	0.307	0.809	**0.072**
Week one to wean	53.9	54.4	53.4	54.9	54.4	53.9	1.1	0.734	0.295	0.727
Birth to wean	63.9	65.2	63.7	65.4	64.2	64.8	1.2	0.422	0.294	0.711
Week one age (d)	5.7	5.9	5.7	6.0	5.9	5.8	0.20	0.300	0.449	0.574
Wean age (d)	25.7	26.0	25.7	26.0	25.9	25.7	0.19	0.225	0.236	0.516
24 hour mortality[4] (%)	2.4	3.1	2.5	3.1	2.6	3.0	0.66	0.429	0.465	0.644
Week one mortality[4] (%)	7.3	7.0	6.2	8.1	7.0	7.3	1.01	0.804	0.157	0.791
Wean mortality[4] (%)	10.4	9.3	9.0	10.7	9.6	10.1	1.13	0.468	0.254	0.761

[1]Data represents a total of 133 litters
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine
[3]Non-normal data, generalised linear model used
[4]Corrected for total number born
[5]Corrected for litter size
[6]Corrected for age

Table 5.13. The effects of β-hydroxy β-methyl butyrate and lignocellulose fibre supplementation to sows in gestation and L-glutamine supplementation to sows in lactation, on piglet performance from 24 hours to weaning on the outdoor production system[1]

Variable	HMB[2]		Fibre		Gln		SEM	*P*-value[2]		
	0 %	0.15 %	0 %	1 %	0 %	1 %		HMB	Fibre	Gln
Average weights (kg)										
24 hour (total)[3,4]	1.62	1.64	1.62	1.64	-	-	0.032	0.554	0.675	-
24 hour (live)[3,4]	1.63	1.65	1.63	1.65	-	-	0.032	0.600	0.621	-
24 hour (post foster)[3,4]	1.64	1.66	1.64	1.66	1.68	1.63	0.032	0.612	0.647	0.236
Week one[5,6]	2.65	2.71	2.66	2.69	2.67	2.69	0.056	0.401	0.618	0.787
Wean[5,6]	7.60	7.74	7.57	7.77	7.68	7.66	0.118	0.349	0.204	0.937
ADGs (g)[2]										
Week one[5,7]	207	205	206	207	203	209	6.0	0.757	0.910	0.483
Week one to wean[5,6]	243	248	242	249	245	246	4.5	0.407	0.242	0.743
Birth to wean[5,6]	240	244	239	245	242	243	4.3	0.521	0.266	0.841

[1]Data represents a total of 133 litters
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine, ADG = average daily gain
[3]Corrected for total born
[4]Corrected for tag age
[5]Corrected for litter size
[6]Corrected for age
[7]Interactive effects of HMB and glutamine are presented in Figure 5.2

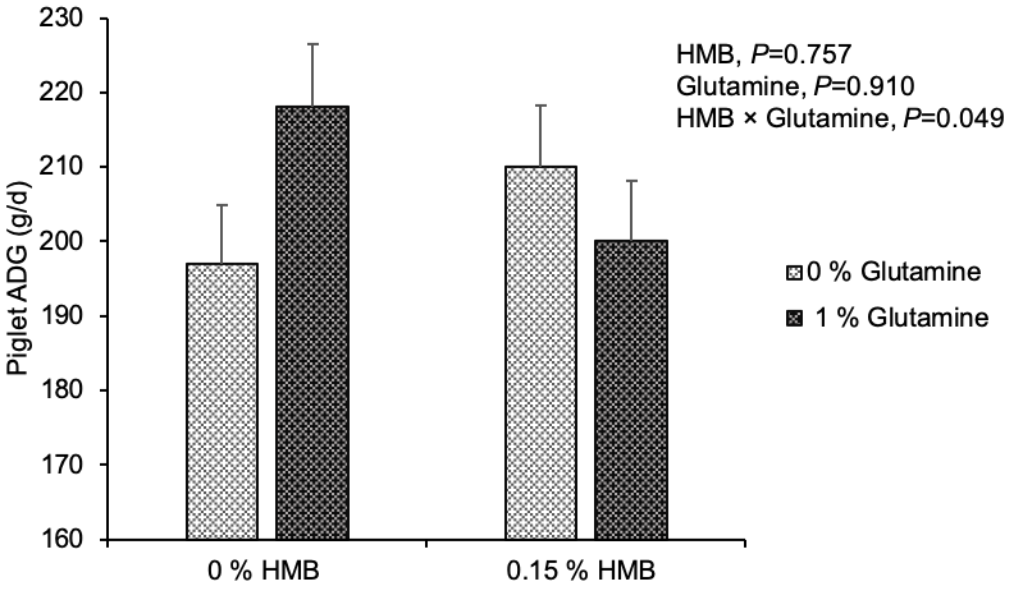

Figure 5.2. The interactive effect of HMB supplementation to sows in gestation and glutamine supplementation to sows in lactation on piglet week one average daily gain on the outdoor production system

Data represent the mean + SEM. Litter size included as a covariate. Glutamine supplementation had no effect on piglet performance to weaning in piglets from sows which had received HMB. However, glutamine improved the ADG of piglets from sows which had not received HMB.

5.4.4. Indoor and outdoor production system data combined

The combined analysis for birth data included 164 indoor and 133 outdoor sows split across the treatments as follows: 0 % HMB, *n* = 148; 0.15 % HMB, *n* = 149, 0 % lignocellulose fibre, *n* = 145; 1 % lignocellulose fibre, *n* = 152; 0 % glutamine, *n* = 147; 1 % glutamine, *n* = 150. The combined analysis for post 24 hour data included 158 indoor (including those which had been effected by scour) and 133 outdoor sows split across the treatments as follows: 0 % HMB, *n* = 144; 0.15 % HMB, *n* = 146; 0 % lignocellulose fibre, *n* = 141; 1 % lignocellulose fibre, *n* = 149; 0 % glutamine, *n* = 143; 1 % glutamine, *n* = 147. All data presented are means ± SEM.

5.4.4.1. Sow characteristics

Sow characteristics are presented in Table 5.14. There were no interactions therefore the data presented are the main effects. On entry to the trial, indoor sows were heavier than outdoor sows (233.2 vs 207.0 kg for indoor vs outdoor, respectively; *P*<0.001) and indoor sows had more back-fat than outdoor sows (13.9 vs 10.9 mm for indoor vs outdoor respectively; *P*<0.001). Lignocellulose fibre supplementation reduced gestation length (*P*=0.020); sows supplemented with lignocellulose fibre had a 0.4 d shorter gestation length than sows that were not supplemented with lignocellulose fibre. Outdoor sows had a longer gestation length than indoor sows (115.6 vs 114.9 d for outdoor vs indoor, respectively; *P*=0.001). Sow weight and back-fat changes from d 109 to weaning were similar across dietary treatments, however outdoor sows lost less back-fat than indoor sows (- 2.1 vs - 3.5 mm for outdoor vs indoor respectively; *P*<0.001).

Table 5.14. The main effects of β-hydroxy β-methyl butyrate and lignocellulose fibre supplementation to sows in gestation and L-glutamine supplementation to sows in lactation on sow characteristics on the indoor and outdoor production systems combined[1]

Variable	HMB[2]		Fibre		Gln		System		SEM	*P*-value[2]			
	0%	0.15%	0%	1%	0%	1%	In	Out		HMB	Fibre	Gln	System
Parity	2.8	2.8	2.8	2.8	2.7	2.9	2.5	3.1	0.14	0.797	0.634	0.263	**0.008**
Sow weights (kg)													
d100	219.5	220.8	220.9	219.4	-	-	233.2	207.0	1.66	0.574	0.517	-	**<0.001**
d100 to d109 change	+5.7	+4.9	+5.6	+5.0	-	-	+7.1	+3.5	0.60	0.345	0.485	-	**<0.001**
d109 to wean change	-33.7	-33.9	-33.1	-34.5	-32.4	-35.2	-34.3	-33.3	1.71	0.933	0.562	0.226	0.674
Sow back-fat (mm)													
d100	12.5	12.3	12.5	12.3	-	-	13.9	10.9	0.23	0.727	0.553	-	**<0.001**
d100 to d109 change	+0.3	+0.2	+0.2	+0.4	-	-	+0.4	+0.1	0.16	0.582	0.337	-	0.302
d109 to wean change	-2.8	-2.7	-2.8	-2.8	-2.8	-2.7	-3.5	-2.1	0.21	0.853	0.998	0.705	**<0.001**
Gestation length(d)[3]	115.4	115.2	115.5	115.1	-	-	114.9	115.6	0.12	0.188	**0.020**	-	**0.001**
Lactation FI (kg)[2]													
Total FI[4]	176.8	179.7	179.8	179.2	179.7	176.8	169.6	186.9	1.55	0.175	0.190	0.162	**<0.001**
ADFI[2]	7.1	7.2	7.1	7.1	7.1	7.1	6.4	7.8	0.06	0.197	0.586	0.343	**<0.001**

[1]Data represents a total of 297 sows for data to parturition and 283 sows for data to weaning
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine, System = production system, In = indoors, Out = outdoors, FI = feed intake, ADFI = average daily feed intake
[3]Corrected for total born
[4]Corrected for litter size

5.4.4.2. Litter performance

The combined litter performance for the indoor and outdoor production systems is presented in Table 5.15. There were no interactions so the data presented are the main effects. Number of piglets born (total live and stillborn) were similar across dietary treatments. However, indoor sows had a higher total number of piglets born compared with outdoor sows (15.9 vs 12.9 piglets/litter for indoor vs outdoor, respectively; $P<0.001$) and a higher number of live born piglets compared with outdoor sows (15.2 vs 12.8 piglets/litter for indoor vs outdoor, respectively; $P<0.001$).

Litter week one weight gain was increased by HMB supplementation (12.0 vs 10.7 kg for 0.15 vs 0 %, respectively; $P=0.029$). In addition, there was a tendency for HMB supplementation to increase total litter weight at week one by 4.3 % ($P=0.101$). There was a tendency for litter week one weight gain to be increased by lignocellulose fibre supplementation (11.9 vs 10.8 kg for 1 for 0 %, respectively; $P=0.062$). In addition, there was a tendency for lignocellulose fibre to increase litter weight gain from birth to weaning (68.0 vs 65.5 kg for 1 vs 0 %, respectively; $P=0.094$). There were no effects of glutamine on litter weights at week one ($P=0.376$) or at weaning ($P=0.876$).

Outdoor sows had smaller litter sizes at week one compared with indoor sows (11.4 vs 12.3 piglets/litter for outdoor vs indoor, respectively; $P<0.001$). Outdoor sows also weaned less piglets than indoor sows (11.2 vs 11.8 piglets/litter for outdoor vs indoor, respectively; $P=0.013$). As lignocellulose fibre reduced sow gestation length pigs in litters from sows supplemented with lignocellulose fibre were 0.5 d older at week one ($P=0.018$) and at weaning ($P=0.011$) than those in litters from sows that were not supplemented with lignocellulose fibre. Percentage mortality was similar across dietary treatments and averaged 12.3 ± 0.10 %. Percentage mortality to weaning was lower on the outdoor production system than the indoor production system (9.4 vs 15.3 % for outdoor vs indoor, respectively; $P<0.001$).

