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Evaluation of physically effective fibre in forages and its interaction with concentrate supplementation on rumen function, performance and health of UK dairy cows

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Preface

And in cattle there is a lesson for you, We give you drink, from their bellies digested food and blood, pure liquid milk for those who drink."

وَإِنَّ لَكُمْ فِى ٱلْأَنْعَٰمِ لَعِبْرَةً ۖ نُّسْقِيكُم مِّمَّا فِى بُطُونِهِۦ مِنۢ بَيْنِ فَرْثٍ وَدَمٍ لَّبَنًا خَالِصًا سَآئِغًا لِّلشَّٰرِبِينَ

Quran 16.66

Declaration

I declare that this thesis has been written and composed by the author and all the work that has been reported is original. Any work reported in this thesis has never been submitted previously to attain an academic qualification. It contains work that has been published by the author at peer-reviewed journals and international conferences.

Usama Tayyab
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List of publications:

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List of Abbreviations

ADF	Acid detergent fibre
ADL	Acid detergent lignin
AIA	Acid insoluble ash
CL	Chop length
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract or crude fat
GS	Grass silage
MS	Maize silage
MR	Mixed ration
NDF	Neutral detergent fibre
OM	Organic matter
pef	Physical effectiveness factor
peNDF	Physically effective neutral detergent fibre
PMR	Partial mixed ration
PS	Particle size
PSD	Particle size distribution
PSPS	Penn state particle separator
SARA	Subacute ruminal acidosis
TMR	Total mixed ration
VFA	Volatile fatty acids

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Abstract

It is important to feed lactating dairy cows a diet with the correct particle size distribution: too long a particle length can reduce milk yield and increase diet selection whereas too short a particle length can reduce milk fat content and possibly lead to sub-clinical acidosis. In order to characterise the particle distribution of UK rations in study 1, the Penn State Particle Separator was modified to include an additional 26.9 mm hole sieve. The majority (58%) of UK dairy farms were found to have sub-optimal or poorly mixed rations. This resulted in a different diet being available along the feed face, which could affect the performance and/or health of individual cows. There was also significant diet selection on many (66%) of herds, with cows selecting for shorter material on some farms and longer material on others. There was little evidence of an effect of mixer wagon type on particle size distribution, but there was considerable variation between farms in mixing protocol. In study 2, a short and long chop length grass silage was fed alone or mixed with maize silage (40:60 DM basis) to 16 cows in 4 periods of 28 days duration. A short chop length (31 mm) grass silage increased the intake of early lactating cows on grass or grass/maize silage-based diets compared to a longer chop grass silage (44 mm). A short chop length grass silage also increased milk production in cows fed grass silage as the sole forage, and improved body condition score and live weight gain in cows when fed as a mixed grass/maize silage-based diet. A short chop length grass silage also decreased DM digestibility and reduced milk fat concentration, but milk fat yield was unaffected. Chop length had little effect on reticulo-rumen pH, whereas the inclusion of maize silage reduced mean and minimum reticulo-rumen pH, although minimum values were above those considered to represent sub-acute ruminal acidosis. Intake, milk production, milk protein concentration and live weight gain were higher when cows were fed diets that contained a mixture of grass and maize silage than grass silage alone. In study 3, feeding cows a high maize silage based diet increased intake, milk yield, nitrogen efficiency, and acute phase proteins but decreased milk fat concentration, rumen passage rate and fibre digestibility compared to when fed a high grass based diet (with 23.6 mm mean particle size). The use of a high starch supplement increased the milk fat concentration compared to a high fibre supplementation in grass and maize silage based diets. The source of concentrate had little effect on intake, milk yield and composition, rumen pH, rumen passage kinetics, nitrogen balance and eating behaviour. In conclusion, the current particle size of UK dairy rations based on grass silage/maize silage on commercial dairy farms in the UK in longer than North American recommendations and will reduce intake and could promote diet sorting. A short chop length grass silage increases intake and milk performance when fed as the sole forage

and milk production and cow performance can benefit from replacing a proportion of grass silage with maize silage, irrespective of the chop length of the grass silage that had a minimal effect on rumen pH. Feeding a high starch diet tends to reduce the length of time ruminating but has no effect on rumen pH and no detrimental short-term effect on the immune response associated with rumen epithelial damage. Consistency of diet mixing, and reduce diet selection may have more of an impact on rumen fermentation than diet composition.

CHAPTER 1: Literature review

1.1. Introduction

The United Nations have predicted that the current world population will increase by 76 million people per year, reaching 9.1 billion by 2050 (Steinfeld et al., 2006). The demand for meat and dairy products will therefore increase. The largest demand for animal products is dairy products, followed by swine (FAO, 2011). It has been predicted that the demand for dairy products will increase to over a billion tonnes consumed per year by 2050 (FAO, 2011). This level of production cannot be maintained sustainably or economically using current farming practices (van Bruchem et al., 1999). Farming has had to intensify to support the current population's demand for food, however options to increase land area for crop production in order to provide animal feed have been restricted (Wirsenius and Berndes, 2010). Indeed, it is no longer environmentally sustainable to increase the area of land used for farming, as doing so will further aggravate changes contributing to climate change, and with an increasing population, arable land is restricted (Steinfeld et al., 2006). In order to support the needs of the world's population, farming will have to intensify so that more products are produced more rapidly from the same amount of land, while still being affordable for all (FAO, 2011).

The increased milk production of dairy cows in many Western countries such as the United Kingdom has required an increase in the level of concentrate supplementation and the production of high-quality forages, with a trend towards lower dietary fibre levels (March et al., 2014). The consequences of these dietary changes include an increased risk of metabolic disorders such as sub-acute ruminal acidosis (SARA), displaced abomasum, milk fat depression, reduced fibre digestion and laminitis (NRC, 2001; Plaizier et al., 2008). The particle size of the diet has been proposed as a key factor, along with forage fibre and non-forage carbohydrate concentration to ensure a healthy rumen function and maintain animal performance (Zebeli et al., 2012a). A shorter forage particle size is associated with improved compaction in the clamp and can result in reduced aerobic spoilage at feed out (McDonald et al., 1991) and may increase DM intake, due to reduced rumen fill and increased fibre digestibility (Thomson et al., 2017). However, too short a forage particle length can increase the rate of volatile fatty acid (VFA) production in the rumen, reduce rumination time, and decrease the production of saliva (Tafaj et al., 2007), with the consequence of inhibiting cellulolytic bacteria activity and increasing the risk of SARA (Tafaj et al., 2007). In contrast, a longer particle size produced a higher milk fat concentration (Mertens, 1997), but can also promote feed

sorting, resulting in some cows receiving excess concentrates and others insufficient (Kononoff and Heinrichs, 2003ab).

Numerous studies have evaluated the particle size of lucerne and maize silage (MS) on performance, rumen function and behaviour in dairy cows, but there is less research on perennial ryegrass silage (Kononoff et al., 2003; Yang and Beauchemin, 2006; Yang and Beauchemin, 2007). The current guidelines for feed particle distribution are primarily based on North American rations that consist of MS and lucerne haylage (Eastridge, 2006), and may therefore not be suitable for the typically wetter (e.g. less than 30% DM) MS and GS commonly fed in Northern Europe (Møller et al., 2000).

1.2. Fibre, definition, characteristics and classification

The carbohydrates (saccharides) are hydrated carbon molecules comprised of carbon, hydrogen and oxygen (Pommerville, 2012). Based on their molecular weight, carbohydrates are classified into four groups, monosaccharides, disaccharides, oligosaccharides and polysaccharides (Sharon and Lis, 1993). The mono and disaccharides, due to low molecular weight, are often referred to as sugars (Nelson et al., 2008). The carbohydrates are often referred to as a group of sugars, starch and cellulose (Sharon and Lis, 1993). In plants, carbohydrates are the main plant structure after water, and can be divided into intra-cellular/cell-contents and cell wall carbohydrates (Pommerville, 2012; Figure 1.1).

Fibre, a polysaccharide, can be described as the structural polymer present in plant cell wall (Keegstra et al., 1973). It contains polysaccharides (hemicellulose, cellulose, galactans, pectin, gums and mucilage), lignified nitrogenous substances, minerals and polyphenols (Dhingra et al., 2012). The different fractions of fibre can be classified into two groups, based on their fermentation ability and water solubility (Table 1.1).

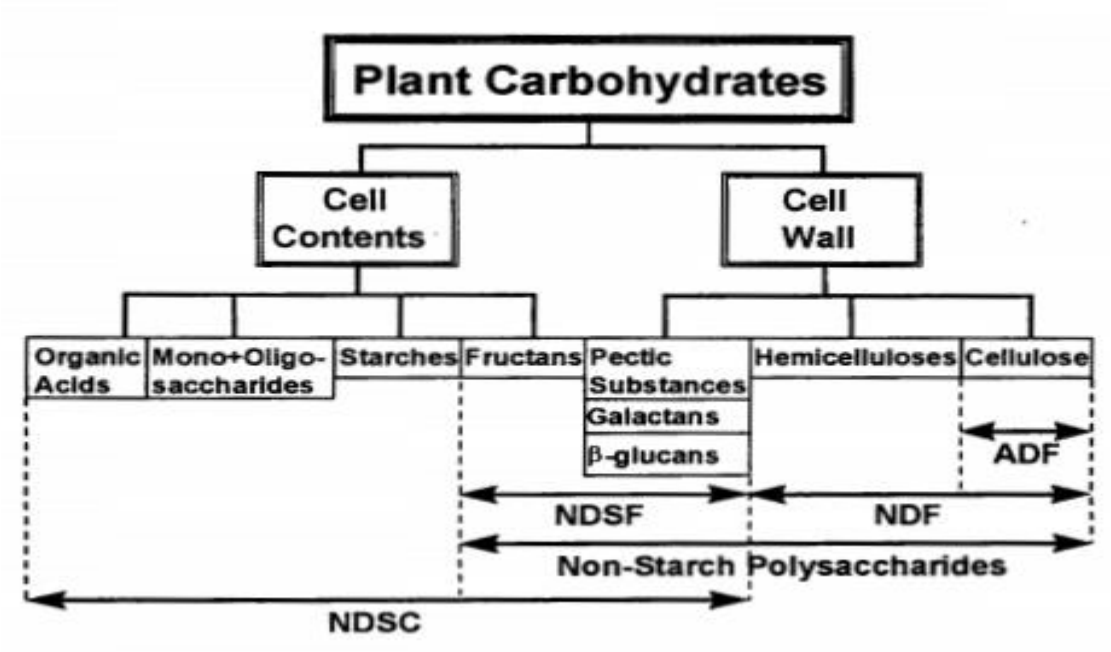

Figure 1.1. Different fractions of plant carbohydrates (Hall et al., 1999). NDSC; neutral detergent soluble carbohydrates, NDFS; neutral detergent soluble fibre, Lignin is part of NDF (neutral detergent fibre) and ADF (acid detergent fibre) is not shown here.

Table 1.1. Plant fibre classification based on solubility (Dhingra et al., 2012)

Water solubility	Component of fibre	Description	Feedstuff source
Soluble	Pectin	Primary cell wall component, composed of D-galacturonic acid	Legumes, sugar beet
	Gums	Secreted by plants after injury	Guar, seaweeds
	Mucilage	Protect seed endosperm from desiccation	Plant extract
	Fructans		Grasses
Insoluble	Cellulose	Long chain cell wall polysaccharide, composed of glucose molecule with β 1-4 glucosidic linkage	Forages
	Hemicellulose	Cell wall component, short chain polysaccharide composed of glucose, xylose, mannose and galactose molecules with β 1-4 glucosidic linkage	Cereal grains
	Lignin	Complex cross-linked polymer of phenyl propane, non-carbohydrate part of cell wall	Mature plants

Cellulose, the main structural component of the cell wall, is made up of long chains of glucose that are linked by β 1-4 glycosidic bonds (Dhingra et al., 2012; Keegstra et al.,

1973). The β 1-4 linkage is resistant to the animal's digestive enzymes but can be hydrolysed by ruminal cellulolytic bacteria (Van Soest, 1994). Hemicellulose is a polysaccharide, formed by polymers comprised of xylose, galactose and mannose (Van Soest, 1994). Pectin is composed of galacturonic acid that is highly digestible by mammalian's enzymes. Lignin, a phenyl-propanoid polymer, is completely indigestible in animals and its concentration in plants varies from 2 to 12% of the DM, and this concentration increases as plants mature (Van Soest, 1994). The lignified proteins present in the cell wall ensure structural integrity, along cell wall fibre and their concentration in plants can reach up to 15% (Nelson et al., 2008). These lignified proteins are divided into four main groups: proline, glycine, proteoglycans and hydroxyproline (Dhingra et al., 2012). Calcium carbonate and silica are the main minerals linked to the cell wall (Van Soest, 1994). All these above-mentioned components of fibre provide rigidity and fragility to the plants (Sharon and Lis, 1993).

According to Keegstra et al. (1973) and NRC (2001) the most important components of fibre found in plants are cellulose (40-45% of the cell wall, 15-40% of total plant DM) and hemicellulose (12-25% of total plant DM). However, the concentration of cellulose and hemicellulose is species specific, and depends upon the age of the plant (Van Soest, 1994). The nutrient quality of plants decreases as they mature (Nelson et al., 2008). However, the decline of plant nutrients is different for legumes and grasses, the two main plant families. The nutrient decline due to maturity within different parts of the same plant also varies (Van Soest, 1994). The stems of legumes provide structural support, and are generally more lignified than the leaves (Dhingra et al., 2012; Van Soest, 1994). With aging, stems become more lignified than leaves. On the other hand, the leaves of grasses have both functions of support and metabolism, and with maturity both stem and leaves become more lignified (Nelson et al., 2008).

The proximate analysis (also called Weende analysis) was developed by Henneberg and Stohmann in 1860 to quantify the macronutrients in the feed (Van Soest, 1994). The crude fibre analysis in Weende's system had many shortcomings e.g. fat free samples are required for analysis, fibre fraction is not completely disintegrated from other feed components, hence resulted in significant errors in the fibre estimation of the feedstuffs (Van Soest et al., 1991). Goering and Van Soest (1970) proposed another method for quantitative evaluation of various fibre fractions of the cell wall, that was widely accepted and still in use. This method characterises fibre into three fractions, the neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) content of the feeds (Figure 1.1). Later, the fibre analysis method of Goering and Van Soest (1970) and

Van Soest et al. (1991) was further improved by Mertens (2002) using heat stable enzymes for starch removal and sodium sulphite for protein disintegration from cell wall, and fibre content was expressed exclusive of residual ash.

The neutral detergent fibre is a collective measurement of the total cellulose, hemicelluloses, lignin and cutin contents (McDonald et al., 2011). It is determined by submitting the feed to a treatment with detergent solution and a chelating agent at neutral pH (Van Soest et al., 1991). The acid detergent fibre is composed of mainly cellulose, lignin, and cutin and is determined by treating the feed with a diluted acidic solution (sulphuric acid) and chelating agents (Van Soest et al., 1991). The acid detergent lignin accounts for lignin and cutin content and is determined by treating the feed with a concentrated solution of sulphuric acid (Van Soest et al., 1991).

1.3. Anatomy and physiology of the ruminant digestive system

Fibre forms a major portion of livestock feed which, due to its physio-chemical nature, is indigestible in the small intestine but is digested through fermentation in the rumen and hindgut to yield energy (VFA) (Table 1.2; Van Soest, 1994). By symbiosis with specific microflora (bacteria, protozoa and fungi), ruminants are capable of digesting fibre using beta glucanases secreted by microbes and releasing the energy contained in plant cell walls (Van Soest, 1994). Plant cell walls are the main components of the ruminant's diet, and not only serve as a nutrient and energy source, but also a regulatory factor determining feed intake and ensuring healthy rumen function (Forbes, 2007).

Table 1.2. The mean nutrient composition of rations used on commercial dairy farms in Canada (Sova et al., 2013).

Nutrient	Amount (g/kg DM)
Organic matter	921
Ash	79
Crude protein	165
Neutral detergent fibre	313
Acid detergent fibre	205
Non-forage carbohydrates	412
Net energy (Mcal/kg)	1.7

Based on their feeding habits, ruminants are divided into two groups, browsers or concentrate selectors (goats) and grass roughage consumers (buffalo and cow) while sheep are placed as intermediate between the two types (Fisher, 2002). Ruminants derive energy from cell wall carbohydrate (fibre), non-fibre carbohydrates (starch and

sugar), protein and fat (Van Soest, 1994). The major task of feeding the ruminant is to find the correct balance of these feed components to promote rumen health and to maximise feed energy intake and gain economic advantage by using a larger amount of forages and fibrous by-product feedstuffs (Vandehaar, 1998). To be successful, a maximum level of energy intake must be maintained in order to provide for maximum production (Forbes, 2007).

1.3.1. Rumen physiology

The ruminant's digestive system begins at the mouth (buccal cavity with salivary glands including parotid, mandibular, and sublingual glands secreting saliva), followed by the oesophagus, a stomach comprised of four compartments (the rumen, reticulum, omasum and abomasum), and the small intestines, caecum, and ending up with the large spiral colon and rectum (Hungate, 1966; Figure 1.2). The whole complex stomach occupies 75% of the total abdominal cavity space in mature ruminants (Van Soest, 1994). The reticulum and rumen are often considered as one single chamber and referred to as the reticulo-rumen, which contains 73% of the total stomach volume in adult dairy cattle (Van Soest, 1994). The reticulo-rumen is also considered as a fermentation vat and absorption chamber, and the ingesta can move freely between the two compartments (Van Soest, 1994). Approximately 50% of all feed digestion occurs in the rumen (Hogan and Weston, 1967). The rumen wall is comprised of small finger like projections called papillae, which enhances the nutrient absorption process by inflating the surface area (Squires, 2010; Van Soest, 1994). The reticulo-omasal orifice acts as a valve that controls the digesta flow from reticulo-rumen to the omasum, and this selective retention and sorting is a main function of the ridges of the reticular wall (Squires, 2010; Van Soest, 1994). All absorbed nutrients are removed from reticulo-rumen blood capillaries by three major veins; reticular, right ruminal and left ruminal vein (Krehbiel, 2014).

The rumen possesses a large population of microbes, which help in feed digestion. For example, rumen fluid contains 10^{10}-10^{11}/ml bacteria, 10^{9}/ml archaea and 10^{4}-10^{6}/ mL eukaryotes including both protozoa and fungi (Hungate, 1966; Gedek, 1991). The rumen bacterial species are usually grouped according to the substrate they ferment (Table 1.3; Cunningham, 2007). The main function of rumen microorganisms is the fermentation of plant polymers (such as cellulose, hemicellulose) and the conversion of non-protein nitrogen into microbial amino acids (Gedek, 1991). Through this process, microbes in the rumen provide nutrients, such as VFA, amino acids and vitamins which are important for meeting the nutrient requirements of the host (Hobson and Stewart, 1997).

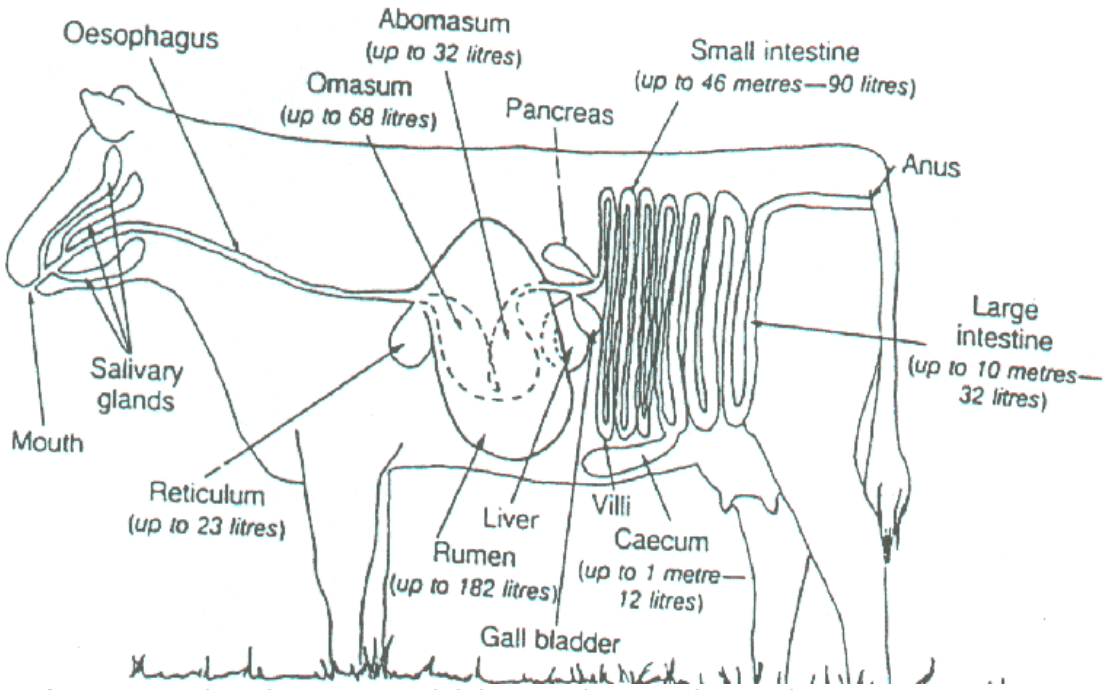

Figure 1.2. Digestive system of dairy cow (Lee et al., 1998).

The VFA (product of anaerobic fermentation) are predominately produced by microbial fermentation of carbohydrates and protein (Dijkstra et al., 1993). The ruminal concentration of VFAs is maintained by their production and absorption rates. Volatile fatty acid absorption depends upon the pH difference and concentration gradient between blood and the rumen (Dijkstra et al., 1993). According to Van Soest (1994), VFA are absorbed in a free form against the alkaline pH of blood (pH of 7.4), and active transport and diffusion are not involved in their absorption. Rumen fermentation produces considerable amounts of hydrogen ions (more than 60,000 mEq/day) which decreases rumen pH (Allen, 1997). A decrease in pH (<5.8) negatively affects fibre digesting microbes (cellulolytic bacteria) and hence fibre digestibility can be reduced (Zebeli et al., 2012), which can decrease intake and animal performance. Therefore, the maintenance of an appropriate rumen pH is necessary for the optimal performance of microbes and an efficient digestive system.

Table 1.3. Major rumen bacterial species found in adult dairy cow (Cunningham, 2007; González et al., 2014; Janssen and Kirs, 2008).

Substrate fermented	Species	Main fermentation products
Cellulose	*Bacteroides succinogens*	Succinate, Acetate, Formate.
	Fibrobacter succinogenes	Succinate, Acetate, Formate.
	Ruminococcus albus	Acetate, Formate, CO_2, H_2.
	Ruminococcus flavefaciens	Acetate, Formate, Butyrate, Lactate, CO_2, H_2.
	Butyrivibrio fibrisolvens	Acetate, Formate, Lactate, Butyrate, CO_2, H_2.
	Clostridium lochheadii	Acetate, Formate, Butyrate, CO_2, H_2.
Hemicellulose	*Ruminococcus* species	Acetate, Formate, CO_2, H_2 Succinate. Butyrate,
	Butyrivibrio fibrisolvens	Acetate, Formate, Lactate, Butyrate, CO_2, H_2.
	Bacteroides ruminocola	Acetate, CO_2, Formate, Succinate.
Pectin	*Butyrivibrio fibrisolvens*	Acetate, Formate, Lactate, Butyrate, CO_2, H_2.
	Bacteriodes ruminocola	Acetate, CO_2, Formate, Succinate.
	Lachnospira multiparus	Acetate, Formate, Lactate, CO_2, H_2.
	Treponema bryantii	Succinate, Acetate, Formate.
	Streptococcus bovis	Lactate.
Amylose	*Bacteroides maylophilus*	Acetate, CO_2, Formate, Succinate.
	Streptococcus bovis	Lactate.
	Succinimonas amylolytica	Succinate, Acetate.
Methane producing	*Methanobrevibacter ruminantium*	Methane.
	Methanobacterium formicicum	Methane.
	Methanobacterium mobile	Methane.
Sugars	*Treponema bryantii*	Succinate, Acetate, Formate.
	Lactobacillus vitulinus	Lactate, Acetate, Ethanol, CO_2, Formate.
	Lactobaccilus ruminis	Propionate.
	Butyrivibrio fibrisolvens	Propionate.
	Bacteriodes ruminocola	Lactate.
Acid	*Selenomonas ruminantium*	Butyrate, Valarate, Caprotae
	Megasphaera elsdenii	

Table 1.3. Cont'

Substrate fermented	Species	Main fermentation products
Protein	*Bacteriodes amylophilus*	Acetate, CO_2, Formate, Succinate.
	Bacteriodes ruminocola	Acetate, CO_2, Formate, Succinate.
	Streptococcus bovis	Lactate.
Lipids	*Anaerovibrio lipolytica*	Propionate, Succinate.
	Eubacterium species	Acetate, Lactate.

1.3.2. Omasum physiology

The reticulum connects with the third chamber, the omasum, a large and more functional organ (than reticulum) with a high surface area in cattle and buffalo (Fisher, 2002; Van Soest, 1994). The reticulo-omasal valve is sensitive to mechanical stimuli and controls the rumen digesta passage rate that ultimately controls the voluntary feed intake and rumination (Forbes, 1995). The omasum is the main absorption site in cattle for 30-60% of total water, 40-69% of total VFAs absorption and most bicarbonate ions (Cunningham, 2007; Van Soest, 1994).

1.3.3. Abomasum physiology

The abomasum in ruminants is often referred to as the true stomach and functions like a monogastric stomach (McDonald et al., 2011). The abomasum produces gastric juice, comprising of pepsin, hydrochloric acid and mucous, with an extremely low pH (<2.0) which is lethal for ruminal microbes and vital for protein digestion (Banerjee, 1991). Dietary proteins and microbial proteins that escapes from the rumen are partially digested here, and most protein digestion takes place in small intestine (NRC, 2001).

1.3.4. Volatile fatty acids production and absorption

Through anaerobic microbial fermentation of carbohydrates and proteins, VFA are produced in the rumen. Acetic acid, propionic acid and butyric acid are the three major VFA found in the ruminal fluid, and their production and relative amount depend on diet composition and feed intake level (Murphy et al., 1982; Sutton, 1985). According to Sutton (1985), VFA are the major energy source in ruminants and provide approximately 70% of the total energy supply.

Acetic acid (acetate) is predominately produced by fermentation of fibre by cellulolytic

and other fibre digesting bacteria in the rumen (Dijkstra, 1994). Acetate is the main energy yielding source that is oxidized in most tissues of the ruminant's body. Acetate provides acetyl CoA, a precursor needed in lipid synthesis and is essential for milk fat production (Van Houtert, 1993).

Propionic acid (propionate) is produced via concentrate (starchy diet) fermentation and is metabolised almost completely in the liver (Van Houtert, 1993). Propionate is a major source of carbon for lactose synthesis through gluconeogenesis in ruminants (Dijkstra, 1994;).

Butyric acid (butyrate) is largely converted to the ketone beta hydroxybutyrate (3-OHB) and mostly utilized as an energy source by ruminant's tissue, and used for lipid synthesis (Dijkstra, 1994; Van Houtert, 1993). Lactic acid is typically formed only when rumen pH level decreases further below pH 6 (Dijkstra, 1994).

Branched chain fatty acids including iso-valerate and iso-butyrate are collectively called iso-acids, and along with n-valerate, are produced by fermentation of proteins (amino acids) in the rumen (Andries et al., 1982). Iso-acids provide essential amino acids supply to fibre degrading bacteria and also have a positive influence on microbial fermentation (Andries et al., 1982).

A small quantity of VFA escapes the non-enzymatic chambers of the fore stomach and passes through the abomasum to the small intestine where they are absorbed (Noble, 1978). Two different kind of chemoreceptor are found in the duodenum of ruminants, one is sensitive to potassium chloride concentration and the second sensitive to VFA (Forbes, 1995), and are only sensitive to the molecular weights of VFAs. The small intestine also possesses tiny finger like projections called villi, which helps in nutrient absorption by increasing the surface area (Banerjee, 1991).

1.3.5. Saliva composition and production

There are three main salivary glands present in the buccal cavity of the dairy cow; parotid, mandibular, and sub-lingual (Table 1.4). The secretions of all these four glands is collectively called saliva, and an adult cow secretes approximately 110-180 L saliva in a day after 6-8 hours of chewing (Bailey and Balch, 1961). The production of saliva depends upon diet intake, fibre content and effective fibre of the diet that stimulates more chewing resulting in more saliva production (Bailey and Balch, 1961; Krause and Oetzel, 2006).

Table 1.4. Salivary glands of cow and type of secretion (Cunningham, 2007).

Gland	Type of secretion
Parotid	Serous watery
Mandibular	Serous and mucus mixed
Sub-lingual	Mucus

Saliva is rich in minerals especially sodium, bicarbonate and phosphate, which act as the main buffering agent in the rumen for helping to maintain a ruminal pH between 6.2 and 6.8 (Bailey and Balch, 1961). Typical composition of bovine saliva is presented in Table 1.5.

Table 1.5. Chemical composition of saliva of an adult dairy cow (Bailey and Balch, 1961)

Component	Quantity (mEq/L)
DM (%)	1.7
Ash (%)	0.8
Sodium (Na)	126
Potassium (K)	6
Phosphate (PO_3^-)	26
Chloride (Cl)	7
Bicarbonate (HCO_3^-)	126
pH	8.6

The physical form of the diet also affects saliva production, with longer chop length diet particles stimulating more saliva through more chewing (Bailey, 1958). Cows eating un-chopped hay produce six time more saliva than cows eating pelleted diet (Table 1.6).

Table 1.6. Effect of diet type and size on eating time and saliva production in an adult dairy cow (Bailey, 1958; Bailey and Balch, 1961).

Feed	Eating rate (g/min)	Saliva production (ml/min)	Saliva production (ml/g of diet)
Pelleted	356.7	241	0.68
Fresh grass	250.4	274	1.11
Silage	247.2	273	1.12
Hay	66.1	255	4.13

1.3.6. Rumen motility and rumination process

Rumination is the process of bringing food back from the rumen to the buccal cavity for further mastication, and this cyclic activity is composed of four distinct phases; a) re-gurgitation, b) re-mastication, c) re-salivation, and d) re-deglutition (Reece, 2009). The wall of the reticulo-rumen is muscular and richly innervated with intrinsic nervous

system supply that helps in coordinated digesta motility (Cunningham, 2007). The selective retention of digesta and release of indigestible residue is achieved by the reticulo-rumen motility (Cunningham, 2007). There are four distinct zones in the rumen based on the ingesta consistency and specific gravity; gas, solid, slurry and liquid (Figure 1.2a). The gas zone is created by fermentation gases, and the solid zone made up of intertwined particles of forages, often referred to as the rumen mat. The rumen mat usually floats due to buoyancy generated by gas bubbles which are produced by bacteria adhered to the mat and also air trapped in ingesta (Cunningham, 2007; Reece, 2009). The bottom of the rumen is referred to as the liquid zone, and between the liquid and solid zone there is a slurry zone, a mixture of both solid and liquid phases (Cunningham, 2007; Reece, 2009). The reticulo-rumen has various divisions or sacs, e.g. dorsal, ventral, cranial, caudo-dorsal blind and caudo-ventral blind sac (Figure 1.3b). These divisions are produced by the projection of muscular pillars into the lumen of the reticulo-rumen that aid in mixing of digesta. These muscular pillars relax and elevate during reticulo-rumen contractions and a healthy adult cow has 1-2 reticulo-rumen contractions/min (Cunningham, 2007; Waghorn and Reid, 1983). There are two patterns of reticulo-rumen motility described by Cunningham (2007); primary or mixing contractions, and secondary or eructation contractions. Primary motility starts with biphasic reticular contractions, first the size of reticulum reduces to half and in second phase the contractions are strong, nearly pumping the reticular lumen (Cunningham, 2007).

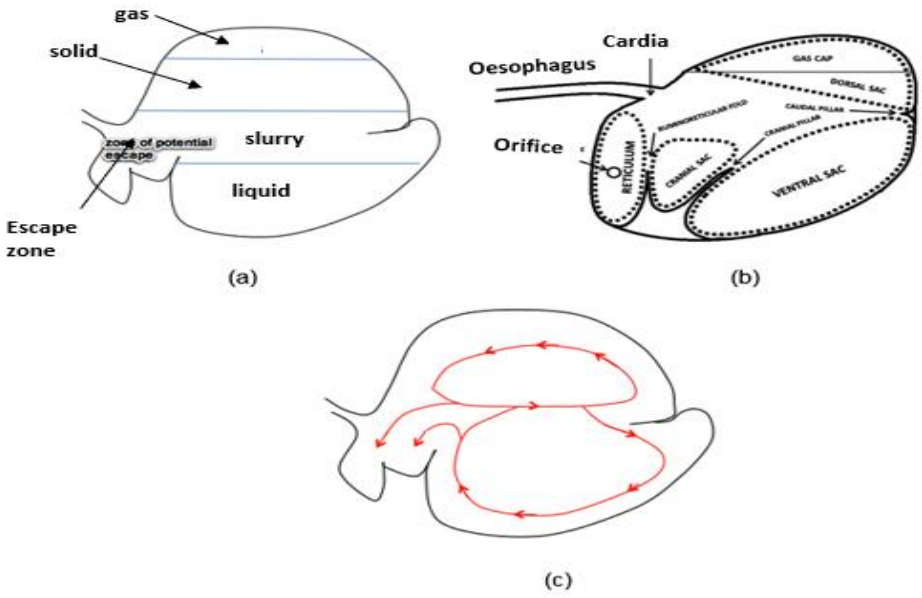

Figure 1.3. a) Stratification of ingesta in the rumen, b) rumen gross anatomy, c) movement of ingesta inside of rumen (Cunningham, 2007; Reece, 2009).

Then, the caudal peristaltic contraction of the dorsal sac starts, that is followed by caudal contraction of ventral sac. The primary contraction completes with cranial peristaltic movements of the ventral sac (Cunningham, 2007). The main functions of primary contractions are to mix ingesta and aiding in separation of large and small particles (Cunningham, 2007; Waghorn and Reid, 1983). Secondary contractions start with cranial movements of the caudo-dorsal blind sac and dorsal sac (Figure 1.3c). With the cranial contractions, gases (CO_2, CH_4) move towards the cardia, the cranial sac relaxes, and the cranial pillar elevates which allows ingesta to move away from cardia, so gases can enter into the oesophagus for eructation (Cunningham, 2007; Waghorn and Reid, 1983).

The reticulo-rumen contractions occur during eating and ruminating, and type of diet and intake level can influence the rate and strength of contractions (Waghorn and Reid, 1983). Cows eating a grass-based diet had 1.92 contractions/min in comparison to 1.77 contractions/min after eating chopped grass (Waghorn and Reid, 1983).

The process of rumination starts with an extra contraction of the reticulum, the cardia relaxes and there is an inspiratory excursion of ribs with the glottis closed (Cunningham, 2007). This contraction produces a negative pressure in the thoracic cavity that results in movement of ingesta into oesophagus from the rumen (Waghorn and Reid, 1983). Unlike monogastric animals, the oesophagus in ruminants is capable of antiperistalsis (Sisson, 1921). As ingesta reaches the buccal cavity the excess water is swallowed and re-mastication of ingesta starts (Cunningham, 2007). The duration of mastication and chewing depends upon diet type. Cows eating a 38% NDF diet spent significantly more time on rumination in comparison to cows eating a 26% NDF diet (Beauchemin et al., 2003; Moon et al., 2004). The ingesta regurgitated usually comes from the slurry zone (Figure 1.3a), which is partially digested by microbes of the solid zone. The coarse material of the solid zone first softens through soaking and undergoes microbial digestion in order to reach the slurry zone, and by rumination the partially fermented ingesta is further broken down and additional substrate is exposed for further fermentation (Cunningham, 2007). The rumination process helps in separation of smaller particles from the slurry phase ingesta, and water and fine particles are squeezed from the bolus and re-swallowed prior to mastication (Cunningham, 2007).

1.3.7. Control of reticulo-rumen motility

The reticulo-rumen motility in controlled by the central nervous system and there is a motility centre located in the brainstem that communicates with the forestomach through the vagus nerve (Cunningham, 2007; Reece, 2009). The fore-stomach is richly

innervated by the enteric nervous system, but mainly the reticulo-rumen motility is controlled by the vagus nerve (Sisson, 1921). The lumen of the reticulo-rumen monitors the ruminal distention, ingesta consistency, VFA concentration, pH, osmolality, and sends numerous signals to the brain (Cunningham, 2007; Reece, 2009). There are stretch receptors present in the reticulo-rumen wall (pillars) that monitor the physical distention (Cunningham, 2007). Any moderate increase in distention stimulates the organ motility and rumination that results in a reduction in particle size of ingesta (Cunningham, 2007). The consistency of the ingesta, which is based on type of diet, also affects the reticulo-rumen motility. Short chopped forage, grains and dry milled diets results in a lower proportion of material in the solid zone (rumen mat) and watery ingesta does not resist the reticulo-rumen pillars movement, thus relatively little force is exerted by the reticulo-rumen musculature to mix the ingesta (Cunningham, 2007). High fluid ruminal ingesta decreases the reticulo-rumen motility (Cunningham, 2007; Reece, 2009). An excess of dry, long chopped forages in the rumen, results in larger interwoven solid phase ingesta and stimulates the stretch receptors that result in a positive feedback on motility (Cunningham, 2007). Hence, diets with longer particle size stimulate motility and rumination. The reticulo-rumen motility rate is directly influenced by rate of particle breakdown, and this mechanism appears to be self-regulatory (Cunningham, 2007; Reece, 2009). Any increase in VFA concentration or decrease in the pH suppresses the reticulo-rumen motility (Forbes, 1995). The normal rumen pH range is pH 5.5 to 6.8, but this depends upon type of diet (Mertens, 1997). Osmolality has less effect on rumen motility, and normal ruminal osmolality is 280 milliosmoles, which rises during the fermentation process (Cunningham, 2007).

Concentrate rich diets are quickly fermented in the rumen and produce high concentrations of VFA (resulting in a low rumen pH) that can reduce the reticulo-rumen motility (Forbes, 1995). A high VFA concentration negatively affects fibre digestibility, and can have a negative influence on rumination that ultimately affects particle size reduction and decreases passage rate and rumen emptying (Forbes, 1995; Van Soest, 1994).

Feed particles do not leave the rumen before they are broken down into smaller size and suspended in the liquid phase (Forbes, 1995). Therefore, longer fibre particles are retained for a longer time in the rumen in comparison to chopped fibre. According to Oshita et al. (2004) the particles leaving the rumen of a high producing dairy cow are in the range of <4 mm. The particle size reduction is accomplished by re-mastication and microbial degradation (Cunningham, 2007). The physical distension of the reticulo-

rumen increases motility which increases the ingesta flow out of the rumen and potentially increases feed intake (Forbes, 1995). The increase in rumen motility starts with the reticulo-rumen contractions that push the ingesta back into mouth for re-chewing and re-mastication that results in particle size reduction, increased passage rate and increased feed intake (Forbes, 1995).

The ingesta, after sufficient fermentation and particle size reduction, enters the omasum during reticular contractions (Cunningham, 2007). The reticulo-omasal orifice enlarges during the second phase of reticulo-rumen contractions and closes shortly after cessation of contractions (Cunningham, 2007). Then, ingesta is moved to the abomasum by omasal contractions (Forbes, 1995).

1.3.8. Development of the rumen

The relative size and movement of stomach compartments in neonatal and adult ruminants are different, and with increasing age, the relative proportion of rumen, omasum and abomasum changes (Johnson et al., 1996; Figure 1.4 and 1.5). The digestive system in young bovines is both physically and functionally different to that of an adult cow. At birth, the digestive system of the calf functions like a mono-stomach animal despite having a four-compartment stomach, and this is due to the presence of an oesophageal groove that help diets to bypass the rumen, reticulum and omasum (Church, 1988; Longenbach and Heinrichs, 1998). The oesophageal groove allows calves to digest and metabolise milk-based diets efficiently via enzymatic digestion in the stomach i.e. abomasum and small intestine (Longenbach and 1998). Milk is a precious commodity and the high cost of feeding calves demands a rapid transition from the mono-stomach to ruminant animal (Gabler et al., 2000). Rumen transition is largely influenced by the type of diet e.g. dry and forage-based diets stimulate muscular development through rumen fill, and VFA stimulate rumen development, thus cereals stimulate rumen development (Baldwin et al., 2004; Harrison et al., 1960).

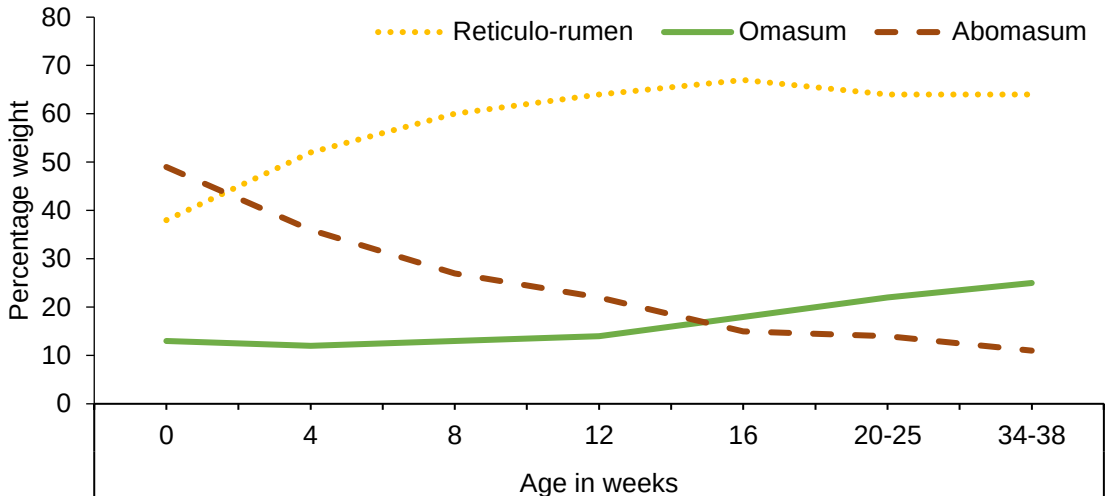

Figure 1.4. Transition of stomach compartments (% total stomach weight) in ruminants in relation to age (Warner and Flatt, 1965).

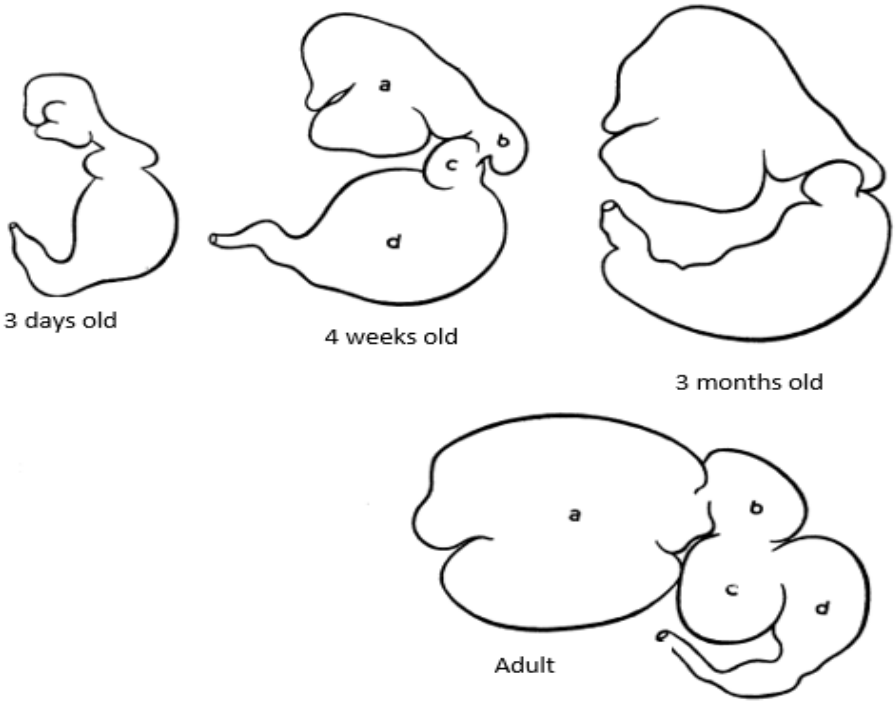

Figure 1.5. Relative size of bovine stomach compartments at various ages, a) rumen, b) reticulum, c) omasum, d) abomasum (Reece, 2009).

The size and number of rumen papillae increase with age, and at birth the rumen volume is lower, and the rumen wall is fine and transparent with limit muscle fibres development and blood vessels (Warner et al., 1956). The rumen wall is lined with squamous epithelial

cells and according to Church (1988), the growth in size and number of squamous epithelial cells results in an increase in length and width of papillae and rumen wall thickness. Both rumen papillae and wall collectively form the ruminal absorptive surface that absorbs the fermentation end-products, especially VFA (Khan et al., 2016; Warner et al., 1956). The fermentation end products such as VFA stimulate rumen transition and also maintain papilla growth and papilla function (Warner et al., 1956). However, the effect of different volatile fatty acids (acetate, propionate and butyrate) on rumen papillae is not consistent; butyrate is most stimulatory followed by propionate (Baldwin and McLeod, 2000). Consequently, different types of diet e.g. milk, forages and concentrate have varying effect on rumen papillae growth.

The provision of a forage diet with a high physical effective fibre has a significant effect on rumen volume and muscularization (Harrison et al., 1960). Similar to the adult rumen, rumen motility is simulated by particle size and physically effective fibre in the young calf (Beauchemin and Rode, 1997). The different physical forms of feed also affect rumen capacity and transition, e.g. coarse diets have been shown to increase rumen volume more than finely chopped diets (Greenwood et al., 1997).

1.4. Carbohydrates digestion and kinetics in the rumen

The rate of digestion of feedstuff in the rumen is the consequence of two competitive processes; degradation and passage rate (Mertens, 2005). The passage rate determines the amount of time feed is retained in the rumen, and the potential extent and rate of degradation determines the digestion that can occur during the retention time (Forbes, 1995). The passage of digesta through the digestive tract of ruminants is a complicated process that includes selective retention, mixing, separation and escape of short particles and liquid from the rumen before they pass through to the abomasum, small and large intestines (Dijkstra et al., 2005). The rate of passage of feedstuffs through the reticulo-rumen, small and large intestine varies, and type and size of feed particles have a great influence (Forbes, 1995). When discussing carbohydrate degradation, three distinctly different types of carbohydrates are distinguished: starch, fibre and soluble carbohydrate (Dijkstra et al., 2005). Soluble carbohydrate is a fraction defined by organic matter minus crude fat, crude protein, starch and fibre, and is highly heterogeneous (McDonalds et al., 2011). As discussed in Section 1.3.7, soluble feed components and fine particles escape from the rumen at a higher rate, while long particles are retained for longer a period of time. Ground forages and concentrates pass out of the rumen more quickly than long fibrous particles (Volden and Larsen, 2011).

The passage rate of liquids, starch in forages and concentrates, and NDF in forages and concentrates differs with level of feed intake (Figure 1.6; Volden and Larsen, 2011). The NDF has a lower rumen passage rate or higher rumen retention time and a higher intake of NDF does not increase the passage rate in comparison to starch and soluble fraction. Overall, forages have a higher rumen retention time in comparison to concentrates.

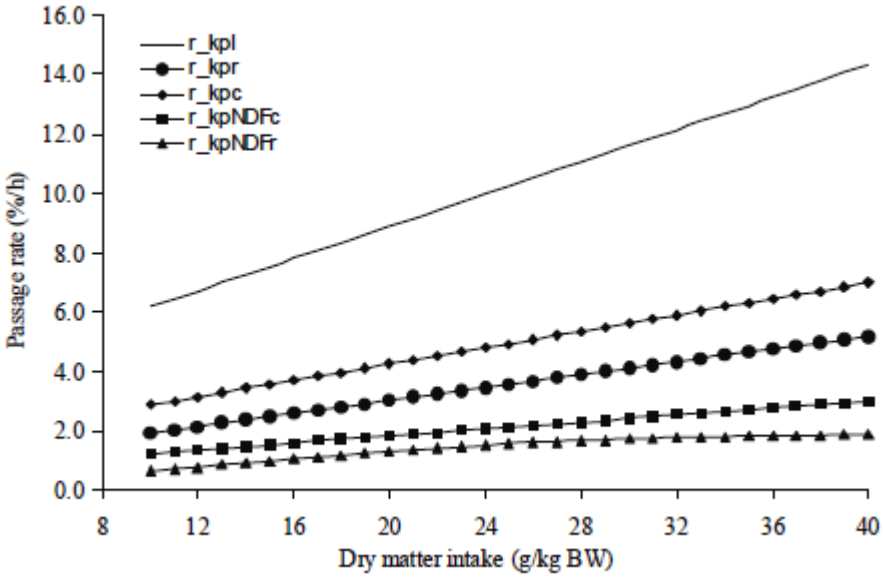

Figure 1.6. Passage rate (% h^{-1}) of liquid (r_kpl), protein and starch in forages (r_kpr), protein and starch in concentrates (r_kpc), NDF in concentrates (r_kpNDFc) and NDF in forages (r_kpNDFr) at different DM intakes (Volden and Larsen, 2011).

1.4.1. Fibre degradation

The main function of the rumen is fermentation of dietary fibre (NDF) by fibre degrading ruminal microbes (Table 1.3; Wang and McAllister, 2002). Ruminal microbes ferment the NDF through a sequential process that includes hydration, adhesion of specific microbial species to the fibre (Figure 1.7), liberation of hydrolytic enzymes and hydrolysis (Bannink and Tamminga, 2005). Hydrolysis converts the fibre into monomers that are further digested intracellularly by the microbes and results in the production of VFAs, carbon dioxide, methane and other fermentation gases (Bannink and Tamminga, 2005). The rate and extent of degradability of fibre of forages is influenced by several factors including maturity of the forage, growing season, chop length and rate of fertilizer used (Jung and Allen, 1995). These factors affect the nutrient composition and lignification of forages that ultimately influence NDF degradation.

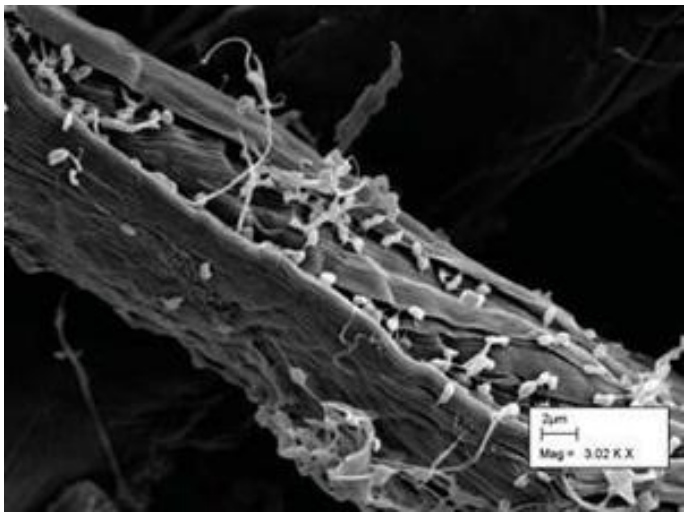

Figure 1.7. Microbes adhered to forage fibre (Weller, 2006)

The degradation of fibre in the rumen is also influenced by several factors including rumen pH, fractional outflow rate of digesta and activity and amount of fibre degrading microbes (Bannink and Tamminga, 2005). The concentration of VFA is negatively related to rumen pH as shown in Figure 1.8 (Allen, 1997). A long period of low pH negatively affects the fibre degrading microbes because most of these microbes function well in the pH range of 6.1-6.8 (Mertens,1997).

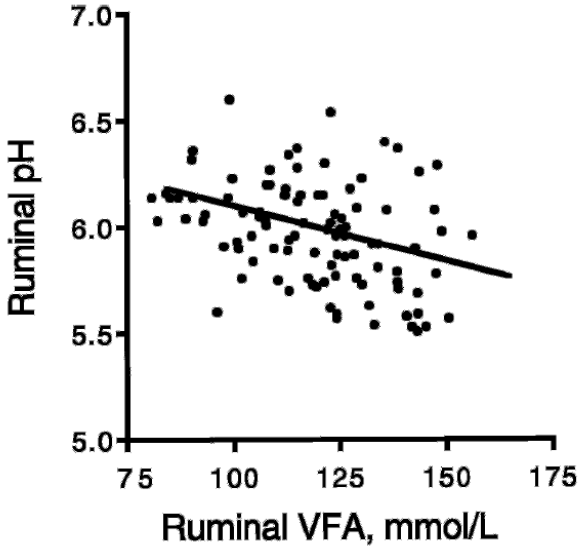

Figure 1.8. Effect of VFA concentration on rumen pH (Allen, 1997).