Table 5.15. The main effects of β-hydroxy β-methyl butyrate and lignocellulose fibre supplementation to sows in gestation and L-glutamine supplementation to sows in lactation on litter performance on the indoor and outdoor production systems combined[1]

Variable	HMB[2]		Fibre		Gln		System		SEM	*P*-value[2]			
	0%	0.15%	0 %	1 %	0 %	1 %	In	Out		HMB	Fibre	Gln	System
Numbers of piglets													
Total Born	14.6	14.1	14.5	14.3	-	-	15.9	12.9	0.26	0.195	0.671	-	**<0.001**
Alive	14.2	13.8	14.1	13.9	-	-	15.2	12.8	0.25	0.179	0.557	-	**<0.001**
Dead	0.4	0.4	0.3	0.4	-	-	0.6	0.1	0.07	0.949	0.523	-	**<0.001**
Week one	11.8	11.9	11.9	11.7	11.7	12.0	12.3	11.4	0.18	0.954	0.408	0.336	**<0.001**
Wean	11.5	11.6	11.6	11.4	11.5	11.5	11.8	11.2	0.16	0.645	0.375	0.871	**0.013**
Litter weights (kg)													
Total born	21.1	21.0	21.0	21.0	-	-	21.8	20.3	0.34	0.783	0.997	-	**0.002**
Live born	20.8	20.5	20.7	20.6	-	-	21.1	20.2	0.33	0.598	0.883	-	**0.050**
Week one	30.2	31.5	30.4	31.3	30.5	31.2	31.2	30.5	0.55	**0.101**	0.263	0.350	0.344
Wean	85.6	86.8	85.1	87.3	86.2	86.3	87.4	85.0	1.15	0.461	0.165	0.934	0.142
Litter weight gains (kg)													
Week one	10.7	12.0	10.8	11.9	11.0	11.7	11.9	10.8	0.44	**0.029**	**0.062**	0.209	**0.097**
Week one to wean	55.4	55.5	54.8	56.1	55.6	55.3	56.4	54.5	0.87	0.983	0.282	0.757	0.136
Birth to wean	66.1	67.4	65.5	68.0	66.6	66.9	68.2	65.3	1.07	0.381	**0.094**	0.870	**0.067**
Age (d)													
Week one	6.3	6.4	6.1	6.6	6.4	6.3	6.7	6.0	0.13	0.731	**0.018**	0.638	**<0.001**
Wean	26.8	26.9	26.6	27.1	26.9	26.8	27.7	25.9	0.14	0.658	**0.011**	0.436	**<0.001**
Mortality[3] (%)													
Day	3.5	3.9	3.4	4.0	3.3	4.1	4.9	2.5	0.53	0.638	0.342	0.287	0.420
Week one	10.4	9.4	9.7	10.0	9.3	10.5	13.1	6.7	0.85	0.398	0.816	0.330	**<0.001**
Wean	13.1	11.5	12.6	12.0	11.4	13.2	15.3	9.4	0.10	0.259	0.681	0.192	**<0.001**

[1]Data represents a total of 297 litters for data to parturition, 290 litters for data to week one and 283 litters for data to weaning
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine, System = production system, In = indoors, Out = outdoors
[3]Corrected for total born

5.4.4.3. Piglet performance

Piglet performance for the indoor and outdoor production systems combined is presented in Table 5.16. There were no interactions therefore the data presented are the main effects. Piglets on the outdoor production system had heavier average 24 hour weights than piglets on the indoor production system (1.57 vs 1.47 kg for outdoor vs indoor, respectively; *P*=0.010). There was a tendency for piglets from sows supplemented with lignocellulose fibre to have a 3.5 % higher birth to wean ADG than piglets from sows that were not supplemented with lignocellulose fibre (235 vs 227 g/d for 1 vs 0 %, respectively; *P*=0.062). Piglets on the outdoor production system also had a higher birth to wean ADG than piglets on the indoor production system (239 vs 223 g/d for outdoor vs indoor, respectively; *P*=0.001).

Piglets from sows supplemented with HMB had 4.3 % higher average week one weights compared with piglets from sows that were not supplemented with HMB (2.66 vs 2.55 kg for 0.15 vs 0 %, respectively; *P*=0.032); this remained a trend at weaning (7.68 vs 7.49 kg for 0.15 vs 0 %, respectively; *P*=0.076). Piglets from sows supplemented with lignocellulose fibre were 3.1 % heavier at weaning compared with piglets from sows that were not supplemented with lignocellulose fibre (7.70 vs 7.47 kg for 1 vs 0 %, respectively; *P*=0.042). Glutamine had no effect on average piglet week one (*P*=0.650) or weaning weights (*P*=0.959). Outdoor piglets were heavier than indoor piglets at week one (2.72 vs 2.51 kg for outdoor vs indoor respectively; *P*<0.001) and at weaning (7.77 vs 7.40 kg for outdoor vs indoor respectively; *P*=0.005).

Table 5.16. The effects of β-hydroxy β-methyl butyrate and lignocellulose fibre supplementation to sows in gestation and L-glutamine supplementation to sows in lactation, on piglet performance from 24 hours to weaning on the indoor and outdoor production systems combined[1]

Variable	HMB[2]		Fibre		Gln		System		SEM	*P*-Value[2]			
	0 %	0.15%	0%	1%	0%	1%	In	Out		HMB	Fibre	Gln	System
Average weights (kg)													
24 hour (total)[3]	1.51	1.52	1.51	1.52	-	-	1.47	1.57	0.019	0.614	0.693	-	**0.010**
24 hour (live)[3]	1.51	1.53	1.51	1.53	-	-	1.47	1.55	0.019	0.635	0.638	-	**0.016**
24 hour (post foster)[4]	1.52	1.53	1.53	1.52	1.54	1.51	1.46	1.59	0.019	0.472	0.790	0.197	**<0.001**
Week one[4,5]	2.55	2.66	2.59	2.64	2.60	2.62	2.51	2.72	0.034	**0.032**	0.304	0.650	**<0.001**
Wean[4,5]	7.49	7.68	7.47	7.70	7.59	7.58	7.40	7.77	0.079	**0.076**	**0.042**	0.959	**0.005**
ADGs (g)[2]													
Week 1[4]	186	188	185	190	185	189	168	206	3.6	0.776	0.333	0.413	**<0.001**
Week 1 to wean[4]	238	242	237	243	239	241	237	243	3.1	0.278	0.146	0.580	0.160
Birth to wean[4,5]	229	233	227	235	230	232	223	239	2.9	0.285	**0.062**	0.687	**0.001**

[1]Data represents a total of 297 litters for data to parturition, 290 litters for data to week one and 283 litters for data to weaning
[2]HMB = β-hydroxy β-methyl butyrate, Fibre = lignocellulose fibre, Gln = L-glutamine, System = production system, In = indoors, Out = outdoors, ADG = average daily gain
[3]Corrected for total born
[4]Corrected for litter size
[5]Corrected for age

5.5. Discussion

Before the results are discussed in detail it should be stressed that ~ 21 % of the indoor litters were affected by scour. Whilst the reason for this illness was unclear, it may have been because within the last year the indoor dry herd had been moved off site for housing during gestation, and thus they were no longer coming into contact with pathogens from the main unit. This may have resulted in new born piglets being susceptible to infection.

Although measures were taken to control the spread of scour certain litters had to be treated with electrolytes. The number of litters that were treated was generally even across treatments and so were included in the analysis. However, as it is well established that the health status of the animal can influence its growth performance (Clapperton et al., 2009, Bedford, 2016) caution should be taken when considering treatment effects post 24 hours. As the scour was an illness that occurred post-birth (~ 3 d), data pre 24 hours should not have been affected. However, an additional analysis was conducted for litter and piglet performance post 24 hours which excluded any litters which had been affected by scour in order to determine whether these litters affected the results and similar treatment effects were observed in both analyses. The outdoor herd was not affected by scour.

5.5.1. The effects of HMB and lignocellulose fibre supplementation on sow characteristics and litter performance up to 24 hours post-partum

There were no significant interactive effects of HMB and lignocellulose fibre on any sow or litter characteristics up to 24 hours post-partum. It was hypothesised that HMB would increase litter weights at birth due to its anti-catabolic effects (Blicharski et al., 2017). However, there were no differences in total born litter weight or total live born litter weight between any of the treatment groups on either the indoor or outdoor production system or when the data from both systems were combined. Previous results regarding the effect of HMB on litter weights have been inconsistent. Experiment 1 (as described in Chapter 3) found a positive quadratic effect of feeding increasing amounts of HMB to sows from d 100 of gestation until parturition on total live born litter weight, with the most beneficial dose being 15 mg/kg BW. However, experiment 2 (as described in Chapter 4) found no beneficial effects of feeding HMB at a dose of 15 mg/kg BW to sows for any duration of time on total live born litter weight. Litter weights are

highly variable and in this study alone they were found to vary from 10.4 to 30.8 kg which is most likely due to the large variation in litter sizes (6 to 24 piglets/litter).

On the indoor production system lignocellulose fibre supplementation to sows was found to decrease gestation length without resulting in a reduction in litter weight. The fermentable fibre fraction of lignocellulose fibre is fermented by bacteria in the large intestine to produce volatile fatty acids (VFAs) (Kroismayr, 2008). These VFAs may have stimulated the production of energy through gluconeogenesis (Theil et al., 2012) which may have been partitioned for in-utero growth. This may have increased the gestational development of the foetuses resulting in them being larger earlier on in gestation. This may have increased sow cortisol levels and higher levels of cortisol reaching the uterus results in the onset of farrowing (van Dijk et al., 2005, Vanderhaeghe et al., 2011).

There are limited studies with observations on the effect of fibre on gestation length, which may be because many sows are currently induced. A study by Guillemet et al. (2007) looked at the effect of feeding a high fibre diet (12.4 vs 3.2 % CF, in % DM) from week five of gestation until parturition. They found no significant difference in gestation length however, sows fed the high fibre diet numerically had a 0.5 d shorter gestation length than the control (114.9 vs 115.4 d, respectively). This is the same difference that was observed in the current study (0.5 d decrease in gestation length with 1 % lignocellulose fibre supplementation). Guillemet et al. (2007) had a much lower number of sows (*n*=21 per treatment) compared to our current study (*n*=79 and *n*=84 in non-fibre and fibre supplemented groups, respectively). Perhaps if they had a higher number of sows the numeric difference would have been significant.

The effect of lignocellulose fibre reducing gestation length was not observed on the outdoor production system, although sows supplemented with lignocellulose fibre numerically had a 0.2 d shorter gestation length. However, when the data from the indoor and outdoor production systems were combined the overall effect of lignocellulose fibre reducing gestation length was significant. The effect of lignocellulose fibre on gestation length may not have been as prominent on the outdoor unit as these sows had continuous access to straw. Once sows were in the farrowing paddocks they had access to a straw bedded arc, whereas indoors once the sows were in the farrowing house they had no access to straw. Therefore, the outdoor sows may have received extra fibre through straw

consumption. The outdoor sows were also a different genotype to the indoor sows and genotype is thought to affect gestation length (Knol et al., 2002, Rydhmer et al., 2008). The average gestation length of the outdoor sows was 1 d longer than that of the indoor sows, therefore perhaps the effect on gestation length was not as prominent though it was still numerically 0.2 d shorter.

On the outdoor production system there was a tendency for HMB to reduce gestation length. This tendency was also observed for indoor sows in both experiments 1 and 2. As previously mentioned in those experiments, circulating cytokines have been linked to the onset of labour (Steinborn et al., 1995, Arntzen et al., 1998). HMB may have increased the level of circulating cytokines resulting in a shortened gestation length. This effect was not observed on the indoor production system or in the combined analysis in this experiment. There were no interactions between HMB and lignocellulose fibre for gestation length so the effect of HMB on gestation length was not negated by lignocellulose fibre. HMB had a reduced immunostimulatory effect on the indoor production system in the present study compared with experiments 1 and 2 which may be because there was a scour related disease going through the herd, and this may have negated the effect of HMB on gestation length.

The number of piglets born alive and born dead were not affected by treatment on either the indoor or outdoor production systems. Indoors, the number of still born piglets was relatively low across the whole study (~ 0.6 pigs/litter) and was lower than commercial figures reported at the time (~ 0.8 pigs/litter) (AHDB, 2019c). This suggests that the herd was performing above average in terms of productivity. Outdoors, the number of still born piglets was also very low across the whole study (~ 0.2 piglets/litter) compared with commercial figures reported for the same time period (~ 0.5 piglets/litter) (AHDB, 2019c). However, it was much more difficult to find any still born piglets on the outdoor production system than on the indoor production system as they were easily lost in the straw arcs. Therefore caution should be taken when interpreting the total number born and the number still born on the outdoor production system.

5.5.2. The effects of HMB, lignocellulose fibre and glutamine supplementation on sow characteristics and litter performance, post 24 hours

It was hypothesised that there would be an additive effect of supplementing HMB, lignocellulose fibre and glutamine to sows on litter performance post 24 hours. However, there were no significant interactive effects of any treatment on sow and litter characteristics post 24 hours on either the indoor or outdoor production systems. HMB increased litter week one weight gain on the indoor production system and there was a tendency for HMB supplementation to increase litter weight at week one compared with the control. As there was no effect of HMB supplementation on litter birth weight this increase at week one was due to post birth growth. The numbers of piglets on each sow were similar across treatments and accounted for in the analysis and thus the increase in litter weight at week one was a result of heavier piglets. In the current study, HMB was found to increase colostrum yield. There was a tendency for piglets from sows supplemented with HMB to have a higher colostrum intake than piglets from sows not supplemented with HMB. Piglet pre-weaning growth is influenced by colostrum intake (Declerck et al., 2016). HMB also increased the lactation ADFI of sows. As the sows were consuming more feed they had more energy for the production of milk (Eissen et al., 2000) resulting in increased litter weight gains and heavier litters at week one. This result agrees with experiment 1 which also found HMB supplementation to increase litter weight at week one.

There was no effect of treatment on litter weights in the outdoor production system. However, there was a tendency for week one litter weight gain to be increased by glutamine supplementation which was not observed on the indoor production system or in the combined analysis. On the outdoor production system litters from sows supplemented with HMB numerically gained 6.9 % more weight at week one than litters from sows which were not supplemented with HMB. When the data from the indoor and outdoor production systems were combined HMB supplementation significantly increased week one litter gain with a tendency for total litter weight to be increased at week one. In addition, when the data from both production systems were combined there were tendencies for litter weight gain at week one and litter weight gain from birth to weaning to be enhanced by lignocellulose fibre. This is likely because litters from sows supplemented with lignocellulose fibre were older than litters from sows that were not supplemented

with lignocellulose fibre on weigh days therefore had a longer period suckling on the sow. Age and litter size were not included in litter performance for the combined analysis as sows on the indoor and outdoor production systems had very different litter sizes therefore covariates interfered with the analysis.