Fractional outflow of the rumen digesta influences the time that NDF is accessible to microbes for degradation (Pellikaan, 2004). The passage rate also influences the amount of microbes and their growth rate (Mertens, 1977). Pellikaan (2004) demonstrated a positive correlation between passage rate and rate of degradation by using ^{13}C as an internal marker for NDF. The reduction in particle size by re-mastication also influences fibre degradability. Poppi et al. (1980) proposed that the majority of particles leaving the rumen of sheep were less than 1.18 mm in size. This landmark particle size was subsequently widely used by various researchers both in sheep and dairy cow studies. Oshita et al. (2004) presented compelling evidence that particles leaving the rumen of high producing dairy cows are in the range of 4 mm size. The smaller particle size of forage fibre offers a larger surface area for microbial adherence and hence enhances degradation.

According to Baldwin et al. (1987) interactions exist between starch and fibre digesting microbes in the rumen. A higher intake of soluble carbohydrates and starch reduce the degradability of fibre in the rumen (Figure 1.9) through lowering rumen pH and also influencing the growth of fibre digesting microbes by reducing the availability of nitrogen sources (e.g. ammonia and proteins; Dijkstra et al., 2005). The rate of NDF degradability has been reported to range from 13 to 82%, and the level of NDF intake has no effect on NDF degradability (Figure 1.10; Bannink and Tamminga, 2005).

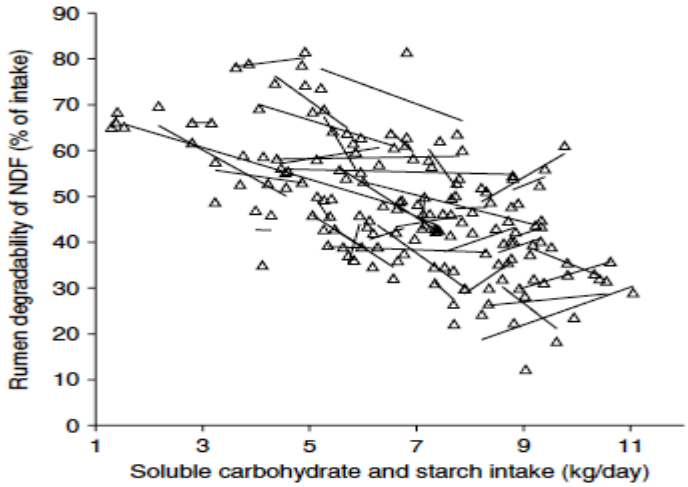

Figure 1.9. Relationship between soluble carbohydrates and starch intake and rumen degradability of NDF (Bannink and Tamminga, 2005).

The degradability of NDF reduced from 65% when no soluble carbohydrates and starch was consumed to 30% when 10 kg of soluble carbohydrates and starch consumed per

day (Figure 1.9). Despite there being no relation between NDF intake and degradability, the consumption of a high amount of NDF (more than 8 kg/day) appears to have an NDF degradability value of between 40-60%, as shown in Figure 1.10.

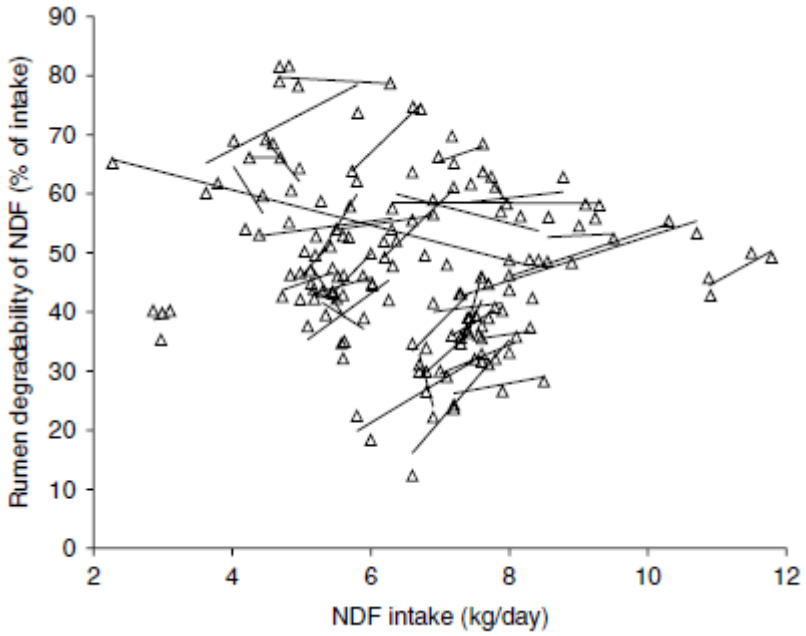

Figure 1.10. Relationship between level of NDF intake and rumen degradability of NDF (Bannink and Tamminga, 2005).

1.4.2. Starch degradation

Starch (composed of amylose and amylopectin) is a major component of grain-based concentrates in the ruminant's diet, and is a high energy dense supplement that is required by high yielding dairy cows for milk production (Svihus et al., 2005). Starch has a rapid rate of degradation in the rumen but a high passage rate that results in less fermentation in the rumen and subsequently more digestion enzymatically in the small intestine and the absorption of glucose (Mills et al., 1999; Oba and Allen, 2003). This rumen escape of starch is an important source of glucose for tissue metabolism and for lactose formation, a major component of milk (Reynolds et al., 1997).

Different sources of starch have different degradation rates, e.g. wheat-starch is more readily fermented than maize-starch (Svihus et al., 2005). The processing (pelleting, extrusion or expansion) of starch also affects starch degradability due to gelatinisation (Svihus et al., 2005). The processing at different temperatures (usually vary between 60-80℃) and moisture influences the extent of starch gelatinization, and treatment of starch with a temperature higher than 120℃ reduces starch degradation due to the Maillard reaction, a process where amino acids reacts with carbohydrates and both become less

digestible (Van Boekel, 2001). The rate of passage controls the availability of insoluble starch for the starch fermenting microbes (Mills et al., 1999). According to Bannink and Tamminga (2005) a considerable quantity of starch can be stored as polysaccharides in rumen microbes (mainly protozoa) and hence digested and absorbed in the small intestine via enzymatic digestion. The level of starch intake has a confounding effect on rumen degradability of starch (Figure 1.11); at starch intakes of less than 2 kg/day there is a decreased rumen degradability of starch, whereas intakes of 2-6 kg/day result in highly variable rumen degradation rate ranging from 10 to almost 100%. However, this variation gets comparatively smaller (40-60%) when starch intake increases above 6 kg/day as shown in Figure 1.11.

Starch and nitrogen metabolism are associated with each other in the rumen since the energy liberated from starch fermentation is used for the incorporation of nitrogen into the microbial mass (Herrera-Saldaña et al., 1990). Insufficient nitrogen availability may limit microbial growth and hence reduces both starch and fibre digesting microbial production. Ørskov et al. (1972) reported a decrease in the duodenal starch flow from 14.2 to 3.4% with an increase in protein intake from 10% to 16.5% in sheep.

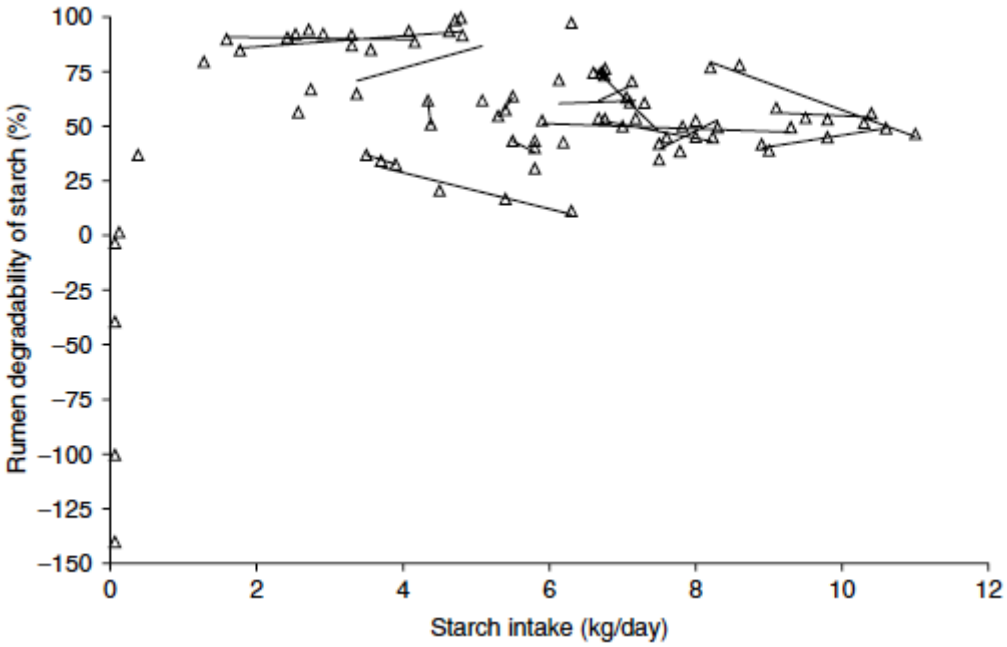

Figure 1.11. Relationship between starch intake and rumen degradability of starch (Bannink and Tamminga, 2005).

1.4.3. Soluble carbohydrates degradation

Water soluble carbohydrates include monosaccharides (e.g. glucose and fructose), disaccharides (e.g. sucrose, lactose and maltose) and fructans, and often comprise a substantial part of a cow's diet. Fructans comprises on average 70% of the water-soluble carbohydrate (WSC) of perennial ryegrass in field conditions (Gallagher et al., 2007; Pollock and Jones, 1978). In the Cornel Net Carbohydrate and Energy System, mono- and disaccharides, collectively called simple sugars have a high rate of degradation (300%/h) in the rumen (Russel et al., 1992; Sniffen et al., 1992). However, the rate of degradation between different sugars (glucose, sucrose and lactose) varies and lactose has a lower rate of hydrolysis (540%/h) than glucose and sucrose (Figure 1.12). The higher rate of degradation of simple sugars is probably due to their high water solubility (Khezri et al., 2009).

With a higher rumen degradation rate of glucose (728%/h) and a fractional passage rate of 15%/h, it can be calculated that only 2.02% [15/ (15+728)] of ingested glucose will escape rumen degradation and enter the small intestine. As a consequence of the rapid rate and extent of fermentation of sugars, a higher intake results in a rapid decrease in rumen pH (Figure 1.13). Khezri et al. (2009) replaced starch with different levels of sucrose in cows' diet and reported that 7.5% sucrose resulted in a rapid decline in rumen pH in comparison to a diet having only starch. This demonstrates that sugars degrade at different rates and have different effects on rumen function.

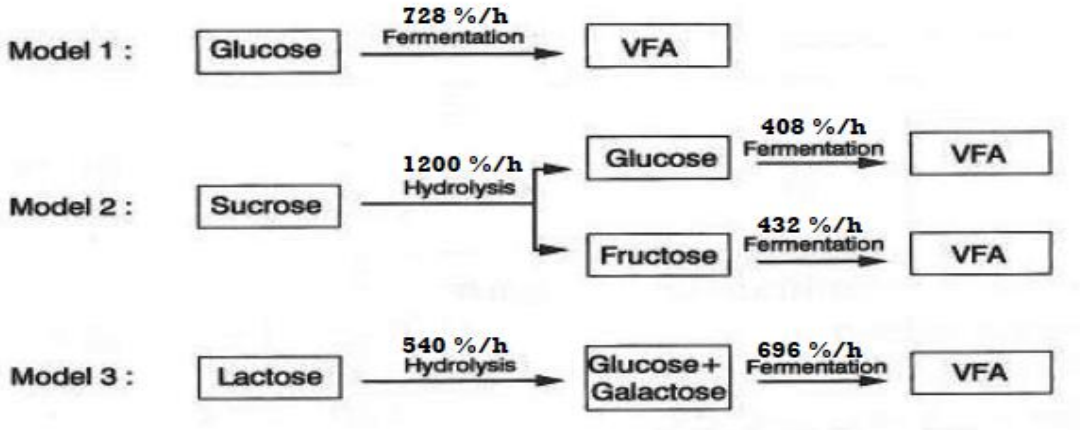

Figure 1.12. Rate of rumen fermentation (%/h) and hydrolysis (%/h) of glucose, sucrose and lactose (Weisbjerg et al., 1998).

Since, a small quantity of starch and glucose escapes the rumen and is digested in the duodenum (less than 10% of cow requirements, Donkin and Armentano, 1995), the main source of glucose production is gluconeogenesis in the liver that uses the VFA propionate as the major substrate (Chow and Jesse, 1992; Overton et al., 1999).

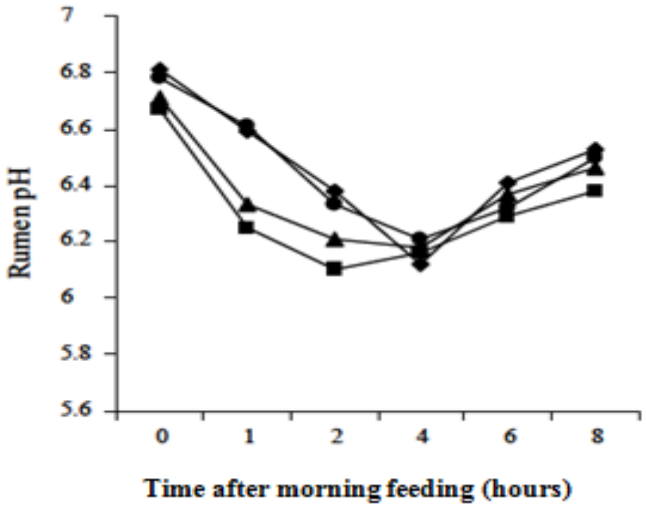

Figure 1.13. Effect of different levels of sucrose (0% ●, 2.5%♦, 5% ▲ and 7.5% ■) intake on rumen pH (Khezri et al., 2009).

1.5. Physically effective fibre concept

The term physically effective fibre (peNDF) was first defined by Mertens (1997) as the fibre fraction that stimulates chewing and forms a floating mat of large fibre particles in the rumen. The particle size of forage and feed has been the primary physical characteristic of peNDF, and is also required to produce a ruminal fibre mat, that helps to retain smaller forage particles, thus increasing their digestion (Zebeli et al., 2006b). In other words, peNDF is the amount of fibre that is large enough to stimulate regurgitation and chewing in dairy cows. The physically effective fibre is described as the amount of a feedstuff's NDF and its physical effectiveness factor (pef), where pef is amount (% proportion) of feedstuff's particle size larger than a size that is considered physically effective for cows (Mertens, 1997). Mertens (1997) proposed that particle size of 1.18 mm is critical for dairy cows and stimulates rumination. The 1.18 mm was considered as the threshold particle size for sheep that was required to prevent particles escaping the rumen and less than 5% of faecal particles were larger than 1.18 mm (Poppi et al., 1980). The first method to calculate the physical effectiveness factor was proposed by Lammer et al. (1996) and consisted of the amount of feedstuff retained on top two screens (8-19 and >19 mm) of the Penn State Particle Separator (PSPS). Kononoff et al. (2003)

proposed the physical effectiveness factor is the sum of particles retained on 19, 8-19 and 1.18 mm sieves of PSPS. Both threshold levels were used widely to calculate the physical effectiveness factor and peNDF for both dairy cows and sheep without taking their production levels into account. However, Oshita et al. (2004) reported that the particles leaving the rumen of a high producing dairy cow are longer than 1.18 mm and are in range of 3-4 mm. There is no ideal particle size for all diets and forages, and recent studies showed that a 4 mm sieve size procedure is more accurate for calculating physical effectiveness factor and peNDF for the high producing dairy cows (Maulfair and Heinrichs, 2013; Kmicikewycz et al., 2015). The peNDF is estimated as the sum of the amount of diet retained on >4 mm screen and multiplying with the diet's NDF content. The physical effectiveness factor of feedstuff varies from 0 (finely ground maize) to 100 (coarse hay). Previously, Sudweeks et al. (1981) introduced the roughage value index and Sauuvant et al. (1990) proposed a fibrosity index; both indexes were expressed as chewing (min/kg of DM). However, peNDF is different from these two indexes, as it is based on the measured NDF content of forage and the effectiveness of NDF to promote chewing (Mertens, 1997).

Forages with a very lower particle size (lower peNDF) do not stimulate chewing activity and result in a lower ruminal pH, and decreased activity of cellulolytic bacteria (Mertens, 1997). A shorter chop length silage is however, often desired by farmers and contractors to improve compaction in the clamp and reduce aerobic spoilage at feed out (McDonald et al., 1991). If the peNDF of a diet is too high (larger particle size, >25 mm), it will decrease the digesta passage rate, decrease fibre degradation owing to a reduced surface area, contribute to rumen fill that decreases feed intake (Zebeli et al., 2012a), and promote sorting of feed by the cow (Kononoff and Heinrichs, 2003). The use of a longer chop length achieved by a different mixing protocol, reduced dry matter intake (DMI) and milk yield in comparison to a control, even though both rations had an identical chemical composition (Humphries et al., 2010)

Achieving the correct particle size and peNDF in a ration can be reflected in the maintenance of a better environment for the growth of rumen microbes, a more efficient degradation of fibre, and as a consequence, an increase in milk fat content in dairy cows (De Brabander et al., 2002; Mertens, 1997). Additionally, more microbial protein synthesis in the rumen is likely to be translated into greater metabolisable protein supply to the small intestine and therefore enhancement of milk protein levels (Sinclair et al., 2014). There are inconsistent results reported in the literature relating to the influence of particle size and peNDF on intake, as shorter particle size may enhance intake by

reducing rumen fill (Zebeli et al., 2007), or conversely may have little effect or reduce intake if a consequence is a depression in ruminal pH (Maulfair and Heinrichs, 2012; Zebeli et al., 2012a). In contrast, there is little data to support an effect of particle size on milk yield, although there is some evidence of an improvement in body energy balance with reduced particle size (Moharrey, 2010; Teimouri et al., 2004). The effects of particle size and peNDF in dairy cow studies are however, complicated by both the level of inclusion and rate of degradability of supplementary concentrates. For example, the dietary response might be completely different when wheat is fed instead of maize, even if the diet contains the same content of peNDF (Zebeli et al., 2010). Current descriptions of peNDF do not include differences in the fermentability of feedstuffs, and there is therefore a requirement to incorporate the rate of degradation of all components of the diet along with particle size and peNDF in dietary recommendations.

1.6. Methods to measure peNDF

The particle size of the diet has been recommended as a key factor along with NDF concentration and non-forage carbohydrate concentration for maintain optimum rumen function (Zebeli et al., 2012a). The precise estimation of particle size for forages used in dairy rations is arduous. In the literature, there have been various methods proposed to analyse feed particle distribution using different sieving methods, and there is no accepted standard. The use of different methods to analyse peNDF makes it difficult to compare the results from different studies. Murphy and Zhu (1997) used nine different methods for the comparative quantitative assessment of particle size of alfalfa haylage, corn silage and concentrate mixtures, and observed inconsistencies in the results. They concluded that particle size analysis is affected by method and type of feed. Murphy and Zhu (1997) divided different procedures into dry and wet sieving methods (Table 1.7). All these methods used separators to separate forage particles based on their length and size. Based on previous studies there are four main particle separators (ASABE Particle Separator, Penn State Particle Separator, Ro-Tap Particle Separator and Z-Box Particle Separator) used by various researchers mainly in the United States (Maulfair and Heinrichs, 2012). Maulfair and Heinrichs (2012) reviewed these methods and concluded that all these separators have their advantages and disadvantages, and the use of each separator depends upon sample type and the hypothesis tested.

Table 1.7. Methods to measure particle size reported in literature (adapted from Murphy and Zhu, 1997).

Method	Description	Reference
Dry sieving		
1	Sample was dried (100°C) after treating with NDS[1], hexane and acetone. Separated by decreasing pore size sieves with mechanical vibration on shaker.	Smith and Waldo, 1969
2	Intact sample separated by decreasing pore size sieves with mechanical vibration on shaker.	Woodford and Murphy, 1988
3	Used vibrating screens for alfalfa haylage and maize silage, while sieve shaker for concentrate mix.	Finner et al., 1978
Wet sieving		
4	Intact sample were flushed with water from top sieve till no visible particle moved, same flushing repeated at each screen when removed and later sample dried at 55°C.	Woodford and Murphy, 1988
5	Intact samples were soaked in artificial saliva, then wet sieving and samples retained on screens dried at 100°C.	Waghorn et al., 1986
6	Samples soaked in water prior to wet sieving, sieves were hanging in water and shaken vertically and later sample on screens dried at 105°C.	Poppi et al., 1981
7	Concentrate mix treated with α-amylase and squeezed with NDS, maize silage squeezed with NDS only and alfalfa haylage without any treatment, were sieved through 20.3 cm screen with vacuum suction and oscillation besides sprinkling water and later sample dried at 60°C.	Allen et al., 1984
8	German system: Samples soaked overnight in detergent solution, sieved with water spray and vibration and later sample dried at 103°C.	Grenet et al., 1984
9	Modified German system: intact sample mixed in water before sieving and water flow was increased than Method 8, afterwards sample dried at 80°C.	Moseley et al., 1984

[1]NDS: neutral detergent solution.

The ASABE (American Society of Agricultural and Biological Engineers), also called the Wisconsin particle separator is the gold standard method to measure peNDF of chopped forages (ASABE, 2007). It is a very large mechanically operated separator, and possesses a pan with five screens of size 19, 12.7, 6.3, 3.96 and 1.17 mm (from top to bottom, respectively). The advantages of this separator are that mechanical operation reduces human error, the moderate number of particle fractions, the greater surface area, and the fact that it can be used for fresh forages (Maulfair and Heinrichs, 2012). The disadvantages of this separator are that it is least portable, requires electricity and is extremely heavy (Maulfair and Heinrichs, 2012). Mostly this separator is used in laboratories.

The Penn State particle separator (PSPS) is a portable, manually operated on-farm tool developed by Lammers et al. (1996) that is based on the ASABE particle separator. The PSPS originally consisted of two screens of 19 and 8 mm sizes that resulted in three particle fractions. Later, Kononoff et al. (2003) added a 1.18 mm screen to separate the <8 mm fraction more efficiently for better characterisation of forages with a large <8 mm fraction. Kononoff et al. (2003) proposed the shaking procedure by setting the PSPS on a flat surface, five times horizontal shaking by hand at a shaking frequency of 1.1 Hz (66 shakes in 1 min) with a stroke length of 17 cm to ensure reproducible results. The same procedure is then repeated after a quarter turn of the separator, with a total of eight sets of five shakes to accomplish a total of 40 shakes in two full turns (Lammers et al., 1996). The PSPS is now the most popular tool worldwide due to its low cost, ease of use, good repeatability and ability to be used for on-farm fresh forages (Maulfair and Heinrichs, 2012). The disadvantage is the fewer fractions (compared to ASABE) and human error during manual shaking, although, human error can be minimised by placing the PSPS on a smooth steady surface (Kononoff et al., 2003). The moisture content of forages and shaking frequency can affect the results of particle size distribution analysed by PSPS (Kononoff et al., 2003). Therefore, to use the PSPS, Maulfair and Heinrichs (2012) recommended that the shaking protocol should be standardised and the variation in moisture content minimised. Several methods of using the PSPS as an on-farm tool for measuring peNDF have been proposed: i) measuring the fraction of particles retained on the >1.18 mm screen (Kononoff et al., 2003), ii) combining the particles retained on the top two screens (19 and 8 mm screens) plus half the particles in the pan, and iii) combining the particles retained on the top two screens (19 and 8 mm screens; Hutjens, 2001).

The Ro-Tap particle separator (RTPS) was developed by Mertens (1997) and contains a series of stacked sieves where the samples are shaken horizontally and vertically with a metal arm which repeatedly taps the top screen. The sample retained on the >1.18 mm screen is multiplied with original sample NDF to calculate peNDF. This method is out-dated now (Maulfair and Heinrichs, 2012). The RTPS contains a screen size of 19, 13.2, 9.5, 6.7, 4.75, 3.35, 2.36, 1.18, 0.6 and 0.3 mm, and these screens are made up of wires (Mertens, 2005). The Ro-Tap only uses a 24 h dried sample, and employs vertical shaking that is different from the ASABE and PSPS, and drying of forages results in smaller and more fragile particles (Kononoff et al., 2003). This technique is time consuming, less portable, and expensive due to sieve cost, while advantages are various particle fractions, mechanically operated and screen sizes that can be customised

(Maulfair and Heinrichs, 2012). The RTPS is used mostly for research purposes and by forage testing labs.

The Z-box particle separator was developed recently by the Miner Agriculture Research Institute (NY, USA) to measure the physically effectiveness factor (pef) of an as-fed TMR and forages (Maulfair and Heinrichs, 2012). Various screen sizes and shaking combinations (vertical or horizontal) were tested in the Z-Box and results were compared with the PSPS. Cotanch and Grant (2006) proposed a 3.18 mm screen size to calculate the pef of maize silage and TMR, with the sample being inverted and forcefully shaken vertically 50 times. The Z-box is a small, portable, cheap, manually operated tool with customised sieves (Maulfair and Heinrichs, 2012). The disadvantage of the Z box is the small sample size (up to 50 g), which potentially increase sampling error (Maulfair and Heinrichs, 2012).

In view of the above discussion, the PSPS and Z-box methods are recommended for on-farm use to measure peNDF of as-fed forages and TMR. However, the PSPS is better correlated with chewing activity due to the use of a large on-farm sample size (Maulfair and Heinrichs, 2012), and also horizontal shaking is preferred because of particle separation at their longest diameter (Mertens, 1997). These recommendations are however, primarily based on comparatively dry North American style diets consisting of alfalfa haylage and corn silage, and may not be suitable for the range of grass and maize silages DM commonly encountered in the UK.