On the indoor production system sows supplemented with glutamine lost more weight from d 109 to weaning than sows that were not supplemented with glutamine. There were no differences in total born litter weights between the sows that received supplemental glutamine and those that did not which suggests that the increased weight loss occurred throughout lactation. It was hypothesised that supplementing sows with glutamine during lactation would reduce the extent to which sows had to mobilise their own body reserves (Manso et al., 2012), therefore it would be expected that they would lose less weight throughout lactation. It is unclear why glutamine had this effect on sow weight change. There was no difference in feed intake or litter weight gains between the treatments. However, in order to make the diets isoenergetic a higher amount of soya oil and vegetable fat were added to the diet containing 0 % glutamine to make up for the energy value of the supplemental glutamine (12.88 MJ/kg) in the diet containing 1 % glutamine. Therefore, the analysed fat content of the diet containing 1 % glutamine was lower (1.3 % lower) than that of the diet containing 0 % glutamine. Therefore, it is possible that sows supplemented with 1 % glutamine lost more weight as they received less fat from their diets. However, this effect was not observed on the outdoor production system or in the combined analysis of both systems. This may be because the outdoor sows had access to herbage and soil which may have supplemented their dietary intake. The outdoor sows were also a different genotype which perhaps had different nutritional requirements.

In addition, on the indoor production system there was a tendency for sows supplemented with lignocellulose fibre to lose more weight from d 109 to weaning than sows than which were not supplemented with lignocellulose fibre. As sows supplemented with lignocellulose fibre farrowed earlier, they had a longer lactation period so would have lost more weight. There was no difference in litter weight gain however, when piglet age was not accounted for in the statistical analysis, piglets from sows supplemented with lignocellulose fibre were significantly heavier at week one and numerically heavier at weaning than piglets from sows that were not supplemented with lignocellulose fibre. This suggests

piglets from sows supplemented with lignocellulose fibre had greater nutritional demands which would have put more strain on the sow and the sows were milking more. The effect of lignocellulose fibre on sow weight loss from d 109 to weaning was not observed on the outdoor production system or in the combined analysis.

It was hypothesised that all treatments would reduce mortality; HMB through improved birth weight, colostrum intake and pre-weaning growth, lignocellulose fibre through reduced farrowing duration and thus improved vitality at birth and glutamine through improved milk quality. It was therefore hypothesised that there would be an additive effect of these treatments on mortality. However, there were no effects of any of the treatments on piglet mortality to weaning on either the indoor or outdoor production system or when the data from both production systems were combined. In experiment 1, HMB reduced mortality which was a likely result of the increased piglet birth weight observed with HMB supplementation, as piglet birth weight is a major determining factor of survival (Milligan et al., 2002a, Quiniou et al., 2002, Baxter et al., 2008, Panzardi et al., 2013). In the current study, neither HMB or lignocellulose fibre improved piglet birth weight. However, on the indoor unit there was a tendency for HMB to increase colostrum intake and improve colostrum IgM concentration. Immunoglobulins in colostrum provide the piglets with passive immunity which reduces their risk of diseases and increases their chances of survival (Theil et al., 2014a). It is also well established that colostrum intake is negatively associated with piglet mortality (Declerck et al., 2016), therefore it is surprising that HMB did not improve mortality levels. It may be that management strategies had a higher influence over mortality. A shorter latency to first suckle is associated with improved survival to weaning (Edwards, 2002, Baxter et al., 2008). Lignocellulose fibre did not reduce farrowing duration or improve time to suckle, therefore it is not surprising that mortality levels were not affected by lignocellulose fibre. Although Wu et al. (2011) found supplementing gilt diets in lactation with glutamine reduced piglet pre-weaning mortality, no effect on mortality was observed in the present study. Glutamine did not improve growth performance to weaning of any piglets in the current study which is likely why improvements in mortality were not seen.

5.5.3. The effects of HMB and lignocellulose fibre on farrowing characteristics and colostrum production on the indoor production system

One of the main aims of the study was to determine whether there was an additive effect of HMB and lignocellulose fibre supplementation to sows on early piglet performance. It was hypothesised that HMB would improve piglet birth weight and lignocellulose fibre would shorten farrowing duration, resulting in a more viable piglet. It was hypothesised that HMB would improve colostrum production in terms of colostrum yield and immunoglobulin concentrations and piglets from sows supplemented with both HMB and lignocellulose fibre would be better able to gain access to this colostrum. However, there were no additive effects of HMB and lignocellulose fibre on any farrowing characteristics or early piglet performance.

5.5.3.1. Farrowing duration

Prolonged farrowing durations are positively associated with the number of still born piglets (van Dijk et al., 2005, Baxter et al., 2009, Oliviero et al., 2010). Longer farrowing durations result in piglets being subjected to more uterine contractions which may cause cord rupture and/or damage to the placenta and may result in deaths due to asphyxia (Herpin et al., 1996, Alonso-Spilsbury et al., 2005). Constipation has been associated with prolonged farrowing durations as hard faeces can cause obstruction by pressing on the birth canal which can lead to difficulty during expulsion (Cowart, 2007, Oliviero et al., 2010). High fibre diets have been found to relieve constipation (Oliviero et al., 2009). As previously mentioned, lignocellulose fibre is a combination of fermentable and non-fermentable fibres (Kroismayr, 2008). The non-fermentable fibre fraction can regulate the digesta passage rate and shift fermentation back from the caecum to the colon. The VFAs produced when the fermentable fibre fraction is fermented by bacteria in the large intestine can improve water absorption in the large intestine which improves the consistency of the faeces (Kroismayr, 2008). Combined, the improved digesta passage rate and consistency of the faeces may result in a reduced incidence of constipation (Kroismayr, 2008). Lignocellulose fibre has previously been found to improve faecal quality in sows in terms of dry matter. A study by Sarandan et al. (2008) found sows supplemented with 3 % lignocellulose fibre throughout gestation had constant faecal dry matter content of ~ 30 %, whereas the faecal dry matter content of the control group varied from very soft (27 %) to very hard (40 %).

Therefore, it was hypothesised that lignocellulose fibre may help relieve constipation and thus lead to a reduction in farrowing duration. However, there was no effect of lignocellulose fibre on farrowing duration. There was no effect of lignocellulose fibre on faecal scores or on the number of sows experiencing constipation, however, as only 15.2 % of sows experienced some signs of constipation, constipation did not appear to be a problem is this study. A study by Oliviero et al. (2009) found that ~ 85 % of sows fed a standard gestation diet experienced some signs of constipation. Lignocellulose supplementation may not have reduced farrowing duration through reduced constipation, as sows in this study did not appear to be affected by constipation prior to parturition.

A study by Enckevort (2013) found supplementing 1 % lignocellulose fibre to sow diets for one week prior to parturition numerically reduced sow farrowing duration by 40 mins. The average parity in the study by Enckevort (2013) was 5.0, whereas the average parity of sows in the present study was 2.6. Parity has been found to be positively correlated with farrowing duration, with higher parity sows having longer farrowing durations than lower parity sows (Björkman et al., 2017). Lignocellulose fibre may have more of an effect on reducing farrowing duration in higher parity sows, although in the current study there was no effect of parity on farrowing duration.

Gestation length has also been negatively associated with farrowing duration (van Rens and van der Lende, 2004, van Dijk et al., 2005). van Dijk et al. (2005) suggested that this may be because sows with a longer gestation length are exposed to a higher level of oestradiol for a longer period. Higher levels of oestrogen are released from the foetal placenta prior to parturition and the concentration in maternal plasma is at a maximum concentration just prior to the onset of parturition (Taverne et al., 1979, Taverne, 1992, van Dijk et al., 2005). Oestrogens are important for parturition as they stimulate myometrial contractility, therefore, increasing the duration of exposure may result in a shorter parturition process (Taverne, 1992, van Dijk et al., 2005). Therefore, the reduction in gestation length caused by supplementation with lignocellulose fibre may be the reason for lack of effect on farrowing duration.

5.5.3.2. Birth weights

In the current study HMB failed to improve piglet birth weight. The results regarding the effects of HMB on piglet birth weight are inconsistent in the

literature and within our own studies. Experiment 1 (described in Chapter 3) found a tendency for HMB to increase piglet birth weight, and that the effects of HMB were dose-dependent when HMB was supplemented to sow diets for the last 15 days of gestation. However, experiment 2 (described in Chapter 4) found no benefit of supplementing HMB to sow diets at a dose of 15 mg/kg BW for different durations of time. The reason for the differences are unknown. Whilst the dose and the duration of supplementation used in the present study was similar to one of those used in experiment 1, there were slight differences in methods of administration which may be the cause of the discrepancies in results. In the current study HMB was formulated into the feed, whilst in experiment 1 HMB was top-dressed. Sows in the current study were also a different genotype (JSR 9T) than the sows in the first study (JSR Genepacker GP90); maternal genetics can influence birth weight (Knol et al., 2002).

Lignocellulose fibre did not affect average piglet birth weight. In fact, as lignocellulose fibre reduced gestation length it may have been assumed that piglets from sows supplemented with lignocellulose fibre would be lighter than piglets from sows that did not receive lignocellulose fibre due to the foetuses having less time develop (Rydhmer et al., 2008). However, this was not the case. As mentioned in Section 5.5.1, lignocellulose fibre may have enhanced the gestational development of the foetuses leading to increased cortisol levels and resulting in earlier parturition (van Dijk et al., 2005, Vanderhaeghe et al., 2011). As the gestational development of the foetuses might have been increased, the reduction in gestation length would not have affected average piglet birth weight.

5.5.3.3. Colostrum production

There was a tendency for HMB to improve colostrum intake of piglets. Birth weight, farrowing duration and time to suckle were similar across treatments, therefore, it is likely that the increase in colostrum intake was due to the higher yield of colostrum available as the colostrum intake of piglets can be limited by the sows' ability to produce it (Devillers et al., 2007). The range of colostrum yield produced in the present study were in line with those found in experiment 1 and those reported by Hasan et al. (2019). As hypothesised, HMB supplementation successfully increased the yield of colostrum. As previously mentioned, it is thought that HMB has lipolytic effects, leading to fat mobilisation and high plasma levels of NEFA (Wilson et al., 2008, Theil et al., 2014a) which could be used for

energy for colostrum production. These results agree with the results of experiment 1 which found HMB supplementation increased colostrum yield in a quadratic manner. In experiment 1 HMB increased colostrum yield by 29 % when supplemented at a dose of 15 mg/kg BW for ~ 15 days. The present study found HMB supplementation at an inclusion level of 0.15 % (as-fed) only increased colostrum yield by 18 %. The most likely reason for the observed percentage difference between the studies is that colostrum production is extremely variable and effected by factors such as parity, sow weights and back-fat changes, farrowing durations and genotype (Devillers et al., 2007, Decaluwe et al., 2013, Hasan et al., 2019). Different sows were used in the study with different colostrum production potentials and thus the overall colostrum yield increase was different.

5.5.3.4. 24 hour weight gain

As predicted, HMB increased the 24 hour weight gain of the piglets by 48 % which is due to the increased yield of colostrum and the tendency for increased colostrum intake caused by HMB supplementation. Again, these results reflect those of experiment 1 which found HMB supplementation at a dose of 15 mg/kg BW increased 24 hour weight gain of piglets. However, the results of the current study are not reflected in percentage mortality. As mentioned in Section 3.5.2, Le Dividich et al. (2005) discussed that the average 24 hour weight gain of piglets that survived to weaning was 70 g. In the current study, all treatment groups had an average piglet 24 hour weight gain of over 70 g/piglet which suggests they all received adequate colostrum for survival to weaning. As lignocellulose fibre did not reduce farrowing duration and thus did not reduce piglet time to suckle, there were no additive effects of HMB and lignocellulose fibre on colostrum intake and piglet 24 hour weight gain.

5.5.4. The effects of HMB, lignocellulose fibre and glutamine supplementation on piglet performance from 24 hours to weaning

It was hypothesised that there would be an additive effect of HMB and lignocellulose fibre supplementation to sows in gestation and glutamine supplementation to sows in lactation on piglet performance from 24 hours to weaning. It was thought that piglets from sows which received the combination of supplements would have improved growth, resulting in higher weights at weaning. However, there was no interaction between any of the treatments on either the indoor or outdoor production systems.

On the indoor production system there was a tendency for HMB supplementation to sows to increase average piglet week one and weaning weights by 4.6 % and 3.2 %, respectively. This is due to the improved colostrum intake and 24 hour weight gain of piglets caused by HMB supplementation as colostrum intake is related to piglet pre-weaning growth (Edwards, 2002, Devillers et al., 2007). Similar results were observed in experiment 1, which found supplementing HMB to sow gestation diets at a dose of 15 mg/kg BW improved average piglet week one weight by 8.1 % and numerically increased average weaning weight by 3.8 %. In addition, Nissen et al. (1994) found that the level of fat in colostrum was enhanced by 41 % with HMB supplementation to sows at a dose of 10 mg/kg BW for 3 - 4 d prior to parturition. If HMB increased fat in colostrum in the current study the piglets would have had more energy for growth which may have also contributed towards the tendency for increased weights at week one and weaning.

This effect was not observed on the outdoor system, however piglets from sows supplemented with HMB were numerically 2.3 % heavier at week one and 1.8 % heavier at weaning than piglets from sows that were not supplemented with HMB. However, when the data from both the indoor and outdoor production systems were combined, HMB supplementation significantly increased average week one weight by 4.3 % and there was a tendency for weaning weight to be increased by 2.5 %.

Outdoor reared pigs have been found to grow faster than indoor reared pigs (Miller et al., 2009, Davis and Miller, 2019). In the combined analysis, piglets reared outdoors were 8 % heavier at week one and 5 % heavier at weaning compared with piglets reared indoors. Piglets reared outdoors also gained an average of 38 g/d more between 24 hours and week one, and 16 g/d more between 24 hours and weaning than indoor reared piglets. Therefore, the outdoor reared piglets were growing faster than the indoor reared piglets. It is possible that the outdoor reared piglets were closer to their genetic potential for growth and thus HMB supplementation to sows had a less prominent effect on increasing piglet weight gains above that of the control piglets.

Lignocellulose fibre had no significant effects on piglet week one or weaning weights on the indoor or outdoor production systems when analysed separately. Piglets from sows supplemented with lignocellulose fibre were older on average

week one and on weaning days than piglets from sows that were not supplemented with lignocellulose fibre, therefore age was included in the model analysing piglet performance. When age was not included in the model there was a tendency for piglets from sows supplemented with lignocellulose to be heavier at week one than control piglets on the indoor production system. In addition, when the data from the indoor and outdoor production systems were combined piglets from sows supplemented with lignocellulose were heavier at weaning regardless of age being accounted for. Dietary fibre to sows in gestation has been found to increase the pre-partum peak in prolactin concentration which is needed for lactogenesis to be initiated (Farmer et al., 1995, Quesnel et al., 2009, Farmer, 2016). Prolactin is also needed for the maintenance of milk production in lactation (Farmer et al., 1998). Fibre alters the rate of passage of digesta which may reduce the level of endotoxins which can supress prolactin and cause lactation insufficiency (Farmer, 2016).

It was hypothesised that supplementing sow lactation diets with glutamine would improve piglet performance from 24 hours to weaning. This is because glutamine has been found to be enhance protein synthesis and inhibit protein degradation through its ability to activate mTOR in skeletal muscle (Meijer and Dubbelhuis, 2004, Curi et al., 2005, Wu et al., 2011). On the outdoor production system there was an interaction between HMB and glutamine supplementation to sows on piglet week one ADG. Glutamine improved piglet week one ADG only when fed to sows which had not received HMB. However, glutamine had no effect on piglet week one ADG when fed to sows which had received HMB. It is unclear why this effect was observed however, it may be that glutamine is not effective when piglets are performing well. In addition, on both production systems piglets from sows supplemented with glutamine had a numerically higher 24 hour to week one ADG suggesting that there may have been a beneficial effect of glutamine supplementation early on in lactation however, this was not significant. When the data from both the indoor and outdoor production systems were combined there were no beneficial effects of glutamine supplementation to sows in lactation on piglet performance to weaning.

The few studies regarding the effect of glutamine on sow and litter performance when supplemented to sow diets in lactation have varying results. Wu et al. (2011) found that supplementing gilt diets with 1 % L-glutamine over lactation (21

d) increased milk glutamine concentration by 12.6 %. They also found glutamine supplementation increased piglet weights at weaning by 6.9 % and reduced mortality to weaning by 78.5 %. The basal level of glutamine in the control diet of present study was 2.94 % (as-fed) whereas it was only 1.72 % (as-fed) in the study by Wu et al. (2011). Therefore, the level of glutamine in the control diet of the present study may have been high enough without supplementation which may explain the lack of effect. The higher basal level of glutamine in the present study may be a result of the different cereals used in the diets. The present study used wheat and barley whereas the study by Wu et al. (2011) used corn. Wheat contains a much higher level of glutamine compared with corn (CVB, 2016, Gholipour et al., 2019). However, as the UK predominately uses wheat and barley-based diets, using a cereal with a lower level of glutamine would not have been relevant to the UK pig industry.

In addition, the discrepancies between results may be due to the fact that Wu et al. (2011) used gilts whereas the present study used multiparous sows. Voluntary feed intake in lactation is insufficient to meet demands for maintenance, growth and milk production (Noblet et al., 1990). As gilts are still growing they partition extra energy into body growth instead of milk production compared with higher parity sows (Pluske et al., 1998). Gilts also produce lower yields of milk compared with higher parity sows; sows of parity 2 to 4 have been found to produce the highest milk yields (Ngo et al., 2012). This suggests that litters from gilts may benefit more from maternal supplementation with glutamine than litters from higher parity sows, as gilt milk production is lower than older sows, therefore piglets may require additional glutamine for growth. In addition, the average weaning weights of piglets in the current study were 7.53 and 7.67 kg for the indoor and outdoor production system, respectively. These weaning weights were higher than the figures reported at the time, 7.15 and 7.33 kg for the indoor and outdoor breeding herds, respectively (AHDB, 2019c). This suggests that piglets in the current study were performing above average, therefore may not have benefited from glutamine supplementation. Milk samples were not collected in the present study, therefore it is not possible to determine whether glutamine supplementation failed to increase milk glutamine concentration or whether the piglets simply did not benefit from additional glutamine in the milk.

5.5.5. The effects of HMB and lignocellulose fibre on immunoglobulin concentrations in sow colostrum on the indoor production system

HMB has been found to increase the IgG concentration of sow colostrum in a linear fashion in terms of both dose and duration of HMB supplementation, when provided as a top-dressing to sows' standard gestation diets as demonstrated in experiments 1 and 2. Experiment 2 also found a duration effect of HMB supplementation on IgA and IgM concentrations in sow colostrum. In the present study, HMB failed to increase the concentrations of IgG or IgA in sow colostrum when provided in the diet at a 0.15 % inclusion level and fed to sows for 15 days prior to parturition. However, there was a tendency for HMB to increase the level of IgM in sow colostrum by 8.8 % compared to sows which did not receive HMB. As previously mentioned, HMB has been found to increase T and B lymphocyte activities (Siwicki et al., 2003); therefore HMB may have increased T cell activation resulting in more plasma cells which release IgM. Approximately 80 % of IgM in colostrum is derived from sow serum and 20 % is synthesised in the mammary gland (Bourne and Curtis, 1973). Therefore, HMB may have stimulated an increase in plasma cells in sow serum which were then translocated to the mammary gland or stimulated an increase in plasma cells in the mammary gland resulting in a higher concentration of IgM in colostrum.

The reasons for the lack of effect of HMB on levels of IgG and IgA in sow colostrum are unclear. Concentrations of immunoglobulins in colostrum can vary with genotype (Inoue et al., 1980); the present study used a different genotype of sow (JSR 9T) compared to the previous two studies (JSR Genepacker GP90). Therefore, the difference in results between studies may be due to the different genotypes used. In the current study, there was an illness in the sow herd so it may be that sows were already producing their maximum potential of IgG and IgA, therefore there were no additional benefits of HMB supplementation on these parameters.

5.6. Conclusions

In conclusion, incorporating HMB into a commercial diet at a concentration of 0.15 % (as-fed) and providing it to sows for ~ 15 days prior to parturition successfully increased the colostrum yield of sows on the indoor production system. This is most likely due to the lipolytic effect of HMB which would have increased the energy for production of colostrum. As a result of this average piglet

weights at week one and at weaning were increased on the indoor production system and increased when the data from both production systems were combined for overall analysis. There was also a tendency for HMB supplementation to increase colostrum concentration of IgM on the indoor production system.

Lignocellulose fibre supplementation to sows at a concentration of 1 % for ~ 5 d prior to parturition reduced sow gestation length on the indoor production system and in the overall analysis of both systems, without reducing average piglet birth weight. In addition, piglets from sows supplemented with lignocellulose fibre were heavier at weaning when the data from both production systems were combined. The heavier weaning weights of piglets may be due to the increased age of the piglets on weigh days, however, as farmers are unlikely to change weaning dates the age of the pig is irrelevant and the increased weights would be seen as beneficial.

Although there were no significant benefits of supplementing glutamine to sows in lactation at a concentration of 1 %, there were indications of improved performance at week one which were more noticeable on the outdoor production system. Therefore, this warrants further investigation.

6.

Supplementing β-hydroxy β-methyl butyrate to sows in late gestation improves overall litter and piglet performance to weaning across four experiments

6.1. Abstract

The experiments in this thesis demonstrated inconsistencies with regard to the effect of supplementing HMB to sows in gestation on litter and piglet performance to weaning. Therefore, this chapter aimed to analyse the overall effects of HMB supplementation to sows at a dose of ~ 15 mg/kg BW for 15 days prior to parturition on piglet performance to weaning and on colostrum immunoglobulin concentrations across the four experiments performed in this thesis. A total of 279 mixed parity sow records were used from the four experiments in this thesis (Chapters 3, 4 and 5). The sow records used were those which had been in the control groups or the groups supplemented with HMB at a dose of 15 mg/kg BW for ~ 15 days (Chapters 3 and 4) or at 1500 mg/kg of feed, as-fed for ~ 15 days (Chapter 5). This created two treatment groups: Control and HMB.

The total number of piglets born and born alive were not affected by maternal dietary treatments. HMB supplementation increased total live born litter weight (P=0.031) and there was a tendency for litter weights at week one and at weaning to be increased compared with the control (P=0.081 and P=0.084, respectively). In addition, there was a tendency for piglets from sows supplemented with HMB to have increased average week one and weaning weights compared with the control (P=0.088 and P=0.054 respectively). Supplementing sows with HMB increased colostrum concentrations of IgG (P=0.015) and IgM (P=0.019).

In conclusion, this study demonstrated that supplementing sows with HMB at a dose of ~ 15 mg/kg BW for ~ 15 days prior to parturition had beneficial effects on litter and piglet performance to weaning and on immunoglobulin concentrations in colostrum across the four trials conducted in this thesis.

6.2. Introduction

The experiments in this thesis examined the effects of supplementing sows with HMB in late gestation on piglet performance and on colostrum production. The

results of the experiments displayed some inconsistencies and many of the reported beneficial effects were trends as opposed to significant findings. Experiment 1 (as described in Chapter 3) supplemented sows with HMB at a dose of 0, 5, 15 or 45 mg/kg BW for ~ 15 days prior to parturition and found that the beneficial effects of HMB on piglet performance and colostrum production were dose-dependent. HMB supplementation increased piglet birth weight, piglet growth rate, and colostrum yield and intake in quadratic dose-dependent manners with the optimum dose identified as 15 mg/kg BW. Supplemental HMB also increased colostrum concentration of IgG linearly up until the highest dose tested. Overall, this experiment identified the optimum dose of HMB supplementation as 15 mg/kg BW.

Experiment 2 (as described in Chapter 4) supplemented sows with HMB for 0, 6, 15 or 22 days prior to parturition at a dose of 15 mg/kg BW. This study found that supplemental HMB increased IgG concentration in colostrum linearly up until the highest duration it was supplemented for. This study also found that supplemental HMB increased colostrum concentration of IgA in a quadratic manner and colostrum concentration of IgM in a linear fashion. However, unlike experiment 1 this study found no effect of supplemental HMB on piglet birth weight or performance. Overall, this experiment identified the optimum duration of HMB supplementation as 15 days prior to parturition.

Experiment 3 (as described in Chapter 5 for the indoor production system) and experiment 4 (as described in Chapter 5 for the outdoor production system) incorporated HMB into a commercial pig diet during manufacture at a dietary concentration of 0.15 % (as-fed) which was fed to sows for ~ 15 days prior to parturition. Neither experiment found an effect of HMB supplementation on piglet birth weight, however experiment 3 found that HMB supplementation increased colostrum yield and there was a tendency for increased colostrum intake. In addition, experiment 3 found a tendency for HMB supplementation to increase average piglet weights at week one and at weaning. However, in experiment 4 although piglets from sows supplemented with HMB were numerically heavier at both week one and weaning compared with piglets from sows that were not supplemented with HMB, this was not significant. When the data from both experiments 3 and 4 were combined, supplementing sows with HMB significantly

increased average piglet week one weight with a tendency towards increased weaning weight.

Sow and litter performance can be highly variable and many studies are often under replicated. Two treatments were repeated across all experiments in this thesis. Combining the data collected from these studies would greatly increase the replication number and validate the effects observed.

6.2.1. Hypothesis

When the data from four experiments are collated, supplementing sows with HMB at a dose of ~ 15 mg/kg BW for 15 days prior to parturition will improve sow, litter and piglet performance from birth to weaning.

6.2.2. Aims

- To determine the effect of HMB supplementation to sows on total born litter weight and litter performance to weaning when the data from four experiments are collated.
- To determine whether HMB supplementation to sows effected average piglet 24 hour weight and overall piglet growth to weaning when the data from four experiments are collated.
- To determine the effect of HMB supplementation to sows on concentrations of IgG, IgA and IgM in colostrum when the data from three experiments are collated.