1.7. Importance of particle size and its effect on animal production performance

1.7.1. Effect of particle size on intake

There are inconsistent effects of particle size on feed intake of dairy cows in the literature. An increase in DM intake was reported when dairy cows were fed a short forage particle size (Zebeli et al., 2012a; Figure 1.14). Kononoff et al. (2003b) also reported a 2.3 kg/day higher DM intake in cows when the particle size of maize silage decreased from 8.8 to 7.4 mm. Similarly, other studies have also shown that feeding a shorter particle size can result in an increase in intake in dairy cows (Alamouti et al., 2009; Tafaj et al., 2007; Zebeli et al., 2009). Contrary to these findings, other studies did not find any effect of forage particle size on the feed intake of cows (Tafaj et al., 2007; Yang and Beauchemin, 2005, 2006a, 2007). For example, there was no effect on DM intake when the particle size of maize silage was increased from 19 to 22.3 mm (Yang and Beauchemin, 2005). As discussed in section 1.4, a higher DM intake can be achieved through decreasing forage particle size due to an increased ruminal surface

area available for fibrolytic bacteria, resulting in an increased rate of digestion (Yang and Beauchemin, 2006b). However, physical rumen fill is not always a limiting factor of dietary intake in high-yielding dairy cows when fed large amounts of concentrate (Allen, 2000). Dry matter intake is controlled by various factors including the rate of digestion and passage rate through the reticulo-rumen, amount and type of concentrates in the diet, and forage source and concentration (Tafaj et al., 2007; Nasrollahi et al., 2015). In a meta-analysis of forage factors influencing intake in dairy cows, Nasrollahi et al. (2015) reported that reducing forage particle size increased DM intake when the forage proportion was greater than 50% of the DM, while intake decreased with decreasing forage particle size at lower proportions of forage in the diet.

1.7.2. Effect of particle size on chewing and rumination

Feeding a longer dietary particle size diet generally results in an increase in eating and rumination time in dairy cows (Beauchemin and Yang, 2005; Nasrollahi et al., 2016; Tafaj et al., 2007). For example, total rumination time increased by 100 min/d when the chop length of hay increased from 6 to 30 mm (Zebeli et al., 2007). The findings of a meta-analysis and meta-regression (covering 46 published experiment) by Nasrollahi et al. (2016) reported a 19 min higher eating time, a 1.1 min longer eating time/kg DM intake, a 44 min higher total chewing time, and a 3.2 min higher chewing time/kg DM intake when forage particle size was increased compared to a shorter forage particle size. Similarly, in another meta-analysis, a positive correlation between forage particle size, chewing time ($R^2 = 0.30$) and rumination time ($R^2 = 0.23$) was found (Tafaj et al., 2007). According to Kononoff and Heinrichs (2003b) particles of alfalfa silage based diets that are longer than 19 mm might be the main factor influencing the chewing activity in dairy cows, while Yang and Beauchemin (2006) proposed that the critical fraction was longer than 8 mm.

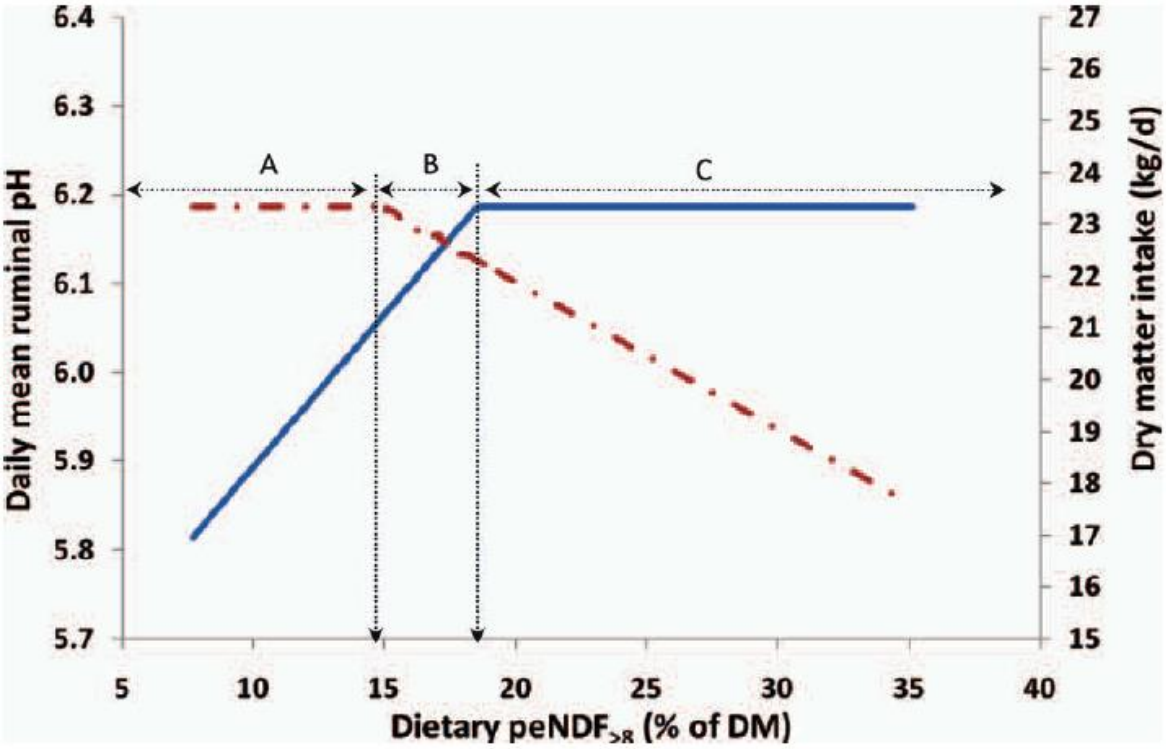

Figure 1.14. Relationship between physically effective fibre, rumen pH (blue line) and dry matter intake (red dotted line) (Zebeli et al., 2012a). Section A describes a higher DM intake when peNDF level is below 15% of DM, section B describes the optimum range of dietary peNDF (14-18% of DM) required to maintain optimum rumen pH and DM intake, and section C describes a higher peNDF maintain a high pH while reduces DM intake in dairy cows.

1.7.3. Effect of particle size on rumen fermentation

Rumen pH primarily depends on dietary composition, forage source, amount of concentrates, fermentability of concentrates and amount of fibre in the diet (Nasrollahi et al., 2016; Zebeli et al., 2012a). On a low forage diet (<50% DM), rumen pH decreased with decreasing particle size, but there was no effect when the forage proportion was high (>50% DM) (Nasrollahi et al., 2016). A weak association (R^2 = 0.14) between particle size and rumen pH was reported by Tafaj et al. (2007) compared to a stronger positive relationship (R^2 = 0.41) between dietary NDF and rumen pH, however, when diets were based on grass silage only the relationship between particle size and rumen pH improved (R^2 = 0.42) compared to when diets were composed of maize silage. Similarly, particle size had no influence on rumen pH with maize silage based diets but the relationship improved (R^2 = 0.28) when both grass and maize silages were included in the diet of dairy cows (Tafaj et al., 2007). There was no effect of particle size on the concentration of VFA or the acetate to propionate (A:P) ratio (Bhandari et al., 2008; Le Liboux and Peyraud, 1999; Zebeli et al., 2008). Contrary to this, a meta-regression study reported a

2.8 mM higher rumen VFA concentration in dairy cows when fed a shorter forage particle size (Nasrollahi et al., 2016). An interaction was also found between forage level and particle size on the A:P ratio, where the ratio decreased when cows were fed a low forage level or when particle size was reduced but increased with high forage diets were fed (Nasrollahi et al., 2016).

1.7.4. Effect of particle size on fibre digestibility and digesta passage rate

The particle size of the diet can influence fibre digestibility. Feeding a shorter chop length diet can enhance fibre degradation in the rumen, possibly by increasing the surface area for microbes to attach (Zebeli et al., 2007). On the other hand, feeding a longer chop length diet may also increase fibre degradation in the rumen, possibly due to higher retention time (Zebeli et al., 2007). Similar to DM intake, the digestibility of the diet can also be influenced by various factors including diet composition, forage level, concentrate level and composition, stage of lactation and particle size (Nasrollahi et al., 2015; Zebeli et al., 2006, 2007, 2012). A meta-regression revealed a positive correlation ($R^2 = 0.41$) between particle size and total tract fibre digestibility (Tafaj et al., 2007) and some other studies (Kononoff and Heinrichs, 2003b; Yang et al., 2002; Yang and Beauchemin, 2005) were in agreement of these findings. In contrast, other studies reported no effect of particle size on diet digestibility (Kononoff and Heinrichs, 2003a; Yang et al., 2001). A short particle size provides a greater surface area for microbes that may potentially increase rumen fermentation and results in a higher VFA concentration, reducing pH which can negatively influence fibre digestibility (Dijkstra et al., 2012; Krause and Oetzel, 2006). Tafaj et al. (2007) reported a concurrent decrease in fibre digestibility, lower chewing activity and low rumen pH following a reduction in particle size. A similar meta-regression analysis revealed an increase in fibre digestibility with decreasing particle size in grass silage based diets but not in maize silage based diets (Nasrollahi et al., 2015).

A shorter particle size of hay resulted in a higher passage rate through the gastrointestinal tract of dairy cows compared to a longer particle size (Tafaj et al., 2001). Similar to DM intake, rumen passage rate is influenced by various factors including diet composition, amount of starch as concentrate and fibre concentrations (Tafaj et al., 2007). However, previous studies have found no relationship between forage particle size and digesta passage rate through the rumen (Beauchemin and Yang, 2005; Tafaj et al., 2007). This lack of an effect of particle size on passage rate may be due to particle

size reduction by chewing and mastication that may potentially increase the rate of finer particles escaping from the rumen (Beauchemin and Yang, 2005).

1.7.5. Effect of particle size on milk yield and composition

There are few studies that have reported an increase in milk yield after decreasing the particle size of the diet (Kononoff and Heinrichs, 2003a; Nasrollahi et al., 2015), with most studies agreeing that there is no effect of particle size on milk yield (Alamouti et al., 2009; Tafaj et al., 2007; Yang and Beauchemin, 2006). The increase in milk yield that has been reported by altering particle size is mainly due to an increase in DM intake (De Brabander et al., 2002; Kononoff and Heinrichs, 2003b; Yang and Beauchemin, 2005). However, an increase in DM intake in mid and late lactation cows may favour building body reserves over milk synthesis, which may explain the lack of an effect of particle size on milk yield in other studies (Zebeli et al., 2012a).

Contrary to previous findings, a meta-regression analysis found a 0.54 kg/d higher milk yield when cows were fed shorter particle size diets compared to longer diets (Nasrollahi et al., 2015). Similarly, milk fat concentration decreased while milk protein concentration increased when cows were fed a shorter particle size diet (Nasrollahi et al., 2015). However, milk composition is less responsive to dietary particle size in early to mid-lactation cows because of negative energy balance and mobilising of body fat reserves resulting in an increase in milk fat content (Zebeli et al., 2006a). Dietary particle size influences the milk fat content only when dietary NDF levels are lower than minimum recommended levels (250 g/kg DM) for dairy cows (Mertens, 1997; NRC, 2001). Milk protein yield was reported to be increased by decreasing particle size only with maize silage based diets were fed (Tafaj et al., 2007). The physical form of forages (e.g. hay versus silage) also influenced the milk fat content, and a hay-based diets increased the milk fat and milk protein content of dairy cows compared to when fed the silage based diets were fed (Tafaj et al., 2007).

The effect of particle size of forages on dairy cows performance are summarised in Table 1.8.

Table 1.8. Summary of the literature on particle size and its effect on dairy cow performance[1].

Reference	Forage	CL or PS (mm)	DMI (kg/d)	Milk (kg/d)	Fat (g/kg)	Rum (min/d)
Beauchemin et al. (2003)	AH	10, 4	ns	ns	ns	-105
Johnson et al. (2003)	MS	40, 11.1	ns	ns	ns	-
Kononoff and Heinrichs (2003a)	MS	22.3, 4.8	ns	ns	-0.9	-51
Kononoff and Heinrichs (2003b)	A haylage	22.3, 4.8	+2.3	ns	ns	ns
Krause and Combs, (2003)	AS	19, 10	-1.7	+1.3	-1.7	-96
Onetti et al. (2003)	MS					
Einarson et al. (2004)	BS	19, 10	+1.6	ns	ns	-
Beauchemin and Yang (2005)	MS	19.1, 11	ns	-	-	-52
Rustomo et al. (2006)	AH	19, 13	ns	ns	ns	-
Couderc et al. (2006)	MS	23, 6	+1.3	ns	ns	ns
Yang and Beauchemin (2006a)	BS	9.5, 4.8	ns	-	-	-77
Yang and Beauchemin (2006b)	MS	28.6, 4.8	ns	-0.6	ns	-97.1
Bhandari et al. (2007)	MS, AH	19, 10	+0.9	ns	ns	-
Yang and Beauchemin (2007)	AS	19.1, 7.9	ns	ns	ns	ns
Cao et al. (2008)	AH	6.3, 2.5	ns	ns	ns	-42.5
Bhandari et al. (2008)	Oat silage	19, 6	+1.8	ns	ns	ns
Alamouti et al. (2009)	AH	40, 20	ns	ns	ns	ns
Yang and Beauchemin (2009)	AS	19.1, 7.9	ns	ns	ns	ns
Behgar et al. (2011)	AH	20, 5	ns	ns	-4.9	ns
Maulfair et al. (2011)	GH					
Kammes and Allen (2012)	GS	19, 10	ns	ns	ns	-23
Nasrollahi et al. (2012)	AH	30, 15	ns	-	-	ns
Kahyani et al. (2013)	AH	30, 15	+1	+1.1	-3.1	-47
Maulfair and Heinrichs (2013)	MS	47.1,	+2	ns	ns	ns
Akbari-Afjani et al. (2014)	MS, AH	15.37, 8.19	-3.9	-3.3	+2.2	-109
Kargar et al. (2014)	MS, AH	13.5, 4	ns	ns	-2.6	ns
Alamouti et al. (2014)	AH, MS	40, 20	+1.6	ns	ns	ns
Kmicikewycz, and Heinrichs (2015)	MS	62.7, 5.33	+3.25	+2.35	ns	-
Esmaeili et al. (2016)	TMR	6.6, 4.12	ns	ns	-3.4	-19
Ramirez et al. (2016)	Timothy hay	76.2, 4.8	ns	ns	-7.5	-103
Thomson et al. (2017)	lucerne hay	14, 19	ns	ns	ns	-13

CL = chop length, PS = particle size, Rum = rumination, AH = alfalfa hay, AS = alfalfa silage, A haylage = alfalfa haylage, MS = maize silage, GS = orchard grass silage, BS = barley silage, ns = non-significant difference.

[1]The long chop is considered as control diet and effects of decreasing CL on cow performance are reported either increased (+) or decreased (-).

1.8. Effect of mixing and mixer wagons on particle size

Mixer wagons and mixing protocols can also influence the particle size and peNDF content of the diet. For example, mixing diets with a vertical mixer wagon resulted in a 2.5 mm longer mean particle size compared to a horizontal mixer model and resulted in a reduction in DM intake and milk yield in dairy cows (Humphries et al., 2010). Heinrichs et al. (1999) also indicated that processing by the mixer wagon prior to feed-out can have a large effect on the particle size and peNDF subsequently fed and the consistency of the mix. Consideration should therefore also be given to the effect of particle size and consistency of mixing on the degree of diet selection and consumed by the cow, and the influence of level and form of supplement on rumen metabolism, cow performance and health under UK conditions (AHDB, 2016).

1.9. Effect of forage source on physically effective fibre

There is a lack of literature on the effects of forage source on peNDF with most studies having investigated the particle size of alfalfa and maize silage (Beauchemin et al., 2003; Kononoff and Heinrichs, 2003a), with less work on ryegrass silage particle size. Tafaj et al. (2007) reported a variable response of particle size of different forage sources, where particle size of grass silage based diets showed more pronounced effects on cow production performance compared to when diets were composed of maize silage or a mixture of both grass and maize silage. Additionally, ryegrass differs in ruminal digestion rate and fermentation characteristics compared to maize silage (Robles et al., 1980). The high starch content of maize silage can alter the rumen environment and leads to confounding results of particle size (De Brabander et al., 2002; Johnson et al., 2002). Therefore, there is a need to consider forage source and its composition when evaluating particle size effects in dairy cows.

1.10. Systemic inflammatory response of sub-acute rumen acidosis

Sub-acute rumen acidosis is a metabolic disorder that is defined as when the rate of production of VFA surpasses the rate of absorption of VFA, subsequently leading to an impairment of the acid base balance of the rumen that lowers pH (Plaizier et al., 2008). A low rumen pH (<5.8 pH) mainly manifested by the accumulation of lactate triggers a cascade in alterations of rumen function, microflora and rumen epithelium that subsequently results in the accumulation of endotoxins, mainly lipopolysaccharides (LPS) released by the microbes (Khafipour et al., 2009; Plaizier et al., 2012; Metzler-Zebeli et al., 2013). This cascade of acidosis results in an inflammation of the gut lumen and disrupts the epithelium of the reticulo-rumen by altering the tight junctions of the

epithelial lining and increases its permeability (Zebeli et al., 2012b; Zebeli and Metzler-Zebeli, 2012). In response to endotoxins, local macrophages release pro-inflammatory cytokines, a stage termed as low degree inflammation caused by SARA. Increases in endothelial permeability allows ruminal endotoxins to enter into the blood circulation and triggers the release of acute phase proteins such as serum amyloid A, haptoglobin and LPS binding protein as an innate immune response of SARA (Ametaj et al., 2010; Plaizier et al., 2012; Metzler-Zebeli et al., 2013). A positive correlation between the amount of concentrates in the diet (> 45% of the diet) and serum amyloid protein has therefore been reported by Zebeli et al. (2012b).

The concentration of free LPS in the rumen increased to 107,152 endotoxin unit/ml when cows were suffering with SARA compared to 28,184 endotoxin units/ml in non-SARA cows (Khafipour et al., 2009). This subsequently led to an increased blood LPS concentration of 0.52 endotoxin unit/ml in SARA suffering cows compared to <0.05 endotoxin unit/ml LPS in the non-SARA cows. Khafipour et al. (2009) reported that the concentrations of haptoglobin (+475.6 µg/ml) and serum amyloid A protein (+271.1 µg/ml) were also higher in SARA suffering cows compared to non-SARA cows.

1.11. Knowledge gap

The increasing demand for dairy products has required an increase in milk production on many dairy farms worldwide. In the UK, achieving high milk production has led many farmers to feed increasing amounts of concentrate feeds and high-quality forages, both of which are associated with a reduced dietary fibre level (Beauchemin et al., 2003). Low dietary fibre levels increase the risk of metabolic disorders including SARA, displaced abomasum, milk fat depression, laminitis, reduced fibre digestion and fat cow syndrome (Krause and Oetzel, 2006; NRC, 2001; Plaizier et al., 2008). Field studies in the USA indicated that 19% of early lactation and 26% of mid-lactation dairy cows suffer from SARA (Garrett et al., 1997). The particle size of the diet has been proposed as a key factor, along with NDF and non-forage carbohydrate concentrations to ensure healthy rumen function (Zebeli et al., 2012a). However, the estimation of particle size for forages in dairy rations is problematic, with most studies being conducted in North America and using diets based on lucerne and maize silage. Additionally, consideration should be given to the effect of processing by the mixer wagon on the particle size consumed by the cow, and the influence of level and form of supplement on rumen metabolism, cow performance and health under UK conditions. The hypothesis of this thesis was that forages and diets used in the UK herds are different than used in North America, their interaction with concentrate may be different under UK conditions

and consequently the current guidelines for particle size may not be suitable for UK rations.

The main objectives of this thesis were;

1- To evaluate and develop methods to more accurately describe forage particle size and functional fibre under UK conditions.
2- To characterise the range of forage particle size and functional fibre of grass and maize silages on commercial UK dairy farms.
3- To determine the influence of mixing and extent of cow selection on commercial UK dairy farms.
4- To evaluate the influence of forage particle size and functional fibre on rumen pH, fermentation, intake, performance and milk composition in dairy cows, and examine the interaction with level and rate of degradation of supplementary sources.
5- To provide recommendations to dairy farmers, nutritionists and contractors on target forage particle size to optimise rumen health and cow performance for housed cows.

A longer term objective is the incorporation of the findings from this study into routine characterisation of particle size by feed laboratories and the inclusion of physically functional fibre levels in feed tables and ration programs.

CHAPTER 2: General materials and methods

2.1. Dry matter (DM)

The TMR, forages, and Penn State Separator sieve fractions were placed in a pre-weighed clean dry silica tray and dried in a hot air oven (Binder, Cole-Palmers, UK) at 105℃ overnight (AOAC, 2012; 934.01). The sample was cooled for 30 min in a desiccator and weighed out. The DM (g/kg) was calculated as;

$$DM\ (g/kg) = \frac{W1-W2}{W} \times 1000 \qquad \text{(Equation 1)}$$

Where,

W = sample weight before drying (excluding tray weight).
W_1 = weight of empty tray + sample before drying (g).
W_2 = weight of tray + sample after drying (g).

Samples were then hammer milled (Crompton Control Series 2000, Wakefield West Yorkshire UK) through a 1 mm screen prior to analysis.

2.2. Crude protein (CP)

The CP values were determined by nitrogen (N) analysis using the Dumas method (AOAC, 2012; 988.05) using a LECO FP528 (LECO Corp, Stockport, UK). Approximately 150 mg of dried ground sample was weighed into aluminium foil, and then placed into the auto analyser. The CP value was calculated as;

$$CP\ (g/kg\ DM) = N\ (\%) \times 6.25 \times 10 \qquad \text{(Equation 2)}$$

2.3. Ash and organic matter (OM)

The ash content was determined after combustion at 550℃ (AOAC, 2012; 942.05). Approximately, 2 grams of dried, milled sample was accurately weighed into a clean dried pre-weighed porcelain crucible. The sample was transferred to a muffle furnace (Gallenkamp Muffle Furnacne, Size 3, GAFSE 620, Gallenkamp, Loughborough, UK) at 550℃ for 5 hours. After ashing the sample was placed in a desiccator for 30 min to cool. The weight of the silica crucible and ash was then recorded. Ash content was calculated as;

$$Ash\ (g/kg\ DM) = \frac{W1-W2}{A} \times 1000 \qquad \text{(Equation 3)}$$

Where,

W_1 = silica crucible weight with ash (g).

W_2 = empty silica crucible weight (g).

A = sample weight (g).

Organic matter (OM) was calculated as;

$$OM\ (g/kg\ DM) = 1000 - Ash\ (g/kg\ DM) \qquad \text{(Equation 4)}$$

2.4. Ether extract (EE)

The ether extract content was determined according to AOAC (2012; 920.39) using a Soxtec apparatus (HT 1043 extraction apparatus, FOSS, Warrington, UK). Approximately 1 g of dried milled sample was accurately weighed into a cellulose extraction thimble (Whatman Plc, Maidstone, UK). The thimble was plugged with fat-free cotton wool and the sample boiled in 25 ml (30-40℃) of petroleum ether (Fisher Scientific, UK) for 1 h. Samples were then removed and rinsed for an additional 15 min and the solvent then evaporated. After cooling, the extraction cup was reweighed and the ether extract content was determined as;

$$EE\ (g/kg\ DM) = \frac{Y-X}{Z} \times 1000 \times \frac{1000}{A} \qquad \text{(Equation 5)}$$

Where,

Y = soxhlet flask weight containing ether extract (g).

X = empty soxhlet flask weight (g).

Z = sample weight (g).

A = sample dry matter (g).

2.5. Neutral detergent fiber (NDF)

The NDF content was determined using Fibertec™ (1020, FOSS, Warrington, UK) system using sodium sulphite and heat-stable α amylase (Sigma, Gillingham, UK) according to the procedure described by Van Soest et al. (1991), and expressed exclusive of residual ash. The NDF reagent was prepared by mixing 93 g of EDTA (di-sodium ethylene diamine tetra acetic acid dehydrate), 34 g sodium tetra borate ($Na_2B_4O_7.10H_2O$), 150 g sodium dodecyl sulphate (SDS), 50 ml tri-ethylene glycol, and 22.8 g anhydrous disodium hydrogen phosphate (Na_2HPO_4) to make 5 L solution with distilled water, and then adjusted to approximately pH 6.9 to 7.1. Alpha amylase solution was prepared by dissolving 2.8 g of α-amylase (α-1, 4-glucan 4-gluconohydralase,

enzyme # 3.2.1.1 ~80EU/mg) from *Bacillus subtilis spp* (Sigma, Gillingham, UK) in 90 ml of distilled water, followed by the addition of 10 ml of tri-ethylene glycol. Approximately 0.5 g of dried milled sample was weighed into a glass crucible (porosity 1, Soham Scientific, Ely, UK) that was tightly fitted onto the Fibretech® (Foss UK Ltd, Cheshire,UK). Neutral detergent reagent (25 ml) and a few drops of octanol (reagent grade, Sigma, Aldrich, Dorset, UK) were added to the sample. The sample was then digested for 30 min. Another 25 ml of neutral detergent reagent and 2 ml of α-amylase solution and 0.5 g of sodium sulphite were added and the sample simmered for 30 min. The sample was then filtered and washed with 20-30 ml of hot distilled water (80℃). Another 2 ml of α-amylase solution and 25 ml of hot distilled water were added to the samples and allowed to stand for 15 min. The sample was then filtered and washed 3 times with hot distilled water, and the crucible removed from the Fibertech® and dried overnight at 105℃. After cooling in a desiccator, the crucible was weighed and placed in a muffle furnace at 550℃ for 4 h. Afterwads, the crucible was cooled in a desiccator to room temperature and reweighed. The NDF content was calculated as;

$$NDF\ (g/kg\ DM)\ = \frac{Y-Z}{X} \times 100 \times \frac{1000}{A} \qquad \text{(Equation 6)}$$

Where,

Y = crucible weight + residue after oven drying (g).

Z = crucible weight + residue after ashing (g).

X = sample weight (g).

A = sample dry matter (g).