6.3. Materials and methods

6.3.1. Data

Data were collated from the control sows and from the sows supplemented with HMB at a dose of 15 mg/kg BW for a duration of ~ 15 days from experiments 1 and 2, and from the control sows and the sows fed a diet with HMB included at 1500 mg/kg of feed (as-fed) from experiments 3 and 4. This created two treatment groups: Control and HMB.

6.3.2. Animals and management and measurements

A total of 279 mixed parity sow records were used from the four experiments (experiment 1 *n* = 69; experiment 2 *n* = 67; experiment 3 *n* = 78; experiment 4 *n* = 65) with parity ranging from 1:9. A total of 4,119 piglet records were used from the 279 sows and of these 188 were still born. Sow performance data used included: sow weights and back-fat thickness measurements on d 100, and sow

weight and back-fat changes from d 100 to d 109 and from d 109 to weaning, gestation length, litter sizes, litter weights to weaning and mortality to weaning. Piglet performance data from 24 hours to weaning was also used. There were no colostrum samples taken from experiment 4 therefore sow colostrum data were used from experiments 1, 2 and 3. See relevant chapters for details (Chapters 3, 4, 5).

6.3.3. Calculations and statistical analysis

Performance data were analysed using the General Linear Model procedure and colostrum immunoglobulin concentrations were analysed using the Mixed Model procedure of SPSS Statistics (version 21.0, SPSS Inc., Chicago IL, USA), with the sow as the experimental unit for all analyses. Data were first tested for homogeneity of variance and normality using the Levene's test and the Kolmogorov-Smirnov test, respectively. Data displaying heteroscedasticity or non-normal data were $\log_{10}$ transformed prior to statistical analysis. Transformed data were back transformed for inclusion in the respective tables. Colostrum IgA and colostrum IgM concentrations required $\log_{10}$ transformation. The statistical model for performance included the effect of diet, experiment and diet × experiment interactions. Non-significant interactions were removed from the model and the main effects were analysed individually. Parity was included in the model as a fixed factor. Total number of piglets born was used as a covariate for average 24 hour weights. Litter size post-fostering and age were used as covariates for piglet performance to wean when significant. The statistical model for colostrum immunoglobulin concentrations included the effects of diet, experiment, diet × experiment interactions and the effect of batch nested within experiment was included as a random effect. If parity was significant it was included in the model as a covariate instead of a fixed factor. Non-significant interactions were removed from the model and the main effects were analysed individually. With regards to all analyses, when significant differences were detected, comparisons between groups were made with fisher's least significant difference test. Data are expressed as least-square means with their pooled standard error of the mean (SEM).

6.4. Results

6.4.1. Sow performance

Sow characteristics are presented in Table 6.1. Sow d 100 weights and back-fat thickness differed between experiments (P<0.001 and P<0.001, respectively). There was no effect of diet on d 100 to d 109 weight change, however weight change differed between experiments (P<0.001). Sow d 109 to wean weight change was not affected by diet (P=0.209), however it was affected by experiment (P=0.037). Gestation length was not affected by HMB supplementation (P=0.352), however gestation length differed between experiments (P<0.001). Sows in experiment 1 had the shortest and sows in experiment 2 had the longest gestation lengths. Lactation FI was not influenced by diet. However, total FI and ADFI differed between experiments (P<0.001 and P<0.001, respectively).

6.4.2. Litter performance

Litter performance is presented in Table 6.2. HMB supplementation to sows increased total live born litter weight (P=0.031); sows supplemented with HMB had 5.0 % heavier total live born litter weights than sows that were not supplemented with HMB (21.1 vs 20.1 kg for HMB for vs Control, respectively). There was also a tendency for sows supplemented with HMB to have 4.6 % heavier litters at week one (31.5 vs 30.2 kg for HMB vs Control, respectively; P=0.081) and 3.8 % heavier litters at weaning (85.5 vs 82.4 kg for HMB vs Control, respectively; P=0.084) compared with sows that were not supplemented with HMB. There was a tendency for total week one litter weight to differ between experiments (P=0.071). Litter weight at weaning was similar between experiments (P=0.312).

Percentage mortality to weaning was similar between diets (P=0.348) and experiments (P=0.113) and averaged 13.1 ± 1.79 % (mean ± SEM). However, there was a tendency for percentage mortality to weaning to be influenced by a diet x experiment interaction (P=0.061). The effect of HMB on mortality was influenced by experiment. Sows supplemented with HMB had lower mortality levels in experiment 1, 3 and 4 however, they had higher mortality levels in experiment 2.

6.4.3. Piglet performance

Piglet performance is presented Table 6.3. There was a tendency for HMB supplementation to sows to increase average piglet 24 hour weight (total) by 2.9 % compared with the control (1.52 vs 1.48 kg for HMB vs Control, respectively; *P*=0.097). Average piglet 24 hour weight (total) was also effected by experiment (*P*=0.006). There was a tendency for piglets from sows supplemented with HMB to have a 3.6 % higher week one to wean ADG than piglets from the control group (237 vs 229 g/d for HMB vs Control, respectively; *P*=0.079). Week one to wean ADG differed between experiments (*P*=0.045).

Piglet week one weights averaged 2.57 ± 0.056 kg (mean ± SEM) across all treatments. There was a tendency for HMB supplementation to sow diets to increase average piglet week one weight (2.61 vs 2.53 kg for HMB vs control, respectively; *P*=0.088). Average piglet week one weight was different between experiments (*P*=0.001). Average piglet weight at week one was 10.8 % heavier in experiment 4 than experiment 1 and 8.4 % heavier in experiment 4 than experiment 3 (*P*=0.001). There were no other significant differences between the average week one weights of piglets in the other experiments. Piglet wean weights averaged 7.29 ± 0.134 kg (mean ± SEM) across all treatments. There was a tendency for HMB supplementation to sows to increase average piglet weaning weight by 3.1 % compared with piglets from control sows (7.40 vs 7.12 kg for HMB vs control, respectively; *P*=0.054). Wean weights were also different between experiments (*P*=0.019). Piglets in experiment 4 were 7.54 % heavier than piglets in experiment 2 at weaning (*P*<0.05).

Table 6.1. The effects of β-hydroxy β-methyl butyrate supplementation to sows at a dose of ~ 15 mg/kg body weight for ~ 15 days prior to parturition on sow characteristics across four experiments[1]

Experiment	Diet[2]	Parity	Weights (kg)			P2 (mm)			Gestation length[3] (d)	FI (kg)[2]	
			D100	D100 to d109 change	D109 to wean change	D100	D100 to d109 change	D109 to wean change		Total FI	ADFI
1	CT	3.2	243.6	9.7	-39.3	14.1	0.3	-1.7	114.4	157.6	5.9
1	HMB	3.2	240.9	10.1	-36.6	13.9	0.1	3.9	114.2	161.3	6
2	CT	3.2	233.2	12.3	-30.2	12.8	-2.8	0.8	116.0	189.8	7.4
2	HMB	3.2	230.6	12.7	-27.5	12.5	-3.0	-0.4	115.8	193.5	7.5
3	CT	3.2	237.1	6.8	-34.3	14.3	0.0	-0.3	115.2	167.2	6.4
3	HMB	3.2	234.4	7.2	-31.6	14.0	-0.1	-0.4	115.0	170.8	6.5
4	CT	3.1	209.6	3.6	-35.5	10.9	-0.1	-2.2	115.8	184.1	7.8
4	HMB	3.1	207.0	4.0	-32.8	10.7	-0.2	-1.6	115.7	187.8	7.9
	SEM	0.05	3.13	0.84	2.45	0.41	0.66	0.65	0.22	2.76	0.11
Main effects											
Diet	CT	3.2	230.9	8.1	-34.8	13.0	-0.7	-0.9	115.4	174.7	6.9
	HMB	3.2	228.2	8.5	-32.1	12.8	-0.8	0.4	115.2	178.4	7.0
	SEM	0.03	1.98	0.05	1.56	0.26	0.42	0.33	0.14	1.75	0.07
Experiment	1	3.2	242.3^{a}	9.9^{a}	-38.0^{a}	14.0^{a}	0.2^{a}	1.1^{a}	114.3^{a}	159.5^{a}	6.0^{a}
	2	3.2	231.9^{b}	12.5^{b}	-28.9^{b}	12.7^{b}	-2.9^{b}	0.2^{b}	115.9^{b}	191.7^{b}	7.5^{b}
	3	3.2	235.8^{ab}	7.0^{c}	-33.0^{ab}	14.2^{a}	-0.4^{a}	-0.4^{c}	115.1^{c}	169.0^{c}	6.5^{c}
	4	3.1	208.3^{c}	3.8^{d}	-34.2^{ab}	10.8^{c}	-0.2^{a}	-1.9^{d}	115.8^{b}	186.0^{b}	7.9^{d}
	SEM	0.05	2.81	0.75	2.20	0.37	0.59	0.46	0.19	2.48	0.10
P-value											
Diet		0.477	0.334	0.589	0.209	0.512	0.776	**0.010**	0.352	0.137	0.162
Experiment		0.667	**<0.001**	**<0.001**	**0.037**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
D × E[2]		0.731	0.522	0.937	0.604	0.648	0.344	**<0.001**	0.270	0.506	0.168

[a-d]Means that do not share a common superscript are significantly different (*P*<0.05)
[1]Data represents a total of 279 sows for data to parturition and 259 sows for data to weaning
[2]CT = Control, HMB = β-hydroxy β-methyl butyrate, D × E = diet × experiment interaction, FI = feed intake, ADFI = average daily feed intake
[3]Corrected for total born

Table 6.2. The effects of β-hydroxy β-methyl butyrate supplementation to sows at a dose of ~ 15 mg/kg body weight for ~ 15 days prior to parturition on litter performance across four experiments[1]

Experiment	Diet[2]	Number of piglets					Litter weights (kg)				Age (days)		Mortality[3] (%)		
		Total born	Alive	Dead	Week one	Wean	Total born	Live born	Week one	Wean	Week one	Wean	24 hour	Week one	Wean
1	CT	14.6	13.9	0.8	11.4	10.9	21.4	19.6	29.1	79.9	7.1	27.1	4.8	14.1	18.2
1	HMB	14.8	14.1	0.7	11.5	11.2	22.3	20.6	30.5	83.0	7.2	27.2	5.1	9.5	13.1
2	CT	15.5	14.2	1.2	12.4	12.0	21.4	20.1	21.9	82.2	6.7	26.7	3.3	7.4	9.5
2	HMB	15.6	14.5	1.1	12.5	12.3	22.2	21.1	33.3	85.3	6.8	26.8	3.7	10.6	13.8
3	CT	15.9	15.2	0.7	12.2	11.6	21.1	20.3	29.7	84.6	6.4	27.4	2.8	12.2	14.7
3	HMB	16.1	15.4	0.7	12.3	119.0	22.0	21.3	31.0	87.7	6.5	27.4	3.2	8.9	11.8
4	CT	12.9	12.8	0.1	11.6	11.3	20.3	20.2	29.9	83.0	5.7	25.7	3.1	8.6	12.5
4	HMB	13.1	13.0	0.1	11.7	11.7	21.2	21.2	31.3	86.1	5.8	25.8	3.5	8.3	11.5
	SEM	0.44	0.40	0.14	0.23	0.24	0.54	0.52	0.87	2.00	0.23	0.23	0.81	1.66	1.79
Main effects															
Diet	CT	14.7	14.0	0.7	11.9	11.5	21.1	20.1	30.2	82.4	6.5	26.7	3.5	10.6	13.7
	HMB	14.9	14.3	0.7	12.0	11.8	21.9	21.1	31.5	85.5	6.6	26.8	3.5	9.3	12.6
	SEM	0.28	0.26	0.09	0.15	0.15	0.34	0.33	0.56	1.27	0.14	0.14	0.51	0.83	0.90
Experiment	1	14.7[a]	14.0[a]	0.8[a]	11.5[a]	11.1[a]	21.9	20.1	29.8	81.5	7.2[a]	27.2[ab]	4.9	11.8	15.7
	2	15.6[ab]	14.4[ab]	1.2[b]	12.5[b]	12.2[b]	21.8	20.6	32.6	83.8	6.8[ab]	26.8[a]	3.5	9.0	11.7
	3	16.0[b]	15.3[b]	0.7[a]	12.3[c]	11.8[bc]	21.6	20.8	30.4	86.2	6.5[b]	27.4[b]	3.0	10.6	13.3
	4	13.0[c]	12.9[c]	0.1[c]	11.7[a]	11.5[ac]	20.8	20.7	30.6	84.6	5.8[c]	25.8[c]	3.3	8.5	12.0
	SEM	0.39	0.36	0.13	0.21	0.22	0.49	0.47	0.78	1.80	0.20	0.20	0.73	1.19	1.29
P-value															
Diet		0.664	0.518	0.616	0.555	0.140	**0.072**	**0.031**	**0.081**	**0.084**	0.621	0.667	0.648	0.284	0.348
Experiment		**<0.001**	**<0.001**	**<0.001**	**0.002**	**0.003**	0.373	0.720	**0.071**	0.312	**<0.001**	**<0.001**	0.242	0.171	0.113
D × E[2]		0.921	0.846	0.647	0.774	0.619	0.330	0.215	0.128	0.379	0.481	0.360	0.136	**0.091**	**0.061**