2.6. Acid detergent fibre (ADF)

The ADF content was determined using a Fibertec™ 1020 (FOSS, Warrington, UK) according to the procedure described by Van Soest et al. (1991), and expressed exclusive of residual ash. The ADF reagent was prepared by mixing 20 g of CETAB (cetyltrimethylammonium bromide; Sigma, Gillingham, UK) in one litre of 1M sulphuric acid (Sigma, Gillingham, UK). Approximately one gram of dried milled sample was weighed into a glass crucible (porosity 2, Soham Scientific, Ely, UK) that was tightly fitted onto the Fibretech® (Foss UK Ltd, Cheshire,UK). Acid detergent reagent (100 ml) was added to the sample and then boiled for 60 min. The sample was then filtered and washed 3 times with hot distilled water (20-30 ml), and the crucible removed from the Fibertech® and dried overnight at 105℃. After cooling in a desiccator, the crucible was weighed and placed in a muffle furnace at 550℃ for 4 h. Afterwads, the crucible was

cooled in a desiccator and reweighed. The ADF content was calculated as;

$$ADF\ (g/kg\ DM)\ =\ \frac{Y-Z}{X}\times 100\ \times\ \frac{1000}{A} \qquad \text{(Equation 7)}$$

Where,

Y = crucible weight + residue after oven drying (g).

Z = crucible weight + residue after ashing (g).

X = sample weight (g).

A = sample dry matter (g).

2.7. Acid detergent lignin (ADL)

The ADL content was determined using a Fibertec™ 1020 (FOSS, Warrington, UK) according to the procedure described by Van Soest et al. (1991), and expressed exclusive of residual ash. Approximately one gram of dried milled sample was weighed into a glass crucible (porosity 2, Soham Scientific, Ely, UK) that was tightly fitted onto the Fibretech® (Foss UK Ltd, Cheshire,UK). Acid detergent reagent (100 ml) was added and the sample boiled for 60 min. The sample was then filtered and washed 3 times with hot distilled water (20-30 ml). Afterwards, 25 ml of concentrated sulphurc acid (99%, Sigma, Gillingham, UK) was added and the crucible left for 3 h on the Fibretech® with the sample mixed hourly. The crucible was removed from the Fibertech® and dried overnight at 105℃. After cooling in a desiccator, the crucible + sample was weighed and placed in a muffle furnace at 550℃ for 4 h, and then cooled in a desiccator and reweighed. The ADL content was calculated as;

$$ADL\ (g/kg\ DM)\ =\ \frac{Y-Z}{X}\times 100\ \times\ \frac{1000}{A} \qquad \text{(Equation 8)}$$

Where,

Y = crucible weight + residue after oven drying (g).

Z = crucible weight + residue after ashing (g).

X = sample weight (g).

A = sample dry matter (g).

2.8. Acid insoluble ash (AIA)

The ash content (from Section 2.3) of the sample was quantitatively transferred to a labelled Kjeldahl tube. After the addition of 100 ml of 2M HCl to tube, the sample was boiled at 175℃ for 10 min (Van Keulen and Young, 1977). The sample was then

filtered through an ash free filter paper (no 541) and the filter paper containing the ash was placed in muffle furnace at 550℃ for 4 h. The sample was then cooled down in a desiccator and weighed. The acid insoluble ash content was calculated as;

$$AIA\ (g/kg\ DM)\ =\ \frac{\mathrm{W1-W2}}{A} \times 1000 \quad \text{(Equation 8)}$$

Where,

W_1 = crucible weight with acid ash (g).

W_2 = empty silica crucible weight (g).

A = sample weight (g) from Section 2.3.

2.9. Starch Analysis

The starch content of the samples was analysed by Trouw Nutrition (Blenheim House, Blenheim Road, Ashbourne, Derbyshire, UK) using the procedure described by McCleary et al. (1997).

2.10. Blood metabolites

Blood samples were collected into fluoride/oxalate and lithium heparin Vacutainers (Ref. 368201 and 367885 respectively, BD Vacutainer, Plymouth, UK) from cows by jugular venepuncture and centrifuged at 3,000 g for 15 min, the plasma extracted and stored at -20℃ prior to subsequent analysis (Sinclair et al., 2015). Plasma samples were analysed for glucose (kit no. GL1611; Randox Laboratories, County Antrim, UK), β-hydroxybutyrate (3-OHB, kit no. RB1008; Randox Laboratories, County Antrim, UK) and urea (kit no. UR221; Randox Laboratories, County Antrim, UK) using a Cobas Miras Plus autoanalyser (ABX Diagnostics, Bedfordshire, UK).

For the second controlled study (Chapter 5), serum samples were diluted 1:20 analysed for haptoglobin (HP) by using an ELISA kit (kit catalogue no. ab157714, Abcam, Cambridge, UK) with the standards concentrations ranging from 1.95, 3.9, 7.8, 15.6, 31.25, 62.5, and 125 ng/ml. Spectrophotometric measurements were undertaken using a BioTeck microplate reader (BioTeck Instruments Ltd, Potton, UK) at 450 nm absorbance.

2.11. Milk composition analysis

Milk samples were analysed for concentrations of milk fat, milk protein, casein, lactose, urea and somatic cell count (SCC) using a Milkoscan Minor analyser (Foss, Denmark) calibrated according to AOAC (2012). For the second controlled study

(Chapter 5), milk sample were analysed by using near midinfrared (MIR) method (National Milk Laboratories, Wolverhampton, UK).

2.12. Fatty acids analysis

The fatty acid methyl ester in hexane was extracted from milk fat by procedure described by Lock et al. (2006). Approximately, 30 g of milk was centrifuged at 17,800 g for 30 min at 4℃ (Heraeus Mulifuge X1R, Thermo Fisher Scientific, Paisley UK). A quantity (0.3 g) of milk fat was accordingly transferred to a clean (pre-rinsed with hexane) 16×150 extraction tube, 5.4 ml of hexane: isopropanol (3:2) added and vortexed for 30 sec. Then 12 ml of sodium sulphate solution (6.67% solution in distilled H_2O) was added, vortexed and left to stand. The top clear layer was then transferred to a new 16×150 extraction tube (containing 1 g sodium sulphate) and left to settle for 30 min. The top layer was then transferred to a new small (16×100) extraction tube and placed in a water bath at 40℃. Hexane was evaporated under continuous nitrogen (N) flushing, and the fat then transferred to an Eppendorf and stored at -20℃. For methylation, the sample was defrosted in a water bath at 40℃ for 20 min and 50 mg of lipid was transferred into a 10 ml pre-rinsed extraction tube. The extraction tube was vortexed for 30 sec after the addition of 2 ml hexane and 40 µl methyl acetate. Then 40 µl methylation reagent was added, and tube vortexed for 2 min and left to stand for 8 min. The methylation process was completed by adding 60 µl termination reagent and sample was vortexed for 30 sec.

Individual fatty acid methyl esters were determined by GC (Hewlett Packard 6890, Wokingham, UK) fitted with a CP-Sil 88 column (100 m x 0.25 mm i.d. x 0.2 µm film). Fatty acid identification and recoveries were determined using pure methyl ester standards (Nu-Chek Prep, Elysian, MN; Natural ASA, Hovdebygda, Norway), and a mixed reference standard was used as a routine check for recoveries and correction factors for individual FA.

2.13. Rumen passage rate

Particle passage kinetics was estimated by using the chromium-mordant technique of the GS-NDF (Cr-NDF) according to Udén et al. (1980). Dried grass silage sample (500 g) was boiled in NDF solution (9 L) for 2 h and then washed thoroughly with water before left in acetone for overnight. Grass sample was rinsed with water until acetone was totally removed and dried at 45℃ for 48-72 h. Sample was then baked in an oven (100℃) in sodium di-chromate solution (172 g sodium di-chromate in 2.5 L of dis H_2O for 500 g of grass-NDF) for 24 h. The cooked grass-NDF was placed in 50% ascorbic acid solution for 18 h, later washed and dried at 65℃. The Cr-NDF was inserted

directly in the rumen via the rumen cannula or fed to the intact cow by mixing with the diet. Faeces were collected at -1 (background concentration of marker), 3, 6, 9, 12, 15, 18, 21, 24, 28, 32, 36, 40, 44, 48, ,52, 56, 64, 72, 80, 88, 96, 108, 120, 132 and 144 h to estimate particle passage kinetics (Hammond et al., 2014). Rumen retention time was calculated according to the procedure described by Dhanoa et al. (1985). The mean retention time (MRT) of digesta phases were determined by a multi-compartmental model, using equations 8 and 12 of Dhanoa et al. (1985) for curve fitting and MRT calculation. Faecal marker concentrations were corrected for individual background concentrations (taken -1 h before administering Cr-NDF).

$$\frac{dXn}{dt} = Ae^{-k1t}\exp[-(N-2)e^{-(k1-k2)t}] \qquad \text{(Equation 9)}$$

$$MRT = \frac{1}{k1} + \frac{1}{k2} + \sum_{i=\emptyset}^{n-1} \frac{1}{k2+(i-2)(k2-k1)}, k2 > k1 \qquad \text{(Equation 10)}$$

Where; k1 is emptying rate of rumen, k2 is emptying rate of intestines, Tp is time to peak marker flow, TT is transit time, R-MRT is rumen mean retention time, TT-MRT is total-tract mean retention time, and cT is clearance time.

2.14. Rumen volatile fatty acid analysis

Rumen VFA concentrations were determined using a GC (3400, Varian Inc.) using procedures as described previously (Erwin et al., 1961). Rumen fluid sample (5 ml) was centrifuged at 25,000 g for 15 min at 5℃. Then 1.2 ml supernatant was transferred into a 2 ml Eppendorf containing 0.3 ml internal standard (25mM 2-Ethylbutyric acid in 25% w/v metaphosphoric acid) in it. Samples were mixed and left to stand for 30 min. After centrifugation at 13,000 g for 10 min, 0.33 ml of sample was transferred to GC vial and 0.8 ml deionised H_2O was added. Individual VFA methyl ester were determined by GC (Varian Star 3400 CX, Varian Inc., Palo Alto, CA, USA) fitted with a Stabilwax-DA column (30 m x 0.25 mm i.d. x 0.25 µm film with 10m guard).

2.15. Chromium analysis

Faecal chromium concentration was analysed using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, NexION® 2000, PerkinElmer, Seer Green, UK) as described by Cope et al. (2009). Dried ground faecal sample (1 g) was combustion at 550℃ for 5 h and then digested in 15 ml 1.5M nitric acid solution. Digested sample was then diluted (1:50) with the deionised water and subsamples were centrifuged at 3,000 g for 10 min. Gallium was used as an internal standard and standard chromium concentrations of 0, 50, 100, 200, 400 µg/kg were used for calibration.

2.16. Modifications to the Penn State Particle Separator

The original Penn State Particle Separator was modified by the addition of 26.9, 33, 44 and 60 mm screen size sieves (Table 2.1). The additional sieve screens were used to provide a more comprehensive particle size distribution for grass silage and ration samples, and used a circular hole, similar to the original Penn State Particle Separator (Lammers et al., 1996), as opposed to the square hole design used in the ASABE/ANSI forage particle separator (ASABE, 2007).

Table 2.1. Details of additional larger screen sizes used to modify Penn State Particle Separator.

	No of holes	Hole area	Gap distance (mm)		
Sieves	(n)	(%)	Vertical	Horizontal	Diagonal
26.9 mm	72	54.5	9.0	41.0	12.0
33 mm	46	43.7	11.5	56.0	16.3
44 mm	27	45.6	20.0	58.4	16.4
60 mm	15	47.1	36.0	60.0	16.8

2.17. Determination of the particle size distribution of forages and mixed rations

The particle size distribution of the forages and TMR/PMR samples were analysed using a modified Penn State Particle Separator (PSPS 2013 version) with three original screens of size 19, 8, and 4 mm (Kononoff et al., 2003; Kmicikewycz and Heinrichs, 2015), and additional screens of 26.9, 33 (ASABE, 2007; Maulfair and Heinrichs, 2010), 44 and 60 mm pore diameter (Section 2.13). The manual shaking procedure for Penn State Particle Separator was adopted after Kononoff et al. (2003) by setting the Penn State Particle Separator on a flat surface with 1.1 Hz shaking frequency (66 full shakes in one minute) and a stroke length of 17 cm, with a quarter turn of the separator resulting in a total of 8 sets of 5 shakes to accomplish a 40 shakes in 2 full turns. Samples were shaken 5 times to provide replication, and a joint sample for each fraction was used to determine an average DM content (Section 2.1).

2.18. Grass and maize silage sampling

Grass and maize silages were sampled separately from their respective clamps. Ten samples of silage were collected from the clamp face in a ‘W’ pattern and thoroughly mixed as described by Sinclair (2006).

CHAPTER 3: Particle size distribution of forages and mixed rations, and their relationship with ration variability and performance of UK dairy herds

3.1. Introduction

Feeding dairy cows with a mixed ration (MR; either total or partial mixed ration; TMR or PMR, respectively) is an effective way to provide a homogeneous and balanced diet throughout the day (Coppock et al., 1981). The composition of MR can vary considerably but perennial ryegrass (GS) and maize silage (MS) are the main forages used in the MR fed to dairy herds in Northern Europe (Johansen et al., 2018; March et al., 2014). In order to maintain animal performance and promote a healthy rumen function the inclusion of forages with an adequate particle size and dietary concentration of non-forage carbohydrate (fibre) in the MR are required (Zebeli et al., 2012a). The physically effectiveness of a ration has been proposed as the product of the particle size multiplied by its neutral detergent fibre (NDF) content, defined as physically effective fibre (*peNDF*; Mertens, 1997). Achieving the correct particle size and *peNDF* in a ration can enhance rumen function leading to an increase in the production of rumen microbes, more efficient degradation of fibre and increased milk fat content (De Brabander et al., 1999; Zebeli et al., 2012a). A shorter forage particle size is associated with improved compaction in the clamp and can result in reduced aerobic spoilage at feed out (McDonald et al., 1991) and may increase DM intake, due to reduced rumen fill and increased fibre digestibility (Thomson et al., 2017). However, too short a forage particle length can increase the rate of volatile fatty acid production in the rumen, reduce rumination time, and decrease the production of saliva (Tafaj et al., 2007), with the consequence of inhibiting cellulolytic bacteria activity and increasing the risk of sub-acute ruminal acidosis (SARA; Tafaj et al., 2007). In a review of the literature, Zebeli et al. (2012) concluded that too low a particle size (and *peNDF),* increases the passage rate of digesta and rate of fibre degradation due to a higher surface area for microbial attachment. In contrast, too long a forage particle size may promote ration sorting and result in some cows receiving excess concentrates and others insufficient (Kononoff and Heinrichs, 2003a).

The estimation of the particle size of forages and MR is problematic, and various methods have been proposed to characterise feed particle distribution using different sieving techniques, with no universally accepted standard. Maulfair and Heinrichs (2012) concluded that the Penn State Particle Separator (PSPS) was the most useful method to use on-farm and proposed dietary guidelines for use on-farm. These recommendations are primarily based on North American rations that consist of MS and

lucerne haylage (Eastridge, 2006), and may therefore not be suitable for the typically wetter (e.g. less than 30% DM) MS and GS commonly fed in Northern Europe (Møller et al., 2000).

Heinrichs et al. (1999) reported that processing by the mixer wagon prior to feeding can also have a large effect on the consistency of the mix, and affect the particle size and *peNDF* concentrations of the ration subsequently consumed. Mixing protocols have been shown to affect feed intake and milk yield, particularly in rations containing longer chop lengths (Humphries et al., 2010; Maulfair and Henrichs 2010). Consideration should therefore also be given to the effect of particle size and consistency of mixing on the degree of diet selection by the cows.

The primary objective of the present study was to characterise the particle size distribution and *peNDF* content of GS, MS and MR fed on UK dairy herds using a modified PSPS, and to compare the observed particle size distributions with current guidelines. The secondary objective of the study was to evaluate the consistency of mixing of MR and extent of sorting of GS and GS/MS based MR, and to determine the relationship between particle size and cow performance on UK dairy herds.

3.2. Materials and methods

3.2.1. Herd characteristics

Fifty commercial dairy herds located throughout the UK (32 in the Midlands of England, 9 in the South of England and 9 in Southwest Scotland) that were feeding GS and/or MS were visited between January and June, 2016. All of the herds were using a MR (PMR or TMR) feeding system and had a high yielding group that contained at least 50 cows. Herds were enrolled onto the study through an initial telephone contact and questionnaire survey to determine suitability and willingness to participate. On the day of the visit a second questionnaire was completed to collect details of herd characteristics, performance levels and frequencies of fresh feed delivery, feed push up and orts removal. In addition, feeding space per cow, feed mixer make and model, forage harvester make and model, and mixing protocol were recorded. The ingredient composition of MR fed to the target group and the mean concentrate quantity fed in the parlour was also recorded.

Out of the 50 herds, 50 fed GS, with 34 using MS in the MR. Other sources of forage being fed were; whole-crop wheat (19), wheat straw (15), fodder beet (5), grass haylage (2), whole-crop triticale (1), whole-crop barley (1), lucerne (1), pea silage (1) and oat

silage (1). Forty-four of the herds had an all year around calving pattern, 4 were autumn block calving and 2 spring block calving. Holstein-Friesian was the major breed on 36 herds, with the predominant breed on the remaining herds being Ayrshire (2), Jersey (1), Brown Swiss (1), or (10) having a mixture of Holstein with other breeds (Brown Swiss, New Zealand Friesian, and Jersey) or crossbred. The main feeding system was TMR which was used on 28 herds, while the remaining 22 herds fed a PMR with additional concentrate fed in the milking parlour. Twenty-four herds used a "tub" type mixer wagon, 18 a "barrel" type, 7 an "auger" design (vertical or horizontal) and one used a forage box.

Total herd size ranged from 75 to 2220 animals, with a mean of 354 (Table 3.1). The number of lactating cows ranged from 67 to 1770 cows/herd, with a mean and median of 310 and 277, respectively. The mean annual milk yield ranged from 6000 to 12500 kg/cow, with a mean of 9199 kg/cow (median = 9200). Annual energy corrected milk yield (ECM, corrected for milk fat and protein; Tyrrel and Reid, 1965) ranged from 7248 to 13209 kg/cow, with a mean of 10011 kg/cow. All herds delivered fresh feed either once or twice daily, with a mean of 1.3 times/d. Of the 50 herds, 20 were feeding the MR in a trough where there was no push up the feed. The average frequency of feed push up in the remaining 30 herds was 4.7 times/d. The mean orts removal frequency was 4.4 times/wk, with a range from 0.25 (monthly) to 7 (daily) times/wk. Feed space per cow ranged from 0.30 to 0.76 m/cow, with a mean of 0.56 m/cow. Length of feed mixing was either manually recorded or provided by the farmer, and ranged from 5 to 60 min. The number of chews per bolus was manually counted for three full bouts for 10 cows randomly selected from the feeding group sampled (Kononoff et al., 2002).

Table 3.1. Herd and feeding characteristics on 50 UK dairy herds.

	Mean	SD	Min	Max	Median
Herd size (n)	354	343.9	75	2220	277
Cows in milk (n)	310	282.3	67	1770	240
Milk yield (kg/cow/year)	9199	1583.2	6000	12500	9200
Milk fat (g/kg)	41.0	0.36	36.2	57.0	40.0
Milk protein (g/kg)	32.9	0.21	29.3	41.0	32.8
FCM yield (4%, kg/cow/year)[1]	9334	1216.4	6895	12025	9111
Frequency of fresh feed delivery (n/d)	1.3	0.46	1	2	1.0
Frequency of feed push up (n/d)[2]	4.7	3.19	1	16	4.0
Frequency of refusals removal (n/wk)	4.4	2.75	0.25	7	5.5
Feed space per cow (m/cow)	0.56	0.098	0.30	0.76	0.61
Length of feed mixing (min/mix)	19	10.2	5	60	15
No. of chews/bolus	66	9.81	44	105	66

[1]Fat corrected milk at 40 g/kg fat.
[2]Herds feeding into a trough (n = 20) have been excluded.

3.2.2. Determination of particle size and physically effective fibre distribution of forages and mixed rations

Where more than one feeding group was present, data were collected from the high yielding group in each herd (n = 40). Where feed was delivered more than once (n = 15), the first (morning) feed was sampled. The feed face of the high yielding group of cows (or all cows if no subdivision was present) was divided into five equal sections to determine the consistency of mixing (Sova et al., 2014). Within each feed face section, a 30 cm × 30 cm quadrat was randomly placed over the MR within 5 min of fresh feed-out, and all material removed and thoroughly mixed (0hMR; Endres and Espejo, 2010). To determine the level of diet selection (feed sorting), the MR was sampled using the quadrat from the same locations along the feed fence again four hours post feeding (4hMR; Leonardi et al., 2005). Prior to fresh feed delivery, refusals (RefMR), where available, were also sampled (n = 33).

The particle size distribution of the forage (GS and MS) and MR samples were analysed on both a fresh and dried basis. A modified Penn State Particle Separator (PSPS) with four screens of 26.9, 19, 8, and 4 mm was used to determine the particle size of GS and GS/MS based MR, and three screens of 19, 8 and 4 mm for MS according to the manual shaking procedure described by Kononoff et al. (2003). Perennial ryegrass (*Lolium perenne*) and MS (*Zea mays* L.) were sampled from first, second or third cut GS and MS clamps as described by Sinclair (2006) and the particle size measured using the modified PSPS described above. The particle size distribution (%) was calculated by dividing the weight of each fraction by the sum of all fractions and multiplying by 100.

The on-farm particle size distribution analysis using one additional PSPS sieve screen (26.9 mm) was found to be insufficient to determine the geometric mean particle size (X_m) of GS and GS based MR. Consequently, two larger sieve screens of size 44 and 60 mm were used to reanalyse particle size of 0hMR and GS using frozen and defrosted samples. The frozen samples were thawed at room temperature for 6h prior to analysis.

3.2.3. Chemical analysis

The DM content (section 2.1) of each fraction of 0hMR, 4hMR, RefMR, GS and MS for each herd was determined. Forage and MR samples were then milled in a hammer mill (Crompton Control Series 2000, Wakefield West Yorkshire UK) fitted with a 1 mm screen. Crude protein, ash and ether extract was analysed as described by AOAC (2012) in Section 2.2, 2.3 and 2.4, respectively . The NDF and acid detergent fibre (ADF) content was analysed according to Van Soest et al. (1991) (Section 2.5 and 2.6). The

starch content of the 0hMR was analysed by Trouw Nutrition (Blenheim House, Blenheim Road, Ashbourne, Derbyshire, UK) using the procedure described by McCleary et al. (1997).

3.2.4. Calculations and statistical analysis

Milk production was standardised to 40 g fat/kg (fat corrected milk [FCM]) as described by Tyrrel and Reid (1965) to allow comparison between herds. The geometric mean particle size (X_m) was calculated using the method described by ASABE (2007). The physical effectiveness factor (*pef*) was determined as the DM proportion of particles longer than 8 mm ($pef_{>8mm}$) or 4 mm ($pef_{>4mm}$, Lammers et al., 1996; Maulfair and Heinrichs, 2010). The $peNDF_{>4mm}$ was calculated by multiplying the NDF content (% DM) of the MR by the $pef_{>4mm}$, and $peNDF_{>8mm}$ by multiplying the NDF content (% DM) of the MR by the $pef_{>8mm}$ (Lammers et al., 1996; Mertens, 1997).

The consistency of ration mixing of each herd was calculated using the co-efficient of variation (CV%) of each particle size fraction of the 0hMR (Buckmaster et al., 2014; Oelberg and Stone, 2014; Sova et al., 2014), with a CV of >5% considered significant (Silva-del-Rio and Castillo, 2012). To characterise the herds for ration variability, the CV of each fraction was weighted for the respective percentage particle size distribution and then the corrected CV (CCV%) summed. Herd-level diet selection was calculated for each fraction by dividing the proportion (DM basis) at 0hMR by the corresponding proportion at 4hMR and RefMR, and presented as a percentage. A sorting value of 100% indicated no sorting, <100% indicated preferential consumption, and >100% indicated selective refusal. To more easily determine the variability of diet selection across herds, the long fractions (>60, 44-60, 26.9-44 and 19-26.9 mm) were summed (>19 mm), and the short (4-8 and <4 mm) fractions summed (<8 mm). Assuming that a sorting value of 100% ± 5 indicated no sorting, >105% indicated selective refusal and a sorting value of <95% indicates preferential consumption.

All data were summarised by herd and tested for normality using the general descriptive statistics component of GenStat 17.1 ® (VSN International Ltd., Oxford, UK). Associations between measures of productivity (energy corrected milk yield, milk fat g/kg, milk protein g/kg), feeding management and ration characteristics were analysed using a standard linear model (i.e. ANOVA) with forage source and shaking technique as fixed effects and herds and location as random effects. A type 1 linear regression model was used to determine the association between X_m and energy corrected milk yield and milk fat using GenStat 17.1 ® (VSN International Ltd., Oxford, UK). For multiple

comparisons, all fractions of the mixed ration were analysed by general ANOVA followed by a Tukey test, with the significant level set at $P < 0.05$.

3.3. Results

3.3.1. Forage proximate and physical characteristics

The mean DM of the GS was 23 g/kg lower ($P = 0.022$) and the CP 54 g/kg DM higher than the MS (Table 3.2). The NDF and ADF content were also 65 and 64 g/kg DM higher in the GS than the MS ($P < 0.001$). The highest % DM retention of GS within PSPS was the 26.9-44 mm fraction (51.6%, $P < 0.001$), with the majority of the DM (80.3%) being longer than 19 mm. In contrast, the highest retention of DM for MS was between 8-19 mm (73.2%, $P < 0.001$). The X_m, $peNDF_{>4mm}$ and $peNDF_{>8mm}$ content were higher ($P < 0.001$) in GS than MS (mean values of 42.6 and 10.6 mm, 48 and 40%, and 47 and 34% for X_m, $peNDF_{>4mm}$ and $peNDF_{>8mm}$ for GS and MS respectively).