[a-d]Means that do not share a common superscript are significantly different ($P<0.05$)

[1]Data represents a total of 279 litters for data to parturition, 266 litters for data to week one and 259 litters for data to weaning

[2]CT = Control, HMB = β-hydroxy β-methyl butyrate, D × E = diet × experiment interaction

[3]Corrected for total born

Table 6.3. The effects of β-hydroxy β-methyl butyrate supplementation to sows at a dose of ~ 15 mg/kg body weight for ~ 15 days prior to parturition on piglet performance to wean across four experiments[1]

Experiment	Diet[2]	Average piglet weights (kg)					Piglet ADGs (g)[2]	
		24 hr (total)[3]	24 hr (alive)[4]	24 hr (post foster)[4]	Week one[4,5]	Wean[4,5]	Week one[4]	Week 1 to wean[4]
1	CT	1.50	1.52	1.51	2.42	7.12	135	231
1	HMB	1.54	1.56	1.55	2.50	7.35	138	239
2	CT	1.44	1.44	1.44	2.56	6.92	169	217
2	HMB	1.48	1.48	1.48	2.64	7.14	171	225
3	CT	1.43	1.44	1.44	2.45	7.22	163	232
3	HMB	1.47	1.48	1.49	2.54	7.44	165	240
4	CT	1.54	1.57	1.57	2.68	7.45	202	234
4	HMB	1.59	1.61	1.62	2.77	7.67	204	242
	SEM	0.028	0.028	0.028	0.056	0.134	5.9	5.2
Main effects								
Diet	CT	1.48	1.49	1.49	2.53	7.18	167	229
	HMB	1.52	1.53	1.54	2.61	7.40	170	237
	SEM	0.018	0.018	0.018	0.035	0.083	3.7	4.7
Experiment	1	1.52^{ab}	1.54^{a}	1.53^{ab}	2.46^{a}	7.24^{ab}	137^{a}	235^{a}
	2	1.46^{ac}	1.46^{b}	1.46^{a}	2.60^{ab}	7.03^{a}	170^{b}	221^{b}
	3	1.45^{c}	1.46^{b}	1.47^{a}	2.50^{a}	7.33^{ab}	164^{b}	236^{a}
	4	1.57^{b}	1.59^{a}	1.59^{b}	2.73^{b}	7.56^{b}	203^{c}	238^{a}
	SEM	0.025	0.025	0.025	0.050	0.120	5.3	3.3
P-value								
Diet		**0.097**	**0.088**	**0.058**	**0.088**	**0.054**	0.662	**0.079**
Experiment		**0.006**	**0.001**	**0.001**	**0.001**	**0.019**	**<0.001**	**0.045**
D × E[2]		0.154	0.164	0.207	0.158	0.326	0.444	0.765

[a-d]Means that do not share a common superscript are significantly different ($P<0.05$)

[1]Data represents a total of 279 litters for data to parturition, 266 litters for data to week one and 259 litters for data to weaning

[2]CT = Control, HMB = β-hydroxy β-methyl butyrate, D × E = diet × experiment interaction, ADG = average daily gain

[3]Corrected for total born

[4]Corrected for litter size; [5]Corrected for age

6.4.4. Colostrum immunoglobulin concentrations

Colostrum IgG, IgA and IgM concentrations are presented in Figure 6.1. Across the three studies colostrum IgG concentration averaged 60.4 ± 4.38 mg/ml (mean ± SEM). Colostrum IgG concentration was increased by 12.6 % by HMB supplementation compared with the control (64.0 vs 56.8 mg/ml for the HMB vs Control, respectively; *P*=0.025). Colostrum IgG concentration was not influenced by experiment (*P*=0.293); however, it was influenced by a diet × experiment interaction (*P*=0.016). Colostrum IgA concentration averaged 11.8 ± 1.56 mg/ml (mean ± SEM). Colostrum IgA concentration was not affected by diet (*P*=0.944) or experiment (*P*=0.888). Colostrum IgM concentration averaged 3.9 ± 0.17 mg/ml (mean ± SEM). HMB supplementation increased colostrum IgM concentration by 9.7 % compared with the control (4.1 vs 3.7 mg/ml for HMB vs Control, respectively; *P*=0.019). IgM concentration was also affected by experiment (*P*=0.029). The concentration of IgM was 30.3 % higher in colostrum from sows in experiment 1 compared with sows in experiment 2 (4.2 vs 3.2 mg/ml for experiment 1 vs experiment 2, respectively; *P*=0.025). Colostrum IgM concentration was 33.5 % higher in sows in experiment 3 compared with sows in experiment 2 (4.3 vs 3.2 mg/ml for experiment 3 vs experiment 2, respectively; *P*=0.014). There was no difference in IgM concentration in colostrum between experiment 1 and experiment 3 (*P*=0.892). Colostrum IgA and IgM concentrations were not influenced by any diet × experiment interactions.

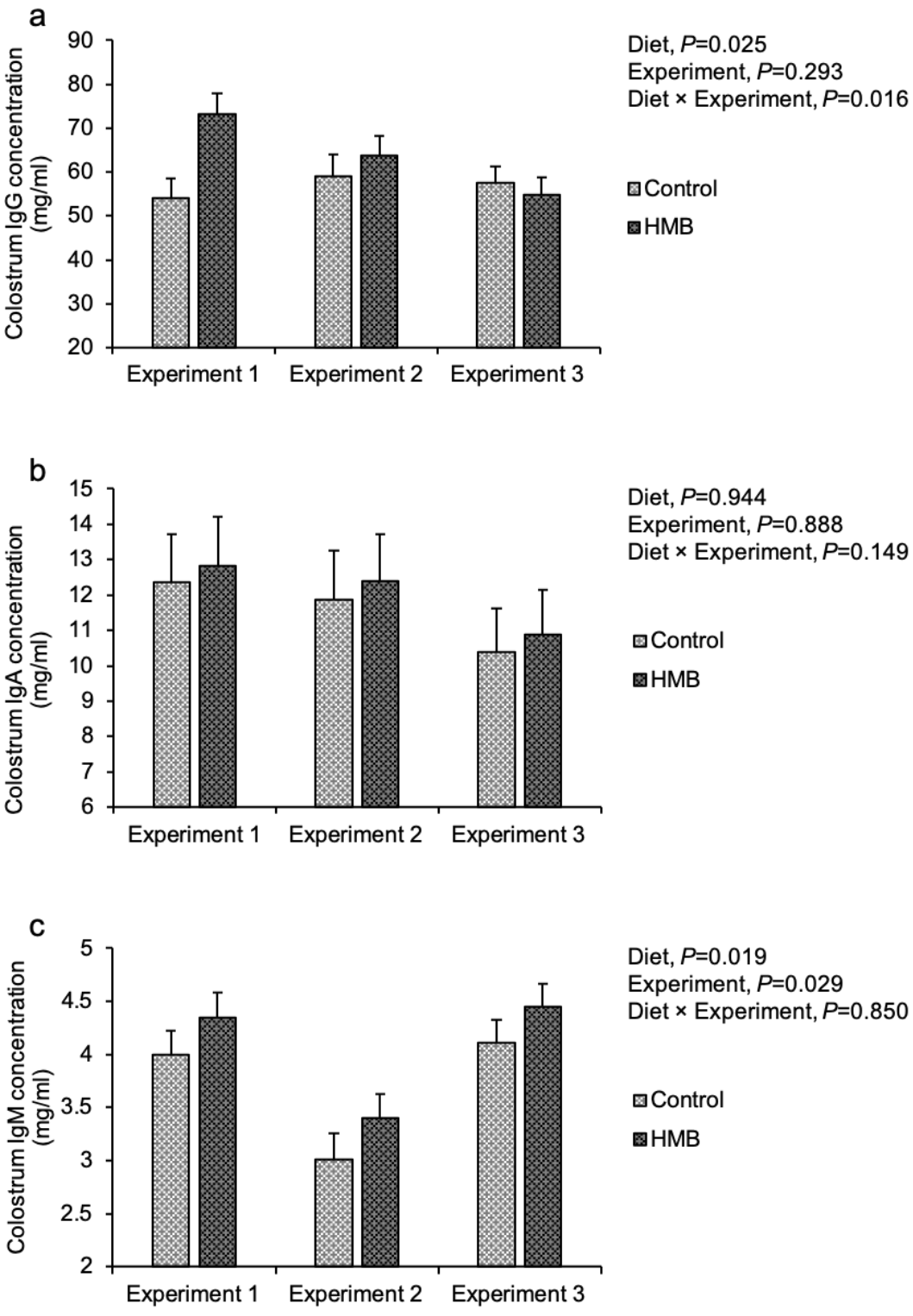

Figure 6.1. The effects of β-hydroxy β-methyl butyrate on sow colostrum IgG, IgA and IgM concentrations (mg/ml) when supplemented to sows at a dose of ~ 15 mg/kg body weight for ~ 15 days prior to parturition across three experiments

HMB = β-hydroxy β-methyl butyrate. Data presented are means + SEM. **a)** Represents concentrations of IgG in sow colostrum. **b)** Represents concentrations of IgA in sow colostrum. Data were $\log_{10}$ transformed and corrected for parity. **c)** Represents concentration of IgM in sow colostrum. Data were $\log_{10}$ transformed and corrected for parity.

6.5. Discussion

As there were discrepancies between the results of individual experiments, this chapter was included to determine the overall main effects of HMB supplementation to sow diets in gestation at a dose of ~ 15 mg/kg BW for 15 days prior to parturition on sow, litter and piglet performance when data from four experiments were collated.

6.5.1. The effects of HMB on sow characteristics and litter performance

This analysis demonstrated that HMB supplementation at a dose of ~ 15 mg/kg BW had no effects on sow weights changes at any of the time points across the experimental periods. Sow back-fat from d 109 to weaning was influenced by a diet × experiment. Sows supplemented with HMB lost less back-fat in experiment 1 and lost more back-fat in experiment 2. This is reflected in how well the litters performed. In experiment 1 litters from sows supplemented with HMB outperformed litters from control sows. In experiment 2 litters from sows supplemented with HMB under performed compared to control litters. Overall, gestation length was not affected by HMB supplementation. However, gestation length differed between the experiments. Sows in experiment 1 had the shortest (14.3 d) and sows in experiment 2 had the longest (15.9 d) gestation lengths. Sow genotype can influence gestation length (Rydhmer et al., 2008). Knol (2001) studied two different dam lines and found they differed in gestation length by two days. This may explain the differences between experiments 3 and 4 as the studies used different breeds (Large White × Landrace [JSR 9T, JSR, UK] vs Large White × Landrace × Duroc for experiments 3 vs 4, respectively). However, experiments 1 and 2 used the same genotype of sow (Large White × Landrace [JSR Genepacker 90, JSR, UK]) so this cannot be the cause of the difference between these two experiments. Gestation length is also affected by parity and litter size. Sows with larger litter sizes often have a shorter gestation length than sows with smaller litter sizes (Rydhmer et al., 2008). However, in the present analysis there were no differences in litter sizes between the experiments therefore the differences in gestation length may be due to external factors that were not controlled for between experiments, such as the time of year the experiments were conducted in. Total FI and ADFI of sows in lactation differed between experiments which may be due to several factors including: litter size, temperature, parity, body weight, genotype and management (Eissen et al., 2000).

One of the main aims of this combined analysis was to determine the overall effects of HMB supplementation to sows on litter performance at a dose of ~ 15 mg/kg BW for 15 days prior to parturition when the data from four experiments were collated. Overall, supplementing sows with HMB increased total live born litter weight compared with the control. HMB has been found to have anti-catabolic effects (Szcześniak et al., 2015); it can enhance protein synthesis via the activation of the mTOR pathway (Smith et al., 2005) and reduce protein degradation through the down regulation of the ubiquitin-proteasome pathway (Ostaszewski et al., 2000). The increases in litter weights at birth resulted in tendencies for heavier litters at week one and at weaning. When experiments were analysed individually the only experiment which found that HMB supplementation significantly increased total born litter weight was experiment 1, however sows supplemented with HMB had numerically heavier litter weights in both experiments 3 and 4. In addition, when experiments were analysed individually, litter weight at week one was significantly heavier in experiment 1 and there was a tendency for heavier litters at week one in experiment 3. Litter weights at birth are highly variable mainly due the large variations in litter sizes (6 - 24 piglets/litter in this analysis). Combining the data from all experiments allowed for a much larger sample size which may have been needed to determine this effect.

Overall mortality across these four trials was ~ 13 % which is similar to commercial figures reported at the time (~ 12 %) (AHDB, 2019c). There was no effect of supplementing sows with HMB on overall piglet mortality to weaning. However, percentage mortality to weaning ranged from 0 - 56 % per litter and the CV was 83 %. Due to the high variation in percentage mortality a larger replication was needed to give this statistical power and so this warrants further investigation.

6.5.2. The effects of HMB on piglet performance from 24 hours to weaning

Average piglet 24 hour weights are highly variable between litters mainly due to the variability in litter sizes (Milligan et al., 2002a). As mentioned above, the litters used in this analysis varied in size from 6 - 24 piglets/litter. Therefore, large sample sizes are needed to detect small effects nutritional supplements may have. The results of experiments 1 - 4 demonstrated inconsistences with regard to the effects of maternal supplementation with HMB on piglet performance.

When the results of these trials were combined there were tendencies for average 24 hour weight (total) and average 24 hour weight (live) to be increased by HMB supplementation to sows. As previously mentioned, HMB has been found to play a role in the regulation of skeletal muscle turnover (Szcześniak et al., 2015). It can reduce protein degradation (Ostaszewski et al., 2000) and stimulate protein synthesis (Smith et al., 2005). HMB has also been found to have a lipolytic effect (Wilson et al., 2008, Theil et al., 2014a); experiments 1 and 3 demonstrated that HMB supplementation to sow diets increased colostrum yield and colostrum intake. The role HMB has in the regulation of skeletal muscle turnover and in colostrum production combined is the likely cause of the increased average 24 hour weight of piglets.

There was a tendency for piglets from sows supplemented with HMB to have an increased week one to wean ADG compared with piglets from control sows. In addition, there was a tendency for piglets from sows supplemented with HMB to have heavier average weights at week one and at weaning. Experiments 1 and 3 (as described in Chapters 3 and 5, respectively) demonstrated piglets from sows supplemented with HMB had increased colostrum intake. Birth weight and colostrum intake are major factors determining piglet pre-weaning growth (Gondret et al., 2005, Edwards, 2002, Devillers et al., 2007) which is the likely cause of the increased weights at weaning.

6.5.3. The effects of HMB on immunoglobulin concentrations in sow colostrum

Whilst experiments 1, 2 and 3 all demonstrated that HMB affected immunoglobulin concentrations in sow colostrum, there were discrepancies in the results. Immunoglobulin concentrations in colostrum are highly variable and it is well established that they are affected by many factors including: time of sampling, sow parity and season (Le Dividich et al., 2005, Hurley, 2015). Therefore, this analysis aimed to determine the overall effect of supplementing HMB to sows at a dose of ~ 15 mg/kg BW for 15 days prior to parturition on immunoglobulin concentrations in colostrum when data from three experiments were combined.

Overall, HMB increased colostrum concentrations of IgG and IgM. As previously mentioned, HMB has been found to increase T and B lymphocytes (Siwicki et al., 2003) and thus the increase in immunoglobulin concentrations in colostrum is

most likely due to T cell activation by HMB. However, there was a diet × experiment interaction with regard to the concentration of IgG as HMB did not increase the concentration of IgG in colostrum in experiment 3. Whilst the reason for this is unclear the litters in experiment 3 were affected by scour and this may have interfered with the mechanism of HMB. In addition, a different genotype of sow was used in experiment 3 compared with the experiments 1 and 2 (Large White × Landrace [JSR 9T, JSR, UK] vs Large White × Landrace [JSR Genepacker 90, JSR, UK] for experiment 3 vs experiments 1 and 2).

Whilst there was a numeric increase in colostrum IgA concentration in all studies, when combined there was no significant effect of HMB on IgA concentration. IgG and IgM may have been increased with HMB supplementation as IgG is the most prominent immunoglobulin in sow colostrum and although IgM is the least prominent, it is involved in primary defence (Hurley and Theil, 2011). These immunoglobulins may therefore have been stimulated first.

6.6. Conclusion

In conclusion, when collating the data collected across the four experiments in this thesis, supplementing sows with HMB in gestation at a dose of ~ 15 mg/kg BW for 15 days prior to parturition successfully increased total live born litter weight. In addition, there was a tendency for average 24 hour weights of piglets to be enhanced. This was then reflected in a tendency for improved total litter weights at both week one and at weaning and in a tendency for improved average week one and weaning weights. This analysis also demonstrated that HMB supplementation increased concentrations of both IgG and IgM in colostrum. This analysis demonstrated that multiple studies or much larger replication may be needed to determine an overall effect of nutritional supplementation in sow studies.

7.
General Discussion

Selection for hyper-prolific sows has resulted in sows commonly producing an average of 14 piglets/litter and up to 33 piglets per year (AHDB, 2017a). However, the drive towards hyper prolificacy has come with negative side effects such as: increased still births, reduced birth weights, reduced individual colostrum consumption and an overall increase in piglet pre-weaning mortality (Canario et al., 2007, Devillers et al., 2011). With the current demand for pork production expected to rise by ~ 5 % (+ 47,000 tonnes) in the UK between 2019 and 2021 (AHDB, 2019a) selection for these large litter sizes is expected to grow. The transition period from gestation to lactation is an area of vital importance for foetal growth and for colostrum production (Theil, 2015). Therefore, the nutritional status of the sow during this period can affect the survival and future growth of her offspring.

β-hydroxy β-methyl butyrate (HMB), a metabolite of leucine, has been shown to have beneficial effects on piglet and litter performance to weaning when supplied to sows in gestation. Potential positive effects have been found in relation to piglet birth weight (Tatara et al., 2007), pre-weaning growth (Nissen et al., 1994, Wan et al., 2017) and colostrum production (Nissen et al., 1994, Krakowski et al., 2002, Flummer and Theil, 2012). However, published research is extremely limited and the majority of these studies only used a limited number of replicates. In addition, the doses and timings of HMB supplementation used in these studies are ambiguous and the results are inconsistent. Therefore, the main aims of this research were to determine the effects of HMB supplementation to sow diets on litter and piglet performance to weaning in a series of large scale experiments and to establish the optimum dose and duration of HMB supplementation which would maximise any beneficial effects on piglet performance and colostrum production.

7.1. The effects of supplementing HMB to sow diets on sow performance

An unprecedented result of providing supplemental HMB to sows in the transition period was the tendency for a reduction in sow gestation length. To our

knowledge at the time of writing, no published studies have observed this effect which may be because a larger replication of sows was needed. In experiment 1 (as described in Chapter 3) there was a tendency for HMB to reduce gestation length in a linear fashion; a higher dose of HMB resulted in a shorter gestation length. When the results were combined for the doses of 5, 15 and 45 mg/kg BW HMB for 15 days prior to parturition gestation length was reduced with respect to the control. However, whilst sows supplemented with HMB at a dose of 45 mg/kg BW numerically had the shortest gestation length, it was not significantly shorter than the gestation length of sows in the other groups. This effect was also demonstrated in experiment 2 (as described in Chapter 4) which found a tendency for HMB supplementation to reduce gestation length in a quadratic manner; the longer the duration of time that the sows were fed HMB, the shorter their gestation length. In experiment 2, only sows supplemented with HMB for the longest duration of time (15 mg/kg BW for 21 d prior to parturition) had a significantly shorter gestation length. Whilst experiment 3 (as described in Chapter 5 for the indoor production system) found no effect of supplementing sow diets with HMB on the gestation length of sows, experiment 4 (as described in Chapter 5 for the outdoor production system) displayed a tendency for HMB to reduce the gestation length of sows. However, when the data from experiments 3 and 4 were combined, there were no overall effects of HMB on gestation length.

HMB has been shown to have immunostimulatory effects. Siwicki et al. (2003) found HMB increased T and B lymphocyte activity in fish. T lymphocytes activate B lymphocytes, releasing interleukins, which cause the differentiation of B lymphocytes into plasma cells (Murphy, 2012). Interleukins are a type of cytokine and circulating cytokines are involved in the onset of labour (Steinborn et al., 1995, Arntzen et al., 1998). HMB increased immunoglobulin concentrations in colostrum in all experiments in this thesis, therefore it may have also increased the level of circulating cytokines in the sow leading to the earlier onset of labour.

Piglet birth weights have been positively associated with gestation length; a longer gestation length is associated with higher piglet birth weights and faster growth rates (Rydhmer et al., 2008). In experiments 1 and 2 sows supplemented with HMB at either the highest dose or for the longest duration of time had either an increased number of low birth weight piglets (experiment 1) or a numeric reduction in average piglet birth weights (experiment 2). There were no negative

side effects for the other doses or durations of HMB supplementation provided. The analysis in Chapter 6 found no overall effect of HMB supplementation to sows on gestation length when data were combined from the control sows and the sows which had received HMB at a dose of ~ 15 mg/kg BW for ~ 15 days prior to parturition. This suggests that the effect of HMB on gestation length was only noticeable when sows were supplemented at a dose above 15 mg/kg BW or for longer than 15 days.

7.2. The effects of supplementing HMB to sow diets on litter and piglet performance

One of the main aims of this thesis was to determine the effects of HMB supplementation to sows in gestation on piglet growth to weaning and to establish the optimum dosage and duration of supplementation. HMB has been found to play a role in skeletal muscle turnover (Szcześniak et al., 2015). It has been found enhance protein synthesis through the activation of the mTOR signalling pathway (Eley et al., 2007, Wan et al., 2017) either directly or indirectly through its effect on the GH/IGF-1 axis (Tatara et al., 2007). HMB has also been found to attenuate protein degradation via the ubiquitin-proteasome pathway (Eley et al., 2008).

Supplementing sow diets with HMB over the transition period had inconsistent effects on litter and piglet performance from 24 hours to weaning in individual experiments in this thesis. Experiment 1 found that HMB increased total born litter weight, total live born litter weight and average piglet 24 hour weight in a quadratic manner with the optimum dosage identified as 15 mg/kg BW. In addition, litter and average piglet weights from sows supplemented with HMB at a dose of 15 mg/kg BW were significantly heavier at week one but not at weaning. However, supplementing sows with the highest dose of HMB (45 mg/kg BW) did not increase litter weight or average piglet weight at 24 hours or at week one.

Experiment 2 found no effect of supplementing sow diets with HMB at a dose of 15 mg/kg BW for any duration of time on litter or average piglet weights. Experiments 3 and 4 found no significant effects of supplementing sow diets with HMB at a dose of 1500 mg/kg of feed (as-fed) on total born litter weight or average piglet 24 hour weight when analysed as separate experiments or as a combined model. However, in experiment 3 there was a tendency for sows supplemented with HMB to have heavier litter weights at week one and heavier average piglet weights at week one and at weaning compared with control sows. When the data

from experiments 3 and 4 were combined, piglets from sows supplemented with HMB were significantly heavier at week one than piglets from sows that were not supplemented with HMB with a tendency for them to remain heavier at weaning.

Overall, when the data from the two treatment groups which were repeated across all four studies were combined in Chapter 6, HMB was shown to have a positive effect on total live born litter weights and average piglet weights at 24 hours. In addition, there were tendencies for litter and average piglet weights to be heavier at both week one and at weaning. Sow litter sizes are highly variable which results in large variations in litter and average piglet weights, therefore combining the results of multiple studies provides a reliable method of determining an overall effect. The lack of an effect of HMB in experiment 2 may have been because the sows were performing at a high level without supplementation. It has been established that if an animal is performing above typical standards the effect of an additive must be interpreted with caution (Bedford, 2016).

The comparison analysis in Chapter 6 used data from control sows and sows which received HMB at a dose of ~ 15 mg/kg BW for ~ 15 days prior to parturition. The inconsistencies between the results of individual experiments may be because supplementing sows with HMB at a dose of 45 mg/kg BW for 15 days, or at a dose of 15 mg/kg BW for 21 days in experiments 1 and 2 respectively, did not have beneficial effects on litter or piglet weights. This may be because these doses reduced gestation length. In addition, Wheatley et al. (2014) found that high doses of HMB were ineffective at stimulating protein synthesis. Stimulation of protein synthesis by HMB has been shown to decrease the release of other amino acids from the muscles to the blood (Holeček, 2017) and in particular, HMB has been shown to reduce plasma levels of glutamine (Holecek et al., 2009), therefore it is possible that the higher doses and the longer duration of supplementation with HMB may have interfered with the metabolism of additional amino acids such as glutamine which are needed for protein synthesis. However, more work is needed to examine this effect.

Published literature has shown inconsistences regarding HMB supplementation to sows on piglet performance (Nissen et al., 1994, Tatara et al., 2007, Flummer and Theil, 2012). Studies by Nissen et al. (1994), Tatara et al. (2007) and Wan et al. (2015) all found positive effects of HMB supplementation to sows on piglet

growth performance to weaning. However, studies by Flummer and Theil (2012) and Flummer et al. (2012) found no effects of HMB supplementation on piglet performance to weaning. The data in this thesis demonstrates that the effects of HMB on piglet performance are dose-dependent which may explain some of the discrepancies observed between studies. In addition, many of the published studies have very low replication and data in this thesis demonstrates that the variation in litter and piglet performance is large and therefore large numbers of sows may be required to detect significant differences.