3.3.2. Mixed ration proximate and physical characteristics

The mean forage to concentrate ratio (F:C) across the 50 herds was 77:23 on a fresh weight basis, and 57:43 on a DM basis, with a GS to MS ratio on the 34 herds that fed both forages of 50:50 (fresh weight basis) or 48:52 (DM basis; Table 3.3). The DM concentration of the MR ranged from 213 to 544 g/kg, with a mean value of 373 g/kg across the 50 herds, whilst the mean CP ranged from 116 to 205 g/kg DM, with a mean value of 160 g/kg DM. The mean and median NDF concentration of the MR was 391 and 381 g/kg DM respectively. For the MR, the lowest proportion of DM was retained in the 60 mm fraction ($P < 0.001$), with the 8-19 mm fraction having the highest proportion ($P < 0.001$), and there was no difference ($P > 0.05$) between the 44-60 and 19-26.9 mm fractions. The $peNDF_{>4mm}$ concentration of the MR ranged from 22 to 47% with a mean of 33%, and the mean $peNDF_{>8mm}$ was 29%. The mean X_m of the MR was 19.5 mm, ranging from 6.2 to 44.9 mm. The starch concentration of MR ranged from 63 to 237 g/kg DM with a mean value of 138 g/kg DM. The mean DM of the 0h, 4h and RefMR did not differ ($P = 0.10$) between sampling times, and the DM concentration of the various fractions of MR did not change over time ($P > 0.05$; data not shown).

Table 3.2. Mean chemical (g/kg DM ± SD) and physical characteristics (%DM ± SD) of grass (n = 50) and maize silage (n = 34) on 50 dairy herds.

	Grass silage			Maize silage				
Chemical composition	Mean	Min	Max	Mean	Min	Max	SED	*P* value
Dry matter (g/kg)	273 ± 46.1	205	390	300 ± 55.8	219	420	11.2	0.022
Organic matter	899 ± 20.0	854	945	961 ± 7.1	942	974	3.6	< 0.001
Ash	101 ± 20.0	55	146	39 ± 7.1	26	58	3.6	< 0.001
Crude protein	136 ± 26.0	81	184	82 ± 9.3	56	98	4.7	< 0.001
Neutral detergent fibre	492 ± 75.0	362	702	427 ± 74.1	276	559	16.8	< 0.001
Acid detergent fibre	331 ± 41.9	242	459	267 ± 44.8	176	347	9.7	< 0.001
Physical composition								
Fractions (mm)[1]								
>60	2.1 ± 5.12[a]	0	31.8	-	-	-	ND	ND
44-60	23.1 ± 13.38[c]	0	53.5	-	-	-	ND	ND
26.9-44	51.6 ± 14.01[d]	5.9	77.2	-	-	-	ND	ND
19-26.9	3.5 ± 3.29[a]	0.7	20.5	6.9 ± 4.55[a]	2.0	22.8	0.75	< 0.001
8-19	15.8 ± 10.01[b]	0.8	39.8	73.2 ± 8.75[d]	37.7	84.1	2.02	< 0.001
4-8	2.4 ± 1.44[a]	0.6	6.9	13.1 ± 5.02[c]	7.7	33.1	0.77	< 0.001
<4	1.6 ± 1.35[a]	0.1	6.0	6.8 ± 4.14[a]	1.4	18.8	0.64	< 0.001
$pef_{>4mm}$ (%)[2]	98 ± 1.5	93	100	93 ± 4.1	81	99	0.6	< 0.001
$peNDF_{>4mm}$ (%)	48 ± 7.0	36	66	40 ± 7.7	24	54	1.7	< 0.001
$pef_{>8mm}$ (%)[3]	96 ± 3.1	86	100	80 ± 8.0	48	90	1.3	< 0.001
$peNDF_{>8mm}$ (%)	47 ± 6.7	35	62	34 ± 7.7	19	48	1.6	< 0.001
X_m[4]	42.6 ± 5.63	17.5	53.9	10.6 ± 1.21	7.4	13.6	0.98	< 0.001

[1]Grass silage was separated into 7 fractions; >60, 44-60, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm. Maize silage was separated into 4 fractions; >19, 8-19, 4-8 and <4 mm.

[a,b,c,d] Within each forage, different superscripts between fractions indicate a significant ($P < 0.05$) difference.

[2]Physical effective factor; % proportion of particles >4 mm.

[3]Physical effective factor; % proportion of particles >8 mm.

[4]Geometric mean particle size.

Table 3.3. Mean chemical composition and physical characteristics of mixed rations (MR) on 50 herds.

	Fresh basis	DM basis		
Forage (kg/cow/d)	40.5	12.2		
Concentrate (kg/cow/d)[1]	11.7	9.5		
Forage to concentrate ratio (F:C)[1]	77:23	57:43		
Grass to maize silage ratio (GS:MS)[2]	50:50	48:52		
Composition (g/kg DM ± SD)[1]	Mean	Min	Max	Median
Dry matter (g/kg)	373 ± 78.6	213	544	380
Organic matter	920 ± 11.5	883	944	922
Ash	80 ± 11.5	56	117	78
Crude protein	160 ± 18.9	116	205	162
Ether extract	28 ± 8.2	11	40	30
Starch	138 ± 44.1	63	237	139
Neutral detergent fibre	391 ± 59.3	290	507	381
Acid detergent fibre	249 ± 42.6	173	329	245
Physical composition (%DM ± SD)				
Fractions (mm)[3]				
>60	0.1 ± 0.29	0	1.4	0
44-60	7.3 ± 9.27	0	32.8	2.4
26.9-44	26.0 ± 15.10	1.6	75.9	24.7
19-26.9	4.4 ± 3.38	0.9	21.8	3.7
8-19	34.9 ± 13.31	3.5	67.8	34.9
4-8	11.8 ± 5.58	0.9	29.6	10.9
<4	15.5 ± 9.72	0.4	37.4	14.9
$pef_{>4mm}$ (%)[4]	85 ± 9.6	63	100	85
$peNDF_{>4mm}$ (%)	33 ± 6.8	22	47	33
$pef_{>8mm}$ (%)[5]	73 ± 12.9	44	99	70
$peNDF_{>8mm}$ (%)	29 ± 7.3	16	43	28
X_m[6]	19.5 ± 12.09	6.2	44.9	13.3

[1]Includes the concentrates offered in the parlour.
[2]Ratio of GS to MS in 34 herds, where both silages were fed.
[3]Rations were separated into 7 fractions; >60, 44-60, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm; SED = 2.72 and $P < 0.001$.
[4]Physical effective factor; % proportion of particles >4 mm.
[5]Physical effective factor; % proportion of particles >8 mm.
[6]Geometric mean particle size.

Herds that fed GS as the main forage had a higher ($P < 0.01$) proportion of the DM retained in the 26.9-44 mm fraction of the 0hMR compared to those that used a mixture of GS and MS (Table 3.4). In contrast, herds that used a mixture of both forages had a higher ($P < 0.01$) proportion of the DM retained on the 8-19 mm fraction. The type of mixer wagon (barrel, tub or auger) had no effect ($P > 0.05$) on the particle size distribution of any fraction of the 0hMR (data not shown). When the PMR or TMR were considered separately, the proportion of longer fractions (26.9-44 and 44-60 mm) was higher ($P < 0.05$) when in the PMR, while the shorter fractions (8-19, 4-8 and <4 mm) were highest ($P < 0.05$) when fed as a TMR (Table 3.5).

Table 3.4. Particle size distribution of mixed rations (0hMR) at feed out containing grass silage (16) and mixtures of grass and maize silage (34) on 50 herds.

	Particle size distribution (%DM)			
Fractions[1] (mm)	GS	GS+MS	SED	*P* value
>60	0.1	0.1	0.08	0.55
44-60	10.6	5.7	2.75	0.08
26.9-44	34.6	22.0	4.25	< 0.01
19-26.9	3.5	4.8	1.01	0.22
8-19	26.4	39.0	3.65	< 0.01
4-8	10.2	12.6	1.67	0.15
<4	14.6	15.9	2.97	0.68
X_m[2]	23.1	17.8	3.63	0.15

[1]Rations were separated into seven fractions; >60, 44-60, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm.
[2]Geomatic mean particle size.

Table 3.5. Particle size distribution of rations fed as TMR (n = 28) or PMR (n = 22) on 50 dairy herds.

	Particle size distribution (% DM)			
Fractions[1] (mm)	TMR	PMR	SED	P value
>60	0.1	0.1	0.08	0.14
44-60	3.8	11.8	2.41	0.002
26.9-44	20.7	32.8	3.99	0.004
19-26.9	3.8	5.1	0.95	0.16
8-19	39.1	29.6	3.57	0.01
4-8	14.1	8.9	1.42	< 0.001
<4	18.4	11.7	2.62	0.014
X_m[2]	14.0	26.5	2.98	< 0.001

[1]Rations were separated into 7 fractions; >60, 44-60, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm.
[2]Geometric mean particle size.

3.3.3. Variability in mixed ration mixing

The coefficient of variation of mixing of MR was highest for the 19-26.9 and >26.9 mm fractions at 15 and 13.7% respectively, while the minimum CV of 6.4% was for the 4-8 mm fraction (Table 3.6). The type of wagon mixer, forage source, TMR or PMR, and X_m had no effect ($P > 0.05$) on ration variability across all five fractions (data not shown).

Table 3.6. Within farm standard deviation (SD) and coefficient of variation (CV) of particle fractions of mixed ration at 5 points along feed face on 50 dairy herds.

Fractions[1]		Standard deviation[3]			CV (%)[4]		
(mm)	Mean[2]	Mean ± SD	Min	Max	Mean ± SD	Min	Max
>26.9	33.4	2.9 ± 2.28	0.1	10.8	13.7 ± 13.25	0.1	10.8
19-26.9	4.4	0.7 ± 1.16	0.1	7.7	15.0 ± 12.56	0.1	7.7
8-19	34.9	2.1 ± 1.60	0.0	7.9	7.3 ± 8.09	0.0	7.9
4-8	11.8	0.7 ± 0.53	0.1	2.9	6.4 ± 4.59	0.1	2.9
<4	15.5	1.1 ± 1.26	0.1	5.9	8.0 ± 7.43	0.1	5.9

[1]Ration was separated into five fractions; >26.9, 19-26.9, 8-19, 4-8 and <4 mm.
[2]Average particle size distribution of MR on 50 herds.
[3]SD of each fraction at 5 sampling points at each farm.
[4]CV = (SD of each fraction at 5 sampling points at each farm/ average value of each fraction) × 100.

3.3.4. Particle size distribution of mixed rations post-feeding and diet selection

Diet selection calculated between 0hMR to 4hMR (0-4h), 4hMR to RefMR (4-24h) and 0hMR to RefMR (0-24h), demonstrated that there was selective refusal of the >26.9 and 19-26.9 mm fractions and a preferential consumption of the 8-19, 4-8 and <4 mm fractions between 0-24h period (Table 3.7), although there was considerable variation between herds. Sorting activity calculated between 0 and 4h showed preferential consumption ($P < 0.001$) for the 4-8 and 8-19 mm fraction of the MR while the >26.9, 19-26.9 and <4 mm fractions were selectively refused. The inclusion of whole-crop wheat (n = 19) and straw (n = 15), the mixer wagon type or X_m had no effect ($P > 0.05$) on the level of feed sorting (data not shown).

Table 3.7. Group level sorting[1] (% ± SD) on 50 dairy herds.

Fractions[2]	Sorting		
(mm)	0-4h	4-24h[3]	0-24h[3]
>26.9	115 ± 59.5	158 ± 98.8	165 ± 113.0
19-26.9	101 ± 10.6	117 ± 47.8	106 ± 9.0
8-19	99 ± 28.0	92 ± 39.1	89 ± 32.4
4-8	99 ± 25.7	85 ± 32.5	83 ± 36.5
<4	103 ± 52.8	96 ± 143.7	93 ± 83.6
SED	8.7	15.7	13.8
P value	<0.001	<0.001	<0.001

[1]Sorting was calculated for each fraction by dividing the proportion (DM basis) at 0hMR by the corresponding proportion at 4hMR and RefMR, and presented as a percentage. A sorting value of 100% indicated no sorting, <100% indicated preferential consumption, and >100% indicated selective refusal.
[2]Rations were separated into 5 fractions; >26.9, 19-26.9, 8-19, 4-8 and <4 mm.
[3]24h sorting activity was calculated across 33 herds, where refusals were available.

3.3.5. Association between particle size and production

There was a positive relationship (R^2 = 0.33; *P* = 0.004) between X_m and mean milk fat content (g/kg) across all herds (Figure 3.1). The relationship was improved when Holstein-Friesian (HF) and HF crosses were analysed separately (R^2 = 0.36; *P* < 0.001), with the R^2 being highest when HF herds were analysed alone, with almost 50% of the variation in milk fat content between herds being accounted for by X_m (R^2 = 0.47; *P* < 0.001). In contrast, there was a negative relationship between X_m and energy corrected milk (ECM) across the 50 dairy herds, accounting for 16% of the variation (*P* < 0.001; Figure 3.2).

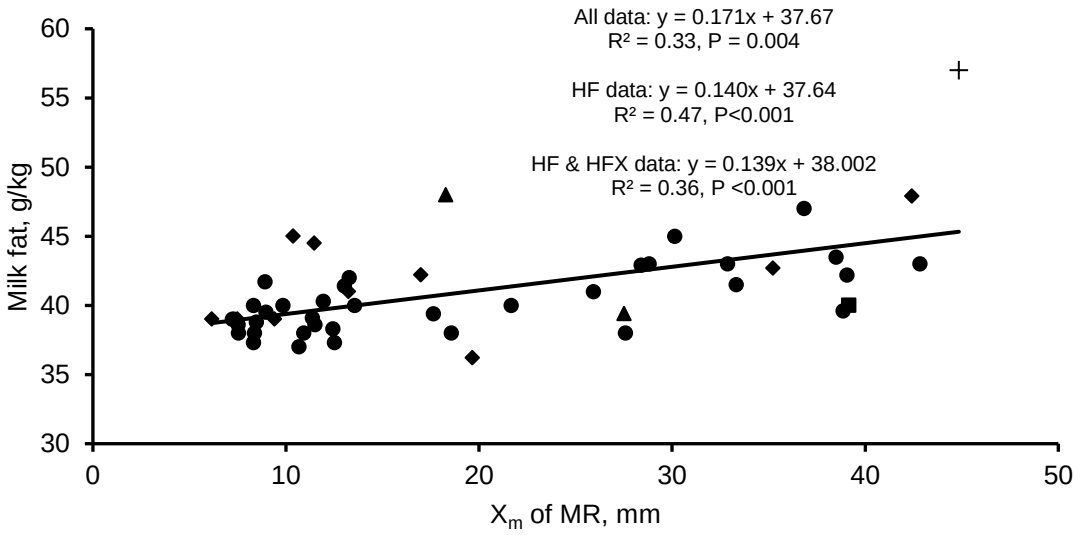

Figure 3.1. Relationship between mean particle size of mixed ration (X_m, mm) and milk fat (g/kg/herd) across 50 herds containing Holstein Friesian (●=36), Ayrshire (▲=2), Jersey (+=1), Brown Swiss (■=1) and Holstein crossbred (HFX♦=10).

3.3.6. Fresh vs dried particle size distribution

When dried prior to separation there was a difference in particle size distribution, with less long material and more short material than when measured fresh and then dried (Table 3.8). For GS the >26.9 mm fraction decreased (*P* < 0.001), while the 8-19, 4-8 and the <4 mm fractions increased (*P* < 0.001) when analysed in a dried form. Similarly, the 4-8 and <4 mm fractions of the MS increased (*P* < 0.001) when analysed in a dried compared to a wet form. For the MR, the proportion of the >26.9 mm decreased (*P* < 0.001), while the proportion of the 4-8 and the <4 mm fractions increased (*P* < 0.01) when analysed in a dried form compared to fresh and then dried.

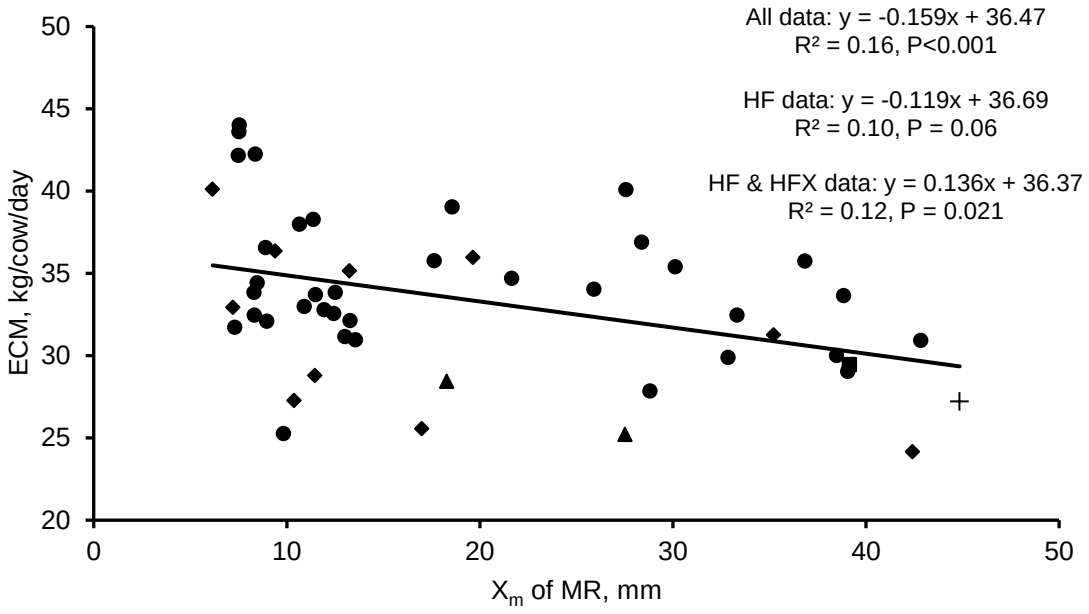

Figure 3.2. Relationship between mean particle size of mixed ration (X_m, mm) and energy corrected milk (Sjaunja et al., 1991) across 50 herds containing Holstein Friesian (●=36), Ayrshire (▲=2), Jersey (+=1), Brown Swiss (■=1) and Holstein crossbred (HFX♦=10).

Table 3.8. Comparative particle size distribution of mixed rations (n = 50), grass silage (n = 50) and maize silage (n = 34) analysed by fresh and dry shaking on 50 dairy herds.

Sample	Fractions[1] (mm)	% Dry matter		SED	*P* value
		Fresh	Dry		
Grass silage	>26.9	78.7	45.7	3.15	< 0.001
	19-26.9	2.7	2.9	0.38	0.75
	8-19	14.3	34.4	2.16	< 0.001
	4-8	2.6	10.1	0.63	< 0.001
	<4	1.7	6.9	0.57	< 0.001
Maize silage	>19	6.9	4.3	0.87	0.004
	8-19	73.2	52.6	2.37	< 0.001
	4-8	13.1	28.8	1.53	< 0.001
	<4	6.8	14.3	1.28	< 0.001
Mixed rations	>26.9	32.8	16.2	4.08	< 0.001
	19-26.9	4.4	3.5	0.54	0.10
	8-19	35.6	38.0	2.58	0.35
	4-8	12.2	21.2	1.25	< 0.001
	<4	15.0	21.1	1.88	0.002

[1]MR and GS were separated into 5 fractions; >26.9, 19-26.9, 8-19, 4-8 and <4 mm. MS was separated into 4 fractions; >19, 8-19, 4-8 and <4 mm.

3.4. Discussion

3.4.1. Herd characteristics and proximate analysis

The mean annual milk yield and herd size recorded in the current study were higher than the values reported for the UK (yield of 8180 kg and 143 cows/ herd, respectively; AHDB, 2016). This difference may be due in part to the selection criteria for the current study, with all herds recruited feeding MR and using GS, MS or a mixture as the main forage source. As a consequence, spring calving, grazed grass-based herds that have a lower mean milk yield (AHDB, 2016; Garcia and Holmes, 1999) were not used, although the trend in the UK is for more continuous housing, indoor feeding rather than grazing (March et al., 2014).

The MS being fed in the current study had a lower DM content at 300 g/kg compared to the 395 g/kg reported by Lammers et al. (1996) in the northeast of the United States of America (USA). The nutrient composition of the GS used in the current study was, however, typical of European ryegrass silage (Møller et al., 2000), with a mean CP of 136 g/kg DM and NDF of 492 g/kg DM. The mean F:C of the MR in the current study (57:43 DM basis) was higher than that reported for 50 herds in Minnesota (52:48, Endres and Espejo, 2010). A higher F:C ratio is more likely to maintain an efficient rumen function (6.2-6.8 pH) and should minimise the risk of SARA (Zebeli et al., 2012a). Twenty four out of the 50 herds fed a lower proportion of forage in the MR than the minimum of 56% proposed by Zebeli et al. (2012a), and may subsequently have been at risk of SARA.

The average DM of the MR in the current study of 373 g/kg was lower than that reported by Eastridge (2006) and Sova et al. (2013) for typical North American rations. In similar cross-sectional studies, Sova et al. (2013) reported a mean TMR DM of 477 g/kg in 22 Canadian herds, while Endres and Espejo (2010) reported a mean of 523 g/kg DM in the TMR of 50 herds in Minnesota, USA. Rations with a high DM content may increase DM intake, but may also encourage cows to sort (Leonardi et al., 2005). The CP content of the MR in the current study was also lower compared to that of 50 herds in the USA (175 g/kg DM; Endres and Espejo, 2010) or 22 herds in Canada (165 g/kg DM; Sova et al., 2013). This difference may be due to the greater use of concentrates and lower use of forages in North American rations as reflected in the lower F:C ratio (Endres and Espejo, 2010). The average NDF content of the MR in the current study was approximately 90 g/kg DM higher than that reported in the USA (298 g/kg DM; Endres and Espejo, 2010) or Canadian rations (313 g/kg DM; Sova et al., 2013). This was probably due to the greater use of forage in the current study, especially GS, which has a higher NDF

concentration than MS or lucerne haylage (Hoffman et al., 1993), but may also be affected by maturity at harvesting which increases NDF concentration (Dawson et al., 2002). The higher concentrations of NDF in the MR along with a sufficient particle size are associated with a more efficient rumen function for fibre degrading microbiota by resisting a depression in rumen pH (Zebeli et al., 2012a). Similarly, the ADF content was approximately 50 g/kg DM higher in the current study compared to that fed in the USA (198 g/kg DM; Endres and Espejo, 2010) or Canadian rations (205 g/kg DM; Sova et al., 2013), but was typical of Northern European rations (Johansen et al., 2018).

3.4.2. Ration physical characteristics

The particle size distribution of MS followed the general guidelines that are based on North American forages (Heinrichs, 2013), although the 8-19 mm fraction of MS in the current study was higher than that reported by Maulfair et al. (2010). This difference may be due to the higher moisture content of MS used in the UK that promotes the adherence of shorter particles, but may also reduce sorting (Leonardi et al., 2005). Overall, the particle size distribution of MS in the UK was similar to the current guidelines for MS based on North America rations, and consequently, there is little requirement for separate recommendations for UK and northern European based MS. Out of the 50 herds used in the current study, the minimum % DM of GS retained on the >19 mm sieve was 49%, considerably higher than the 10-20% guidelines for lucerne haylage in the USA (Heinrichs, 2013). Feeding a longer particle size may result in a higher rumen pH and avoid SARA, but is also associated with a reduction in feed intake due to a greater rumen fill (Tafaj et al., 2007; Zebeli et al., 2012a).

The mean particle size distribution of the 0hMR in the current study differed from the guidelines based on North American rations (Heinrichs, 2013), with the long (>19 mm) particle size distribution being 38%, approximately 50% higher than that reported by Sova et al. (2013), DeVries et al. (2011) or Hosseinkhani et al. (2008), and approximately 4 times higher than that reported by Heinrichs (2013), Endres and Espejjo (2010) or Miller-Cushon and DeVries (2009), or Wisconsin University Guidelines (Heinrichs and Kononoff, 1996; Table 3.9). The difference in particle size distribution of MR in the current study reflected the high inclusion of GS that contained a very long particle size (>19 mm = 80% DM, X_m = 42.6 mm). The use of other forages (e.g. whole-crop wheat, wheat straw, fodder beat) in the MR did not significantly affect the particle size distribution of the MR in the current study, and supports that the high proportion of GS in the ration was the major factor causing the differences. The higher proportion of the 26.9-44 and 8-19 mm particle fractions in the MR may also be explained by the high moisture content, as

4-8 and <4 mm particles may have adhered to longer particles (Leonardi et al., 2005). However, the current findings suggest a need for more specific particle size distribution recommendations when wetter GS is the major forage in the MR, or an alternative method of particle size evaluation is required.

When GS was the sole forage in the MR, rations had a higher proportion of the 26.9-44 and 44-60 mm fractions which may promote ration sorting (DeVries et al., 2007), although in the current study there was no relationship between X_m and degree of sorting after 4 or 24 h. The additional 26.9, 44 and 60 mm pore size sieves used in the PSPS in the current study allowed a more even distribution of particle size for GS and MR samples than the traditional PSPS. However, as a very small proportion of particles was retained on the 19-26.9 mm screen, a screen larger than 26.9 mm may be more appropriate.

Table 3.9. The particle size distribution (%DM) of North American rations.

Fractions (mm)	Sova et al. (2013)	Endres and Espejjo (2010)	DeVries et al. (2011)	Hosseinkhani et al. (2008)	Miller-Cushon and DeVries (2009)	White et al. (2017)
>19	19.8	10.9	18.4	18.1	8.8	11.4
8-19	34.3	41.5	33.6	32.3	46.6	31.9
4-8	35.5	47.6	48.0	49.6	44.5	56.7
<4	10.5	-	-	-	-	-

3.4.3. Variability in ration mixing

Feeding MR is an effective method to provide all the required nutrients to dairy cows, and a properly mixed ration ensures a uniform delivery of all feed ingredient to the animal (Coppock et al., 1981). Mixer wagons and mixing protocols can however, influence particle size distribution and result in differences in feed intake and milk yield, particularly for rations with longer chop lengths (Humphries et al., 2010). Heinrichs et al. (1999) also reported that processing by the mixer wagon prior to feed-out can have a large effect on the particle size and *peNDF* subsequently fed and the consistency of the mix. In a survey of Iranian herds, Esmaeili et al. (2016) reported a high variability (CV >10%) in particle size distribution of MR with the highest variation recorded for the >19 mm fraction, a finding in agreement with the current study. There were 42% of herds that had a CV ≤5% (indicating a well-mixed ration; $P < 0.001$), 26% that had a CV of between 5-10% (moderately mixed; $P < 0.001$), and 32% that had a CV >10% (poorly mixed ration; $P < 0.001$). There was no effect ($P > 0.05$) of mixer model on overall ration variability across all herds. In contrast, Heinrichs et al. (1999) reported that MR processing by the

mixer wagon can have a significant effect on the ration consistency, particle size and *peNDF* concentrations of the ration subsequently consumed.