The only study which found a positive effect of supplementing HMB to sows on piglet mortality was experiment 1. Chapter 6 found no overall effect on mortality level when the studies were combined. Mortality levels across all the sows used in this thesis were ~ 14 % which is slightly higher than industry figures reported over the three year period (~ 12 %) (AHDB, 2019a). However, the average numbers of piglets born alive across the experiments on the indoor unit was 14.5 piglets/litter which is higher than commercial figures reported for Great Britain at the time (~ 13.3 piglets/litter) (AHDB, 2019a). Mortality levels to weaning were relatively high in the control group of experiment 1 (19.3 %) and lower in the control group of experiment 2 (10.9 %) which suggests that the control group of experiment 1 were under performing and the control group of experiment 2 were over performing. This supports the idea that HMB may have differing effects depending on how well the individuals are performing. An overall reduction in mortality may not have been observed because the variation in mortality was so high. In the combined analysis in Chapter 6 the percentage mortality to weaning ranged from 0 to 56 % per litter and the CV was 83 %. Therefore, more sows may be needed.

7.3. The effects of supplementing HMB to sow diets on piglet birth weight and colostrum production

Average piglet birth weight is a major factor determining piglet pre-weaning growth and survival (Gondret et al., 2005). Exact birth weights were measured in experiments 1 and 3 for a smaller sample of litters. In experiment 1 supplementing sows diets with HMB significantly enhanced average piglet birth weight in a quadratic fashion; piglets from sows supplemented with HMB at a dose of 15 mg/kg BW had the highest average birth weight. However, piglets from sows supplemented with HMB at doses of 5 and 45 mg/kg BW did not have

increased birth weights. In experiment 3 piglets from sows supplemented with HMB had numerically higher birth weights than piglets from the control sows, however this was not significant which may be because larger replication was needed. As mentioned above, the effect on piglet birth weight may be due to the effect HMB has on protein metabolism (Szcześniak et al., 2015).

To our knowledge there is limited research into the effect HMB has on colostrum production in sows. Flummer and Theil (2012) demonstrated supplementing HMB to sows at a dose of 2500 mg/d for 7 days prior to parturition increased colostrum yield as measured per piglet. In agreement with this, experiment 1 found that HMB increased the colostrum yield of sows at all doses tested. Colostrum yield was highest in sows supplemented with HMB at a dose of 15 mg/kg BW. In support of these findings, experiment 3 found that when HMB was included in the diet at 1500 mg/kg of feed (as-fed) sow colostrum yield was increased. The majority of colostrum is synthesised prior to parturition (Quesnel et al., 2015). In experiment 1 sows supplemented with HMB at a dose of 45 mg/kg BW farrowed earlier than sows supplemented with HMB at a dose of 15 mg/kg BW, therefore they had less time to synthesise colostrum which may be why there was no beneficial effect of supplementing sows with HMB at a dose of 45 above 15 mg/kg BW on colostrum production. As a result of the increased colostrum yield, HMB increased average piglet colostrum intake and average piglet 24 hour gain in both experiments 1 and 3. This may offer an explanation as to why piglet performance post 24 hours was enhanced. As previously mentioned, the increase in colostrum yield may be attributed towards the lipolytic effects of HMB which cause fat mobilisation providing energy for the production of colostrum (Wilson et al., 2008, Flummer and Theil, 2012).

7.4. The effects of supplementing HMB to sow diets on immunoglobulin concentrations in sow colostrum

Pigs are born immunologically naive and so are reliant on acquired immunity from colostrum (Quesnel, 2011). Therefore, another key aim of this thesis was to determine the optimum dose and duration of supplementing sow diets with HMB on immunoglobulin concentrations in sow colostrum. HMB has been shown to have immunostimulatory effects and Krakowski et al. (2002) found that it enhanced the level of IgG in sow colostrum when supplemented to sows at a dose of 15 mg/kg BW for 21 d, from six weeks prior to parturition. This effect may

be through T cell activation by HMB. However, the study by Krakowski et al. (2002) only used five sows per treatment. To our knowledge at the time of writing no published studies have repeated this finding or examined the effect of HMB supplementation on colostrum concentrations of IgA and IgM in sows.

Experiment 1 demonstrated that HMB increased the concentration of IgG linearly up until the highest dose tested. There was no significant effect of supplementing HMB at a dose of 45 over 15 mg/kg BW. Whilst concentrations of IgA and IgM were numerically higher in colostrum from sows supplemented with HMB there were no significant effects. Experiment 2 supported these results by also demonstrating that supplementing sow diets with HMB increased the concentration of IgG in colostrum in a linear fashion up until the highest duration of supplementation, when time of sampling was accounted for. In contrast to experiment 1, experiment 2 found that HMB also enhanced colostrum levels of IgA and IgM. With regard to duration of supplementation, HMB increased IgA in a quadratic and IgM in a linear fashion. However, in experiment 3 when HMB was incorporated into a commercial pig diet during manufacture at a dose of 1500 mg/kg of feed (as-fed), whilst the concentration of IgM in colostrum was enhanced there were no effects on colostrum concentrations of IgG and IgA.

Chapter 6 showed that when the data were combined HMB had an overall positive effect on both IgG and IgM concentrations in colostrum but not on the concentration of IgA. The lack of effect on IgG concentration in experiment 3 may be due to disease pressure as there was an illness in the herd. In addition it may be due to the different sow genotype used as colostrum immunoglobulin concentrations are affected by genotype (Inoue et al., 1980). More research is needed in order to examine this.

7.5. The effects of supplementing HMB to sow diets over the transition period in combination with lignocellulose fibre and the effect of glutamine supplementation in lactation on sow, litter and piglet performance

Experiments 3 and 4 aimed to determine whether the beneficial effects HMB had on piglet birth weights and colostrum production could be optimised through additional supplements to the sow in the transition period and in lactation. It was hypothesised that supplementing sows during the transition period with lignocellulose fibre would increase the speed of parturition thus reduce piglets'

latency to suckle. Piglets from sows supplemented with a combination of HMB and lignocellulose fibre would therefore be more viable, have a shorter latency to first suckle and thus be better adapted to consume colostrum with a higher concentration of immunoglobulins. Lignocellulose fibre had no effect on farrowing duration therefore there were no additive effects of supplementing sows with a combination of HMB and lignocellulose fibre on piglet performance. Interestingly, lignocellulose fibre reduced gestation length with no negative effect on piglet birth weight. Whilst the reason for this is unclear, it would appear the lignocellulose fibre may have increased nutrient availability to the foetus thus enhancing the development of the foetuses. This may then have led to an earlier increase in cortisol levels causing the sows to farrow earlier (van Dijk et al., 2005, Vanderhaeghe et al., 2011). However, more research is needed to examine the mechanism behind this effect.

As a result of the effect of lignocellulose fibre on gestation length, piglets from sows supplemented with lignocellulose fibre were older than piglets from the control sows at week one and weaning therefore age was accounted for when analysing piglet performance data. Piglets in both experiments 3 and 4 were numerically heavier at week one and weaning than piglets from control sows. In experiment 3, when age was not accounted for, piglets were heavier at week one than piglets from sows that were not supplemented with lignocellulose fibre. In addition, when the data from experiments 3 and 4 were combined, piglets from sows supplemented with lignocellulose were significantly heavier at weaning than piglets that were not supplemented with lignocellulose fibre even with age accounted for in the model. This finding could improve farm productivity as piglets would be heavier at weaning.

The high concentration of glutamine present in milk is associated with the neonatal piglets' rapid growth and cell division requirements (Manso et al., 2012). However, the uptake of glutamine by the mammary gland from the blood is not adequate to support the synthesis of milk proteins, therefore additional glutamine is synthesised by the mammary gland (Trottier et al., 1997, Haynes et al., 2009, Wu et al., 2011). Supplemental glutamine has been shown to enhance the level of glutamine in sow milk (Wu et al., 2011, Manso et al., 2012). In addition, HMB supplementation has been found to decrease plasma levels of glutamine (Holecek et al., 2009), therefore piglets from sows supplemented with HMB in

gestation may benefit from additional glutamine in the milk throughout lactation. Therefore, it was hypothesised that supplementing sow diets with glutamine in lactation would increase the concentration of glutamine in their milk and thus improve the pre-weaning ADG of the piglets. Furthermore, piglets from sows supplemented with a combination of HMB in gestation and glutamine in lactation would have a higher ADG and weight at weaning. Whilst there were some indications that piglets from sows supplemented with glutamine had improved week one performance, the only beneficial effect of glutamine observed was an HMB × glutamine interaction with piglet week one ADG on the outdoor production system. Glutamine improved piglet week one ADG only when fed to sows which had not received HMB. However, glutamine had no effect on piglet week one ADG when fed to sows which had received HMB. It is unclear why this effect was observed but it may be that glutamine has no beneficial effects when piglets are already performing well. In addition, this effect was not observed on the indoor production system, or when the data from production systems were combined which suggests there were no beneficial effects of glutamine on overall pre-weaning performance. This may be because the basal level of glutamine in the control diet was already high enough without supplementation. In addition, glutamine concentration in milk was not measured so it cannot be confirmed whether supplemental glutamine failed to increase the level of glutamine in sow milk or whether piglets did not benefit from a higher concentration of glutamine in milk.

7.6. Conclusions

In conclusion, this research aimed to determine how piglet pre-weaning performance and colostrum production could be enhanced through supplementing sow diets across the transition period with HMB. It aimed to determine the optimum dose and duration of supplementation with HMB on piglet performance and colostrum production. Whilst the results of individual experiments were inconsistent, overall this research found that HMB improved piglet growth performance from birth to weaning when provided at a dose of ~ 15 mg/kg BW for ~ 15 days prior to parturition. In addition, this research found that HMB supplementation increased colostrum yield and colostrum intake of piglets in a dose-dependent manner. Experiments 1 and 2 combined provided evidence that the effect of HMB on immunoglobulin concentrations was dose-dependent and overall this research provided evidence that HMB increased both IgG and

IgM concentrations in colostrum but there was no overall effect on the concentration of IgA.

Experiments 1 and 2 both fed sows a pre-weighed individual amount of HMB as a top-dressing to the sows' standard diet. It was fed dissolved in apple squash to encourage intake. On a commercial farm it may be difficult to hand feed each sow an individual amount of HMB therefore incorporating HMB into the diet during manufacture provided an easy, practical method of HMB administration on farm. However, many farmers only feed one diet throughout gestation and switching to a new diet at the end of gestation may be difficult to accommodate. Therefore, more research is needed to determine adequate methods of HMB administration. With the recent increased use of precision feeding technology, it may be possible to feed each individual sow a quantity of HMB based on their body weight and over a specific time period.

Supplementing sows with 1 % lignocellulose fibre from d 110 of gestation until parturition reduced gestation length without resulting in a reduction in average piglet birth weight which ultimately resulted in heavier piglets at weaning. This finding suggests that the sow may limit piglet birth weight due to farrowing earlier if the foetuses get too big. More research is needed to determine the mechanism behind the enhanced development of the foetuses.

Providing sows with 1 % glutamine in lactation demonstrated some indications of improved piglet performance at week one which were more prominent on the outdoor production system and these require further examination. However, there were no improvements in overall pre-weaning performance. The reasons for this are unclear but it may be because the diets were wheat and barley based which are higher in glutamine concentration than corn so the basal level of glutamine was high enough without supplementation. However, as most UK pig diets use wheat and barley it was most relevant to see the effect of glutamine in diets containing these cereals.

Overall, this research highlights the potential to improve piglet growth and colostrum quality through maternal supplementation with HMB in late gestation, and the potential to improve piglet performance through maternal supplementation with lignocellulose fibre for five days prior to parturition. More

research into these areas should be conducted in order to help exploit these findings.

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Appendix A

A.1 Feed analysis

Feed analysis methods provided by Sciantec Analytical Ltd (Cawood, UK).

Crude Fibre: The sample was de-fatted and digested successively with 0.1275 M sulphuric acid and 0.313 M sodium hydroxide. The organic material which remained insoluble was recorded as crude fibre.

Crude protein: The sample was weighed into nitrogen free foil parcel and dropped into a hot furnace. It was then flushed with pure oxygen to produce rapid combustion. The combustion products were passed through filters and a thermoelectric cooler to remove water and then collected in a ballast tank and allowed to equilibrate. An aliquot of the gaseous mixture was swept through hot copper to remove O_2 and reduce NO_X to N_2. Carbon dioxide and water were removed by chemical absorption and the remaining nitrogen was measured by a thermal conductivity cell. Nitrogen content (x 6.25) was used to estimate protein content.

Oil A: The Oil was obtained by the continuous extraction of the sample with warm light petroleum, boiling range 40 - 60 °C. The solvent was removed and the dry oil weighed.

Amino acids: The sample was oxidised with hydrogen peroxide/formic acid/phenol solution, which converts any methionine to methionine sulphone and any cystine to cysteic acid, as some of the cystine and methionine would otherwise be lost upon hydrolysis. Excess oxidation reagent was decomposed with Sodium Metabisulfite. The oxidised sample was hydrolised with 6 M hydrochloric acid for 24 hours at 110°C. The hydrolysate is adjusted to pH 2.20 and the amino acids were separated by ion exchange chromatography (Biochrom instrument) and determined by post column reaction with Ninhydrin reagent using photometric detection at 570 nm.