3.4.4. Herd level diet selection

Herd level diet selection was calculated as the proportional change in each fraction of the MR over time post-feeding. Feed sorting activity is usually associated with the preferential consumption of fine starch or protein rich particles in the ration (DeVries et al., 2007). However, in the current study, there were selective refusals for the >19 mm fraction and preferential consumption for the <8 mm fraction. Of the 50 herds, 82% had either selective refusal or did not show preferential consumption ($P < 0.001$) for the >19 mm fraction which may be associated with the inclusion of long particles of GS. There was no sorting activity observed for the <8 mm fraction in 46% of the herds. As discussed previously, this may have been due to the comparatively high moisture content of the MR in the current study that caused the cohesion of smaller particles to larger particles making it more difficult to sort (Beauchemin, 1991; Fish and DeVries, 2012; Leonardi et al., 2005).

3.4.5. Associative effects of particle size and production

Several authors have reported a relationship between *peNDF* and milk performance (Tafaj et al., 2007; Zebeli et al., 2012). In the current study there was also a positive relationship between $peNDF_{>4mm}$ or $peNDF_{>8mm}$ and milk fat ($R^2 = 0.14$ and $R^2 = 0.16$; $P < 0.01$, respectively) although these were not as strong as with X_m. The positive relationship between X_m and milk fat content, and the negative relationship with milk yield is in agreement with De Brabander et al. (1999). A long fibrous particle size is associated with an increase of acetic acid production in the rumen that can subsequently lead to a higher milk fat content (Merten, 1997). Alternatively, a higher fibre ration may increase rumen pH and reduce the ruminal production of *trans*-10, *cis*-12 conjugated linoleic acid that has been associated with milk fat reduction (Harvatine and Bauman, 2011). Contrary to our findings, Tafaj et al. (2007) reported no correlation between particle size and milk yield or milk components and suggested that any effect of particle size on milk yield mainly depends on its influence on DM intake, which was not measured in the current study.

3.4.6. Comparison of fresh and dry separation

Compared with when measured fresh, the particle size distribution of dried forages and MR differed, with the proportion of longer fractions decreasing while short fractions increased after drying of samples (Kononoff et al., 2003). This difference may

be attributed to the wetter forages and rations used resulting in adherence of short particles to larger particles, or the physical reduction in particle size due to the shaking when undertaken dry. It is therefore recommended to partially or completely dry the forages and MR before analyses in order to overcome the moisture variation (Heinrichs, 2013). However, this may not be a practical way of measuring particle size of wetter forages and MR on-farm.

3.5. Conclusions

The particle size distribution of GS and MR based on GS in UK dairy herds was found to be considerably higher than current guidelines that are based on North American forages and rations. This suggests that the particle size of UK dairy rations is either too long, or that new guidelines or methods of particle size evaluation for GS and GS/MS based MR in Northern Europe are required. The poor consistency of mixing and high degree of selection recorded on the majority of herds is of concern, and further research into reasons for this variation and its impact on cow performance is required. Finally, the high use of concentrates by 50% of the herds in the current study is a potential threat to SARA and reiterates the need for more appropriate means of particle size characterisation and guidelines for wetter, GS based dairy rations, with further controlled studies required to determine the optimal particle size distribution of these rations.

CHAPTER 4a: Grass silage particle size when fed with or without maize silage alters performance, reticular pH and metabolism of Holstein-Friesian dairy cows

4a.1. Introduction

The increased milk production of dairy cows in many Western countries such as the United Kingdom (UK) has required an increase in the level of concentrate supplementation and the production of high-quality forages, with a trend towards lower dietary fibre levels (March et al., 2014). The consequences of these dietary changes include an increased risk of metabolic disorders such as sub-acute ruminal acidosis (SARA), displaced abomasum, milk fat depression, laminitis, reduced fibre digestion and fat cow syndrome (Plaizier et al., 2008). The particle size of the diet has been proposed as a key factor, along with forage fibre and non-forage carbohydrate concentration to ensure a healthy rumen function and maintain animal performance (Zebeli et al., 2012a). Additionally, optimal rumen fermentation can lead to an increase in the microbial protein and metabolisable protein supply to the small intestine and therefore enhance milk protein yield (Sinclair et al., 2014).

A short forage particle size when included in total mixed rations (TMR) based on lucerne and maize silage has been shown to increase DM intake (DMI) and milk protein yield (Tafaj et al., 2007; Zebeli et al., 2012a), but may result in a reduction in rumination, eating and total chewing time, as well as rumen pH (Tafaj et al., 2007). In contrast, a longer particle size produced a higher milk fat concentration (Mertens, 1997), but can also promote feed sorting, resulting in some cows receiving excess concentrates and others insufficient (Kononoff and Heinrichs, 2003). However, the effects of particle size in grass silage (GS) based TMR on intake and milk production are inconsistent, mainly due to differences in the particle size and physically effective fibre (peNDF; particles long enough to stimulate rumination, Mertens, 1997) measurement procedure.

In a recent study to determine the range of particle size of grass and maize silages and MR fed to dairy cows on commercial farms in the UK (Chapter 3), it was reported that the MR fed on UK dairy herds had more longer (>19 mm) particles than recommended for North American diets, and that the difference in particle size distribution was principally due to the inclusion of GS (Chapter 3). There is however, a lack of information on the effects of particle size of GS based diets on dairy cow performance. Additionally, the greater inclusion of wheat and barley that are more commonly fed in Europe (AHDB, 2017) and are rapidly degraded in the rumen (Offner et al., 2003) enhances the risk of SARA and increases the importance of particle size and peNDF. The hypothesis of the current study was that dairy cows fed diets with a short compared to a long particle size

of GS when fed with or without maize silage (MS) would increase intake and milk production but decrease rumen pH and milk fat content. The objectives of the study were to determine the effect of chop length of GS when fed at different ratios of GS:MS on the intake, performance, reticular pH, diet digestibility, metabolism and eating behaviour in Holstein-Friesian dairy cows.

4a.2. Materials and Methods

All the procedures involving animals were conducted in accordance with the UK Animal (Scientific Procedures) Act (1986; amended 2012) and received local ethical approval from Harper Adams University.

4a.2.1. Animals, housing, forages, diets and experimental routine

Sixteen early lactation (60 ± 10.6 days in milk) multiparous Holstein-Friesian dairy cows producing 41.9 ± 3.86 kg (mean ± SD) of milk per day and weighing 675 ± 60.9 kg at the beginning of the study (16th January 2017) were used in a 4 × 4 Latin square design with four periods, each of 28-days duration, with measurements undertaken during the final 12-days of each period. At the start of the experiment, cows were blocked according to milk yield and randomly assigned to one of four dietary treatments. The cows were housed in a building containing free stalls fitted with mattresses and had free access to water.

A first cut perennial ryegrass (*Lolium perenne*) sward was mown at a leafy stage on the 25th May 2016, wilted for 24 h and then alternate windrows harvested using a precision chop self-propelled forage harvester (John Deere 7840i, Nottinghamshire UK) at two different settings to provide a theoretical chop length of 10 mm (short chop) or 44 mm (long chop). An additive (Axphast Gold, Biotal, Worcestershire, UK) was applied at the rate of 2 litres per tonne to each GS which were ensiled in separate roofed concrete clamps. Maize silage (*Zea mays*) was harvested on the 10th October 2016 using the same forage harvester as the GS to provide a theoretical chop length of 15 mm. A silage additive (Maizecool Gold, Biotal, Worcestershire, UK) was applied at 2 litres per tonne, and the MS ensiled in a concrete clamp.

The two GS (short or long) and two ratios of GS:MS (100:0 or 40:60 respectively, DM basis) were used to formulate four diets (Table 4a.1). The dietary treatments were: long chop GS (LG); short chop GS (SG); long chop GS and MS (LM) and short chop GS and MS (SM). All diets were fed as a TMR with a forage to concentrate ratio of 54:46 (DM basis) to provide a similar metabolisable energy and protein content using Feed into Milk formulation software (Thomas, 2004). Diet mixing and feeding protocol was adopted after

Sinclair et al. (2015) using 16 Hokofarm roughage intake feeders (RIC feeders, Marknesse, Netherlands). Fresh feed was offered daily at 1000 h at the rate of 1.05 of *ad-libitum* intake, with refusals collected 3-times/week prior to feeding. Forages were sampled twice weekly (Sinclair, 2006); one sample was oven dried at 105℃ and the ratio of GS to MS adjusted to the desired level, while the second sample was stored at -20℃ for subsequent analysis. Samples of all four TMR were collected from feed bins daily during the final week of each period and stored at -20℃ for subsequent analysis.

Table 4a.1. Dietary inclusion (kg/kg DM) and predicted nutrient composition for diets fed to cows that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM).

Ingredients	LG	SG	LM	SM
Maize silage	0	0	0.323	0.323
Short grass silage	0	0.537	0	0.214
Long grass silage	0.537	0	0.214	0
Rapeseed meal	0.017	0.017	0.064	0.064
Wheat distillers	0.017	0.017	0.064	0.064
Palm kernel cake	0.005	0.005	0.018	0.018
Molasses	0.001	0.001	0.005	0.005
Caustic wheat	0.175	0.175	0.122	0.122
Soyhulls	0.105	0.105	0.083	0.083
Soybean meal	0.055	0.055	0.086	0.086
Megalac[1]	0.015	0.015	0.004	0.004
Sopralin[2]	0.068	0.068	0.009	0.009
Minerals/vitamins[3]	0.007	0.007	0.007	0.007
Predicted composition				
ME (MJ/kg DM)[4]	12.0	12.0	12.1	12.1
MPN (g/kg DM)[5]	121	121	119	119
MPE (g/kg DM)[6]	103	103	103	103

[1]A rumen protected source of fat (Volac, Royston, UK).
[2]A rumen protected source of soybean (NWF Agriculture, Cheshire, UK).
[3]Mineral/vitamins premix (KW Alternative Feeds, Leeds, UK) providing (g/kg) 220 calcium, 30 phosphorus, 80 magnesium, 80 sodium, (mg/kg) 760 copper, 30 selenium, 1000000 IU vitamin A, 300000 IU vitamin D_3, 3,000 IU vitamin E, 2.5 mg/kg vitamin B_{12}, 135 mg/kg biotin.
[4]ME, metabolisable energy.
[5]MPN, metabolisable protein-rumen nitrogen limited.
[6]MPE, metabolisable protein-rumen energy limited.

Cows were milked twice daily at 0700 and 1700 h with milk yield recorded at each milking and samples taken during the final week of each period (two morning and two evening milkings) for subsequent analysis. Body condition score (BCS, Ferguson et al., 1994)

and live weight were recorded after the evening milking during the week prior to commencing the study and then at the end of each period. Whole tract apparent digestibility was estimated using acid insoluble ash as an internal marker (Van Keulen and Young, 1977) with faecal samples collected at 1000 and 1600 h for five consecutive days during the final week of each period, and stored at –20℃ prior to subsequent analysis.

4a.2.2. Reticular pH and blood collection

To determine reticular pH, pH boluses (eCow® Devon Ltd, Exeter Devon, UK) were administered orally to all cows one week prior to data collection. Boluses were calibrated prior to administration by immersing in warm water (39℃) for 30 min according to the manufacture's instructions. Data were recorded every 15 min, and downloaded at the end of each period. A second set of pH boluses were administered to all cows during the first week of the 3rd period to monitor reticular pH during periods 3 and 4. Blood samples were collected from 12 cows (3 per treatment) by jugular venepuncture over 2-days during the collection week at 0900, 1100, 1230 and 1400 h, centrifuged at 3000 g for 15 min, the plasma extracted and stored at -20℃ prior to subsequent analysis.

4a.2.3. Particle size distribution and eating behaviour

The particle size distribution of the fresh TMR was measured by collecting samples 5 min post-feeding on days 20 to 25 of each period and using a modified Penn State Particle Separator (PSPS) with 5 sieve screens of size 44, 26.9, 19, 8, and 4 mm at 0, 4, 8 and 24 h (Section 2.16). A manual shaking procedure was adopted (Kononoff et al., 2003), and each diet was separated into six fractions; >44, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm. The geometric mean particle size (X_m) was calculated using the method described by ASABE (2007) as;

$$Geometric\ mean\ length\ (Xm) = \log^{-1} \frac{\sum(Mi\ \log mXi)}{\sum Mi} \qquad \text{(Equation 1)}$$

With the standard deviation of X_m determined as;

$$Standard\ deviation\ (SDgm) = \log^{-1} \left[\frac{\sum Mi\ (\log mXi - logXg)2}{\sum Mi}\right]^{1/2} \qquad \text{(Equation 2)}$$

Where; X_i is diagonal of screen opening of the i^{th} screen, $X_{(i-1)}$ is diagonal of screen opening in the next larger than the i^{th} screen, X_m is geometric length (particle size), mX_i is mean geometric length of particles on i^{th} screen = $[X_i \times X_{i-1}]^{1/2}$, M_i is mass on i^{th} screen.

Jaw movement (eating, ruminating and idling) was visually recorded for 48 h commencing at 0530 h on day-18 of each period by instantaneous scan monitoring of all cows at 5 min intervals (Martin and Bateson, 2007). All observers were trained for 1 h before the start of the study with a 96% similarity index achieved. Observations were conducted using 2 observers for a duration of 4 h to minimise fatigue and enhance accuracy (Martin and Bateson, 2007).

4a.2.4. Chemical analysis

Forage and TMR samples were analysed for DM (934.01), CP (intra-assay CV of 2.3%) and ash, while NDF (using heat-stable α-amylase; Sigma, Gillingham, UK), ADF and ADL (intra-assay CV of 1.4 and 1.3% for NDF and ADF respectively) were analysed according to Van Soest et al. (1991) and expressed exclusive of residual ash (Section 2.1 to 2.6). Starch concentration was analysed using the procedure described by McCleary et al. (1997). Milk samples were analysed using a Milkoscan Minor analyser (Foss, Denmark). Plasma samples were analysed for glucose, β-hydroxybutyrate (3-OHB) and urea (Randox Laboratories, County Antrim, UK; kit catalogue no. GL1611, RB1008 and UR221 with an intra-assay CV of 0.6, 4.5 and 2.3%, respectively) using a Cobas Miras Plus autoanalyser (ABX Diagnostics, Bedfordshire, UK; Section 2.10). Faecal samples were pooled for each cow within each period, dried and analysed for acid insoluble ash (Section 2.8), nitrogen, NDF and ADF. Forage pH was determined using a pH meter (HI 2210, Hanna Instruments, Bedfordshire UK) after suspending 50 g forage in 100 ml distilled water for 30 min. Milk FA were determined according to procedure described in Section 2.12. Feed FA were determined by the procedure described by Jenkins (2010).

4a.2.5. Calculations and statistical analysis

All data were tested for normality using the general descriptive statistics and analysed as a Latin Square Design with a 2 × 2 factorial treatment structure using GenStat 17.1 (VSN International Ltd., Oxford, UK), with main effects of chop length (C), forage ratio (F) and their interaction (C × F). The model used was: $Y = \mu + C_i + F_j + C{\times}F_{ij} + P_j + A_k + €_{ijk}$, where Y is the observation, μ the overall mean, C_i is the chop length effect, F_j is the forage ratio effect, $C{\times}F_{ij}$ is the interaction between chop length and forage ratio, P_j the fixed period effect, A_k the random effect of animal and $€_{ijk}$ the residual error. Blood plasma, rumen pH and sorting activity data were analysed as repeated measures ANOVA. Results were reported as treatment means with SED, with the level of significance set at $P<0.05$ and a tendency stated at $P<0.1$.

4a.3. Results

4a.3.1. Forage and feed composition

The nutrient composition of the long and short chop GS were similar with a mean DM, CP and NDF concentration of 201 g/kg, 121 and 487 g/kg DM respectively, whilst the GS had a lower DM concentration, but a higher NDF and CP concentration than the MS (Table 4a.2). The mean particle size (X_m) of the long chop GS was 13.3 mm more than the short GS, with the MS having the shortest X_m. The MS based diets (LM and SM) had a higher DM compared to the GS based diets (LG and SG), but all four diets had a similar CP content, with a mean value of 174 g/kg DM. The GS based diets had a higher ash, NDF and ADF content compared to the MS based diets. The mean X_m of the GS based diets was 10.5 mm greater than the MS based diets, and was 9 mm less for the short chop than the long chop GS based diets. The $peNDF_{>4mm}$ was also higher for the GS than the MS based diets.

4a.3.2. Production performance

Average DMI was 3.2 kg/day lower ($P < 0.001$) in cows when fed the GS than the MS based diets (Table 4a.3). The short chop length diets resulted in a 0.9 kg DM/day higher ($P = 0.035$) intake in cows compared to the long chop length diet. Cows fed the GS based diets produced 2.4 kg/day less ($P < 0.001$) milk than when fed diets containing grass and maize silages (Table 4a.3). There was an interaction ($P = 0.011$) between chop length and forage ratio on milk yield, with a short chop length increasing yield in cows when fed GS but not MS based diets. There was a tendency ($P = 0.09$) for a higher milk fat content in cows when fed the long chop length diets. Live weight change was 0.85 kg/day higher ($P < 0.001$) in cows when fed the MS compared to the GS based diets, and there was a tendency ($P = 0.065$) for a lower live weight gain in cows when fed long chop compared to the short chop length diets.

Table 4a.2. Nutrient composition (g/kg DM), fatty acid profile and particle size of grass silage (long chop, LCG and short chop, SCG), maize silage (MS) and diets fed to diets that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM) or short chop grass and maize silages (SM).

	LCG	SCG	MS	LG	SG	LM	SM
DM (g/kg)	198	204	350	308	307	368	380
CP	120	122	81	170	176	176	175
Ash	71	73	39	92	92	71	68
OM	929	927	961	908	908	929	932
NDF	484	490	366	392	384	342	339
ADF	327	331	229	256	261	211	209
ADL	-	-	-	24	25	29	28
Starch	-	-	291	127	133	201	197
ME (MJ/kg)	10.9	10.8	12.0				
Fermentation characteristics (g/kg)							
pH	4.13	4.06	3.80				
NH_3-N (g/kg total N)	71	68	62				
Acetate	62.6	26.5	34.6				
Propionate	0.3	0.1	1.1				
Iso-butyrate	0.0	0.0	0.1				
Butyrate	0.3	0.3	0.1				
Lactate	114	140	48				
Fatty acids (g/100 FA)							
C16:0	4.0	3.8	4.8	14.1	15.6	9.1	9.6
C18:0	0.5	0.4	1.2	1.5	1.7	1.3	1.4
C18:1*c*9	0.3	0.3	3.4	3.5	3.9	4.2	4.4
C18:2*n*-6	0.5	0.5	1.5	1.2	1.3	2.4	2.4
C18:3*n*-3	4.7	5.5	0.9	3.3	3.7	2.3	2.3
∑ FA	13.2	13.6	17.4	26.2	28.4	28.2	28.3
Fractions (%DM)							
>44 (mm)	28.6	4.1	-	15.6	-	0.1	-
26.9-44 (mm)	54.7	25.3	-	32.9	16.3	21.0	3.0
19-26.9 (mm	3.9	5.7	14.0	4.9	4.5	3.7	3.3
8-19 (mm)	9.2	54.1	76.3	17.2	48.2	32.6	52.1
4-8 (mm)	2.3	8.5	8.3	17.1	18.7	19.5	19.6
<4 (mm)	1.3	2.3	1.4	12.3	12.3	23.1	21.9
X_m (mm)	44.2	30.9	12.8	26.9	10.4	8.9	7.5
SD_{gm}	1.15	1.89	1.57	2.5	2.3	2.7	2.2
$pef_{>4mm}$ (%)	98.7	97.7	98.6	87.7	87.7	76.9	78.1
$pef_{>8mm}$ (%)	96.4	89.2	90.3	70.6	69.0	57.4	58.5
$peNDF_{>4mm}$ (%)	47.7	47.8	36.1	34.4	33.6	26.1	26.7
$peNDF_{>8mm}$ (%)	46.6	43.7	33.0	27.7	26.5	19.5	20.0

OM = organic matter; NH_3-N = ammonia nitrogen; ME = metabolisable energy; X_m = geometric mean particle size; SD_{gm} = SD of X_m; *pef* = physical effectiveness factor; *peNDF* = physically effective fibre

Table 4a.3. Intake and performance of dairy cows fed diets that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM).

	Treatments					*P*-value		
	LG	SG	LM	SM	SED	C	F	C × F
DM intake (kg/day)	20.0	20.5	22.8	24.0	0.56	0.035	<0.001	0.335
Milk yield (kg/day)	37.3	39.1	41.1	40.5	0.63	0.179	<0.001	0.011
Feed efficiency[1]	1.89	1.92	1.81	1.69	0.058	0.272	<0.001	0.071
4% FCM[2] (kg/day)	37.3	37.5	40.1	38.9	1.11	0.477	0.012	0.376
Milk fat (g/kg)	40.1	38.5	39.5	38.6	0.93	0.090	0.560	0.418
Milk fat (kg/day)	1.49	1.50	1.60	1.55	0.044	0.477	0.012	0.376
Milk protein (g/kg)	30.9	30.7	32.3	32.4	0.28	0.738	<0.001	0.461
Milk protein (kg/day)	1.16	1.20	1.33	1.31	0.023	0.432	<0.001	0.085
Milk lactose (g/kg)	45.8	46.2	45.5	45.7	0.26	0.095	0.058	0.709
Milk lactose (kg/day)	1.72	1.81	1.87	1.85	0.033	0.122	<0.001	0.029
Live weight (kg)	668	671	683	693	4.6	0.065	<0.001	0.339
Live weight change (kg/day)[3]	-0.35	-0.41	0.15	0.79	0.277	0.144	<0.001	0.078
Body condition score	2.41	2.52	2.51	2.74	0.060	<0.001	<0.001	0.138
Body condition score change[2]	-0.07	-0.09	-0.12	0.16	0.120	0.145	0.256	0.088

C = chop length; F = grass to maize silage ratio; C×F = interaction between C and F.
[1]Feed efficiency = kg milk/ kg DMI.
[2]FCM = fat corrected milk.
[3]Change over the 28-day period.

4a.3.3. Estimated whole tract digestibility

There was an interaction for DM (P = 0.019) and OM (P = 0.022) digestibility, where the short chop length increased digestibility in cows when fed the GS but not the MS based diets (Table 4a.4). There was also an interaction (P = 0.003) for N digestibility, where a short chop length increased N digestibility when cows were fed the GS based diets, and decreased digestibility when fed the MS based diet. Digestibility of NDF was 0.228 kg/kg higher (P < 0.001) in cows when fed the GS compared to the MS based diets, and there was an interaction (P = 0.014) between chop length and forage ratio on ADF digestibility, where a shorter chop length GS increased digestibility for the GS based diet, and decreased digestibility for the MS based diet.

Table 4a.4. Digestibility in dairy cows fed diets that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM).

	Treatments					*P*-value		
	LG	SG	LM	SM	SED	C	F	C × F
DM (kg/day)								
Intake	20.0	20.5	22.8	24.0	0.56	0.036	<0.001	0.335
Faecal output	6.74	5.36	8.19	8.89	0.518	0.363	<0.001	0.008
Digest.(kg/kg)	0.659	0.739	0.639	0.629	0.0257	0.063	0.001	0.019
OM (kg/day)								
Intake	18.2	18.6	21.2	22.4	0.51	0.025	<0.001	0.275
Faecal output	5.80	4.58	7.25	7.93	0.485	0.435	<0.001	0.009
Digest.(kg/kg)	0.677	0.754	0.656	0.645	0.0262	0.084	0.001	0.022
N (g/day)								
Intake	554	554	634	671	16.9	0.134	<0.001	0.133
Faecal output	161	127	166	189	10.00	0.477	<0.001	<0.001
Digest.(kg/kg)	0.709	0.772	0.737	0.719	0.0177	0.082	0.326	0.003
N efficiency[1]								
NDF (kg/day)								
Intake	8.09	7.89	7.77	8.28	0.245	0.374	0.846	0.049
Faecal output	3.08	2.65	4.50	4.92	0.244	0.993	<0.001	0.020
Digest.(kg/kg)	0.614	0.666	0.418	0.407	0.0290	0.323	<0.001	0.140
ADF (kg/day)								
Intake	5.10	5.36	4.85	5.01	0.154	0.062	0.010	0.659
Faecal output	1.93	1.71	2.83	3.03	0.127	0.957	<0.001	0.103
Digest.(kg/kg)	0.582	0.681	0.417	0.389	0.0243	0.149	<0.001	0.014

C = chop length; F = grass to maize silage ratio; C × F = interaction between C and F; DM = dry matter; Digest = digestibility; OM = organic matter; N = nitrogen. [1]N efficiency = milk N output/ total N intake.

4a.3.4. Reticular pH and eating behaviour

Reticular pH was highest prior to the morning feeding in all treatments and then declined with time (P < 0.001; Figure 4a.1). There was a time × forage ratio interaction on reticular pH, which was lower in cows fed MS for most of the day except around fresh feed delivery, but there was no effect of GS chop length. When cows were fed the GS based diets the mean minimum reticular pH was 0.1 higher (P = 0.001) than when fed the MS based diet (Table 4a.5). Cows fed the MS based diets also spent a longer time at reticular pH levels below pH 6.2 and 6.5 (P = 0.003) compared to the GS based diets. Cows spent 1.1 h/day longer eating (P < 0.001) when offered the GS compared to the MS based diets and 0.9 h/day longer (P = 0.003) eating the long chop compared to the short chop GS (Table 4a.5). Similarly, eating time (ET) was 4.7 min/kg DM higher when cows were fed the GS compared to the MS based diets (P < 0.001), and 2.4 min/kg DMI higher (P < 0.05) when fed the longer compared to the shorter GS. There was an interaction (P < 0.05) for rumination time (RT; h/day), with the shorter chop length increasing the RT in cows when fed the GS but not the MS based diets, whereas when expressed on a min/kg DMI, a shorter chop length increased RT on the GS and decreased RT on the MS based diets. The particle size distribution of fractions 8-19 and 4-8 mm decreased (P < 0.05) with time post-feeding, and the DM proportion of the 26.9-44 mm fraction was higher (P < 0.001) for diets that contained long chop GS or when mixed with MS (Table 4a.6).

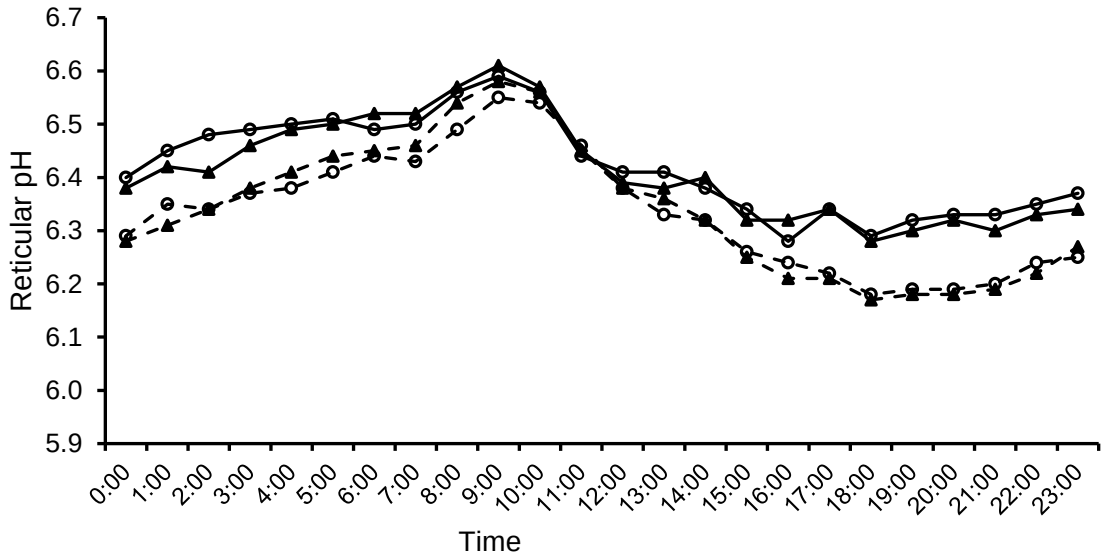

Figure 4a.1. Hourly reticular pH in cows fed diets that contained long chop grass silage (LG;--o--); short chop grass silage (SG; –▲–); long chop grass and maize silages (LM; --o--), or short chop grass and maize silages (SM; --▲--). (SED, 0.042; Time, *P*<0.001; forage ratio, *P*=0.003; Time×F, *P*<0.001).

Table 4a.5. Reticular pH and eating behaviour of dairy cows fed diets that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM).

	Treatments					*P*-value		
	LG	SG	LM	SM	SED	C	F	C x F
Daily minimum pH	5.99	5.98	5.90	5.87	0.039	0.421	0.001	0.594
Daily maximum pH	6.82	6.84	6.76	6.82	0.038	0.175	0.128	0.497
Mean pH	6.42	6.41	6.33	6.34	0.035	0.998	0.001	0.775
% time <5.8 pH[1]	0.93	0.41	0.42	0.37	0.471	0.401	0.422	0.492
% time <6.0 pH	4.91	3.85	5.42	6.37	2.863	0.979	0.460	0.622
% time <6.2 pH	14.5	17.1	27.0	27.8	5.11	0.643	0.003	0.795
% time <6.5 pH	63.9	65.9	81.0	77.9	6.27	0.902	0.003	0.572
Eating (h/d)	5.8	4.9	4.6	4.0	0.30	0.003	<0.001	0.463
Eating (min/kg DMI)	17.3	14.5	12.2	10.3	0.96	0.021	<0.001	0.520
Rumination (h/d)	9.3	10.0	10.1	10.0	0.23	0.084	0.013	0.029
Rumination (min/kg DMI)	28.1	29.2	26.8	25.3	0.79	0.709	<0.001	0.026
Chews/bolus (n)	54	65	59	69	2.3	<0.001	0.011	0.768

C = chop length; F = forage ratio; C×F = interaction between C and F

[1]Average percentage of time cows spent below each pH level

4a.3.5. Milk fatty acids and blood metabolites

Cows fed the short chop length diets had a 0.04 g/100g higher milk fat C18:3*n-3* concentration (P < 0.001), whereas, those receiving the long chop length diets had a 0.05 g/100g higher concentration of *cis*-9, *trans*-11 conjugated linoleic acid (CLA; P = 0.032; Table 4a.7). For cows fed the GS based diets, milk concentrations of C16:0, C16:1*n-7*, C18:1*c*9 and C18:3*n-3* were higher (P < 0.05), compared to when the MS based diets were fed. In contrast, milk from cows fed the MS based diets had a higher (P < 0.01) concentration of C10:0, C12:0, C14:1, C18:0, C18:1*trans*-8, C18:1*trans*-9, C18:1*trans*-12, C18:2*n-6* and total polyunsaturated FA (P = 0.015) compared to when fed the GS based diets.

Plasma glucose concentration decreased (P < 0.001) post feeding (Figure 4a.2a) and was 0.17 mmol/l higher (P = 0.008) in cows when fed the MS compared to the GS based diets. Plasma 3-OHB concentrations increased (P < 0.001) with time post-feeding, but there was no effect of chop length or forage ratio (Figure 4a.2b). Similarly, plasma urea concentration increased (P = 0.004) post-feeding to a maximum at 1230 h, with cows fed the MS based diets having a 0.86 mmol/l higher (P < 0.001) concentration than when fed the GS based diet (Figure 4a.2c).

Table 4a.6. Particle size distribution of diets fed to cows that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM) or short chop grass and maize silages (SM) at 0, 4, 8 and 24h post feeding.

	DM %					*P*-value			
Fractions[1]	LG	SG	LM	SM	SED	Time	C	F	C x F
>44 mm									
0h	15.6	-	0.1	-	0.87	0.234	<0.001	<0.001	<0.001
4h	17.7	-	0.1	-					
8h	17.5	-	0.1	-					
24h	19.3	-	0.2	-					
26.9-44 mm									
0h	32.9	16.3	21.0	3.0	1.15	0.107	<0.001	<0.001	0.051
4h	32.0	16.4	22.2	3.3					
8h	33.4	17.1	22.8	3.4					
24h	33.0	19.5	23.9	2.4					
19-26.9 mm									
0h	4.9	4.5	3.7	3.3	0.28	0.056	0.008	<0.001	0.475
4h	5.3	4.3	3.9	3.4					
8h	5.1	4.1	3.5	3.4					
24h	5.3	4.7	4.4	3.4					
8-19 mm									
0h	17.2	48.2	32.6	52.1	0.73	0.035	<0.001	<0.001	<0.001
4h	16.9	48.4	32.6	50.5					
8h	16.4	47.7	31.2	50.4					
24h	16.1	47.3	31.0	52.4					
4-8 mm									
0h	17.1	18.7	19.5	19.6	0.66	<0.001	0.019	<0.001	0.217
4h	16.0	19.2	18.2	20.0					
8h	15.3	18.9	17.3	19.6					
24h	14.4	18.0	15.6	19.1					
<4 mm									
0h	12.3	12.3	23.1	21.9	0.63	0.123	0.542	<0.001	0.128
4h	12.2	12.8	22.1	22.8					
8h	12.4	13.7	23.5	23.2					
24h	12.3	13.0	22.8	22.6					

C = chop length; F = forage ratio; C × F = interaction between C and F.
[1]Diets were separated into 6 fractions; >44, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm.

Table 4a.7. Milk fatty acids profile (g/100 g FA) in cows fed diets containing long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM) or short chop grass and maize silages (SM).

	Treatments					*P*-value		
	LG	SG	LM	SM	SED	C	F	C x F
C4:0	1.36	1.70	1.32	1.33	0.103	0.021	0.009	0.031
C6:0	1.20	1.38	1.22	1.22	0.073	0.070	0.198	0.084
C8:0	0.89	0.94	0.98	0.96	0.039	0.436	0.069	0.224
C10:0	2.11	2.13	2.58	2.50	0.050	0.389	<0.001	0.224
C12:0	3.01	3.08	3.93	3.80	0.090	0.730	<0.001	0.111
C14:0	12.26	12.44	13.62	13.18	0.199	0.350	<0.001	0.034
C14:1	1.02	1.03	1.16	1.11	0.039	0.627	<0.001	0.240
C15:0	1.32	1.28	1.38	1.31	0.050	0.131	0.268	0.733
C16:0	42.20	42.78	40.53	39.83	0.716	0.905	<0.001	0.215
C16:1	0.52	0.52	0.50	0.52	0.015	0.324	0.257	0.392
C16:1*n*-7	1.23	1.19	1.15	1.15	0.036	0.551	0.026	0.441
C17:0	0.66	0.60	0.59	0.60	0.027	0.278	0.093	0.106
C17:1	0.21	0.21	0.21	0.21	0.014	0.890	0.934	0.696
C18:0	9.15	9.86	9.38	9.94	0.298	0.381	0.008	0.084
C18:1 *t*8	0.23	0.22	0.26	0.27	0.009	0.855	<0.001	0.146
C18:1 *t*9	0.16	0.15	0.20	0.20	0.007	0.799	<0.001	0.229
C18:1 *t*10	0.35	0.24	0.37	0.39	0.052	0.280	0.030	0.092
C18:1 *t*11	0.80	0.77	0.76	0.80	0.057	0.771	0.846	0.392
C18:1 *t*12	0.27	0.28	0.36	0.36	0.011	0.576	<0.001	0.988
C18:1 *c*9	17.33	16.47	15.60	16.31	0.546	0.847	0.020	0.052
C18:2 *n*-6	2.02	2.02	2.20	2.29	0.067	0.383	<0.001	0.373
C18:3 *n*-3	0.46	0.51	0.37	0.40	0.014	<0.001	<0.001	0.397
C20:0	0.19	0.19	0.18	0.19	0.005	0.701	0.447	0.464
C20:3 *n-3*	0.27	0.07	0.28	0.21	0.077	0.027	0.167	0.260
C20:3 *n-6*	0.12	0.11	0.11	0.12	0.009	0.753	0.949	0.716
C22:0	0.04	0.04	0.03	0.03	0.003	0.686	0.118	0.580
CLA *c*9, *t*11	0.50	0.42	0.45	0.43	0.032	0.029	0.367	0.202
CLA *t*10, *c*12	0.04	0.05	0.05	0.07	0.010	0.069	0.037	0.788
EPA	0.05	0.06	0.04	0.05	0.008	0.120	0.065	0.746
DHA	0.08	0.14	0.20	0.20	0.065	0.503	0.050	0.588
∑SFA	74.4	75.5	75.7	74.9	0.70	0.754	0.469	0.054
∑MUFA	22.1	21.1	20. 6	21.3	0.62	0.791	0.149	0.051
∑PUFA	3.5	3.4	3.7	3.8	0.16	0.720	0.015	0.352

C = chop length; F = forage ratio; C × F = interaction between C and F; CLA = conjugated linoleic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; ∑SFA = total saturated FA ∑MUFA = total monounsaturated FA; ∑PUFA = total polyunsaturated FA

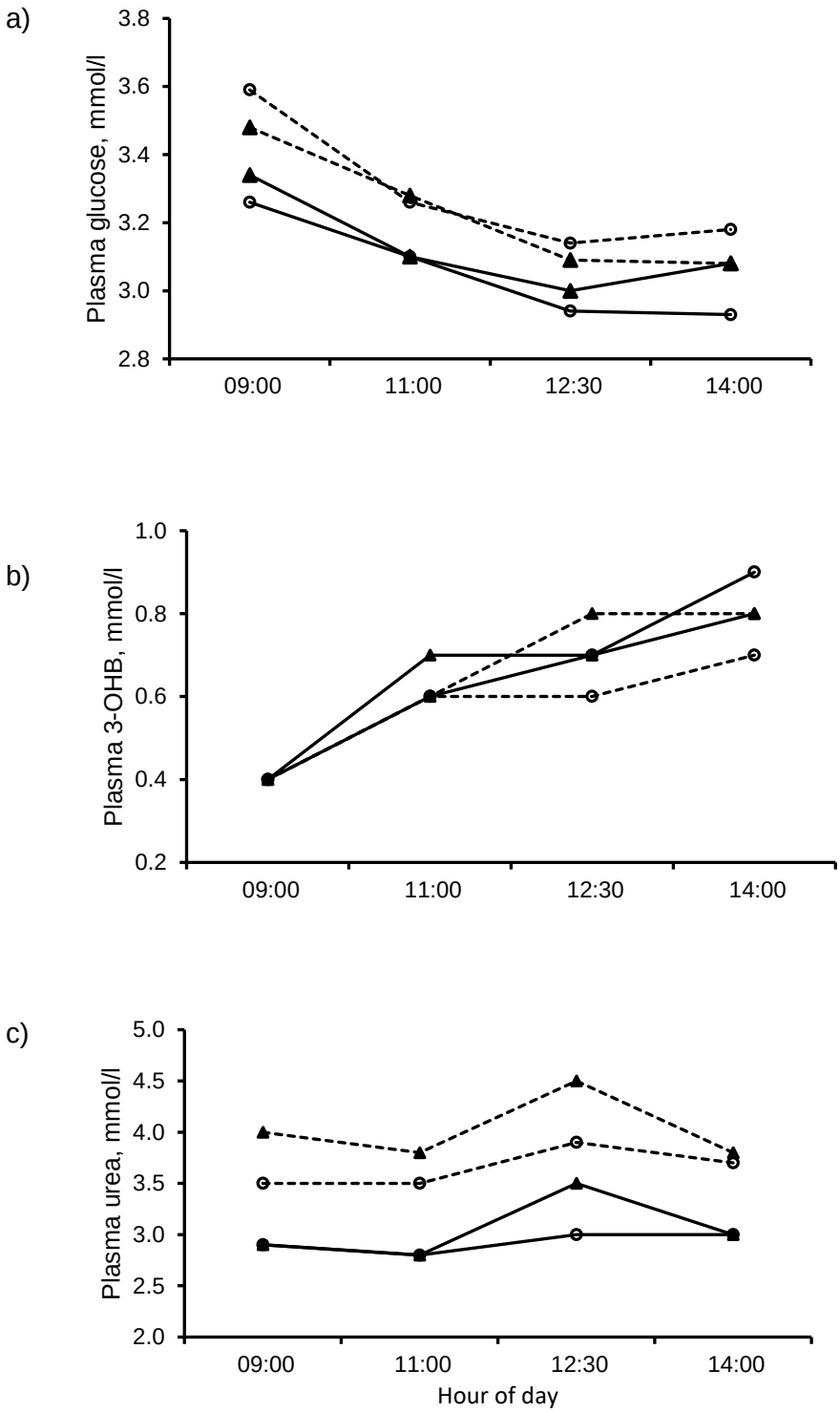

Figure 4a.2. Plasma glucose (a), plasma β-hydroxybutyrate (3-OHB) (b) and plasma urea (c) concentrations in cows fed diets containing long chop grass (LG; —o—); short chop grass silage (SG; —▲—); long chop grass and maize silages (LM; --o--), or short chop grass and maize silages (SM; --▲--). For plasma glucose; SED, 0.108; Time, $P < 0.001$; F, P=0.008. For plasma 3-OHB; SED, 0.112; Time, P<0.001. For plasma urea; SED, 0.265; Time, P=0.004; chop length, P=0.093; forage ratio, P<0.001.

4a.4. Discussion

4a.4.1. Nutrient composition and particle length

The current study was conducted to determine the effect of chop length of grass silage when fed alone or mixed with maize silage on cow performance, rumen pH, eating behaviour and blood metabolites. The particle size of the long chop length grass silage and maize silage used in the current study were similar to the mean values fed on UK dairy farms reported in Chapter 3 (43 and 11 mm respectively), whereas the short chop length grass silage was within the shortest 5% of the grass silage surveyed. The DM content of the grass silage was lower than typically reported for 1st cut ryegrass silages (Sinclair et al., 2015), although the chemical composition of both chop length grass silage was similar, a finding in agreement with previous studies that have altered forage chop length prior to ensiling (Kononoff and Heinrichs, 2003; Yang and Beauchemin, 2007). The lactic acid content was however, higher and the acetic acid content lower in the short chop compared to the long chop length grass silage, a finding in agreement with others who have reported that a shorter chop length can enhance consolidation in the clamp and improve the fermentation profile (McDonald et al., 1991).

4a.4.2. Animal performance

The increase in DMI when cows were fed the maize silage compared to the grass silage based diets is in agreement with previous studies that have investigated the effect of including maize silage (Hart et al., 2015; O'Mara et al., 1998). Mulligan et al. (2002) reported an increase in intake of 3.5 kg/d DM when grass silage was replaced by maize silage in the diet of late lactation dairy cows, whereas a linear increase in DMI was observed when maize silage replaced grass silage in the diet of mid-lactation dairy cows (Kliem et al., 2008). However, a higher acetate content of the long chop grass silage coupled with its low DM content may have resulted in a lower quality and subsequently decreased DMI and milk production (McDonald et al., 1991). Feeding cows with diets containing a short chop length grass silage increased DMI in the current study, possibly due to less time required for chewing prior to swallowing, a finding in accordance with other studies that have investigated the effect of chop length (maize silage or alfalfa) on DMI in dairy cows (Nasrollahi et al., 2015). The increase in the DMI of cows fed the short chop length diets in the current study could be attributed to a reduced rumen fill and lower rumen retention time, both of which are associated with an increased intake (Zebeli et al., 2007).

The current finding of a higher milk yield in cows when maize silage replaced grass silage is in agreement with O'Mara et al. (1998) and Hart et al. (2015), and is most likely to be the result of the higher DMI in cows fed the maize silage based diets. There was an interaction between chop length and forage ratio on milk yield in the current study, with a short chop length grass silage increasing yield in cows when grass silage was the sole forage, but not when grass silage was fed along with MS. This difference may be explained by the mean particle size of the diets, with the LG diet having a substantially longer particle size than any of the other 3 diets. Longer particles in LG may have disappeared from the rumen at a slower rate, resulting in a lower DMI and subsequent milk production (Kononoff and Heinrichs, 2003; Zebeli et al., 2012a). Milk fat production was not affected by chop length in the current study, possible due to a sufficient dietary $peNDF_{>4mm}$ content of all four diets (minimum of 26%), as it has been suggested that milk fat content is only influenced by chop length when dietary peNDF levels are lower than the recommended level of 18-22% DM (Zebeli et al., 2012a). Cows receiving the maize silage based diets in the present study gained live weight whereas when they received the grass silage based diets they lost weight, which may be attributed to differences in DM and ME intake as a consequence of feeding mixed forage diets as suggested by O'Mara et al. (1998). In contrast, chop length did not significantly alter body weight or body weight change, a finding in agreement with that reported by Kononoff and Heinrichs (2003), and reflects that in the current study DMI was less affected by chop length than the GS:MS ratio.

The estimated digestibility co-efficients of the dietary components in the current study were similar to previous studies that have evaluated grass silage and maize silage in the diet of dairy cows (Sinclair et al., 2015). In a review of the literature Khan et al. (2015) concluded that increasing stage of maturity was one of the major factors influencing fibre digestibility in maize silage, and the comparatively high DM of the maize silage used in the current study (350 g/kg DM) may have resulted in a more resistant fibre structure, reducing the digestibility of the fibre in the maize silage compared to the grass silage diets. Alternatively, the decreased rumen pH due to the higher concentration of non-structural carbohydrates in the maize silage diets may have had a negative impact on the fibre degrading microbiota, decreasing diet digestibility (Nasrollahi et al., 2015; Tafaj et al., 2007).

4a.4.3. Reticular pH and eating behaviour

Similar to previous studies (Yang and Beauchemin, 2007), the highest reticular pH was recorded prior to feeding, with a nadir reached at approximately 9 h after fresh

feed delivery. Cows fed the maize silage compared to the grass silage based diets had a lower mean and minimum reticular pH, which may be associated with the higher concentration of starch and lower concentration of peNDF$_{>8mm}$ in the maize silage diets (130 vs 199 g starch/kg DM and 27.1 vs 19.1% peNDF$_{>8mm}$, for the grass silage and maize silage based diets respectively). In contrast, chop length had no effect on reticular pH, a finding in agreement with Tafaj et al. (2007). In contrast, Yang and Beauchemin (2007) reported an increase in mean rumen pH when a longer chop length forage was fed, although the results were based on lucerne silage rather than the ryegrass silage used in the current study.

Chop length did influence eating time in the current study, with cows spending more time eating the long than the short chop diets, a finding in agreement with Kammes and Allen (2012), who reported a tendency for a longer daily eating time when cows were offered a long versus short chop length orchard grass silage. Kammes and Allen (2012) reported no effect of chop length on ruminating time, but in the current study the effect of chop length was unclear, with a decrease in ruminating time per kg DMI in cows when fed grass silage, and increase when fed the maize silage based diets, although there was a clear effect of forage source, with cows fed the maize silage diets (which had the shortest particle size), spending significantly less time ruminating.

4a.4.4. Metabolism and milk fatty acids

The higher plasma glucose concentration in cows fed the MS diets in the current study may be due to the higher dietary content of sugar and starch (Oba and Allen, 2003), whereas the lower plasma urea concentration in cows fed the grass silage based diets may reflect a lower content of rumen degradable N as a greater proportion of dietary N was from rumen-protected protein sources in these diets, although all diets were formulated to have a similar excess of rumen degradable nitrogen. Alternatively, the grass silage based diets may have resulted in a more suitable rumen microbial environment for the capture of degraded N, as demonstrated by the higher reticular pH.

Overall, the inclusion of maize silage in the diet altered the FA profiles of the milk more than the grass silage chop length. Chilliard et al. (2000) reviewed the literature on diet and milk FA profile and concluded that cows fed maize silage based diets had a higher concentration of C10:0, C12:0 and C18:2*n-6*, due to the higher concentrations in maize silage compared to grass silage, a finding in agreement with the current results. Hart et al. (2015) also reported a 0.99 g/100g higher milk fat content of C16:0 in cows when fed a 70:30 (DM basis) grass to MS based diets compared to those receiving a 30:70 GS:MS

diets, a finding in agreement with the current findings. Milk FA profile of cows when fed the grass silage only diets were similar to previous studies (Moorby et al., 2009). Soita et al. (2005) reported no effect of chop length on milk FA, but in the current study a shorter grass silage chop length increased the milk fat proportion of C18:3*n-3*, which may be related to a lower rate and extent of biohydrogenation in the rumen, possibly due to a shorter rumen retention time.

4a.5. Conclusions

The short chop length grass silage used in the current study was within the shortest 5% of that fed in the UK but had no effect on reticular pH compared to an average chop length grass silage, but increased intake and milk performance when fed as the sole forage. Milk performance can also benefit from replacing a proportion of grass silage with maize silage in a TMR when fed to high producing dairy cows, irrespective of the chop length of the grass silage, but with a reduction in reticular pH and fibre digestion. The effects of a shorter chop length grass silage when fed at a high concentrate to forage ratio, or with a greater dietary content of rapidly fermentable starch, requires further investigation.

CHAPTER 4b: The particle size of ryegrass silage when fed with or without maize silage influences eating behaviour and activity of Holstein-Friesian dairy cows

4b.1. Introduction

Ryegrass silage (GS) is the most common forage fed to dairy cows in Northern Europe, and it also provides a major source of forage fibre to meet the nutritional demands of high yielding dairy cows throughout the year (March et al., 2014). Forage fibre is an essential part of a cow's diet that stimulates chewing and ruminating and subsequently promotes saliva production (Allen, 1997; Yang and Beauchemin, 2007). Rumination activity of dairy cows depend on various factors including forage proportion in the diet, forage particle size, concentration of NDF in the diet, and physically effective fibre (peNDF) content (Mertens, 1997; Yang and Beauchemin, 2006). Feeding cows a diet containing a higher content of these factors may reduce diet intake and promote sorting that may subsequently reduce milk yield (Kononoff and Heinrichs, 2003; Tafaj et al., 2007). Thus, it is important to find an optimum particle size of diets so that it is effective enough to stimulate rumination but does not restrict diet intake and reduce sorting. In a recent meta-analysis, White et al. (2017) reported a range of 141 to 507 min/d eating time with a mean of 284 min/d in dairy cows. Forage particle size is a major factor along with its NDF content that affects eating time (De Brabander et al., 2002). Similarly, Kammes and Allen (2012) found that long chop orchard grass silage increased eating time (+1.4 min/kg DMI) and ruminating time (+2.2 min/kg DMI) for cows compared to short particles.

Cows prefer to ruminate whilst lying (Hedlund and Rolls, 1977), however, low concentrate diets can increase rumination whilst standing and increase idle lying periods (Nielsen et al., 2000). Lying down is a high-priority activity for dairy cows (Krohn and Munksgaard, 1993) and essential to maintain good health, welfare and high productivity levels (Tucker et al., 2004). Lying times of between 8.7–13.2 h/d have been reported for dairy cows in cubicle housing (Charlton et al., 2014). However, management practices such as feeding and milking (Overton et al., 2002; DeVries and von Keyserlingk, 2005) can influence the duration of lying.

Lying behaviour is an indicator of lameness in cows that is a serious animal welfare and economic problem in the dairy industry (Ito et al., 2010; Espejo et al., 2006). Lameness undermines the welfare of cows and can decrease milk yield, increase infertility, and

elevate the risk of premature culling (Bicalho et al., 2008; Garbarino et al., 2004; Whay et al., 2003). Housed cows spend 12-13 h/d lying down to maintain good hoof health and locomotion (Galindo and Broom, 2000; Jensen et al., 2005). Cows spending less time lying usually have a higher plasma cortisol concentration that triggers the development of hoof lesions (Friend et al., 1979; Gonzalez et al., 2003; Leonard et al., 1996).

Numerous studies have evaluated the chop length (CL) of lucerne and maize silage (MS) on eating behaviour in dairy cows, but there is less research on ryegrass silage (Kononoff et al., 2003; Yang and Beauchemin, 2006; Yang and Beauchemin, 2007). It was hypothesized that feeding a long chop GS when fed with or without MS would increase eating and ruminating time but promote sorting. The main aim of the current study was to investigate the effect of GS CL and GS:MS ratio on the activity, eating behaviour and sorting activity of dairy cows.

4b.2. Material and methods

This study was part of the larger study reported in Chapter 4a. This study was conducted in accordance with the UK Animal (Scientific Procedures) Act (1986; amended 2013) and attained local ethical approval.

4b.2.1. Animal, diets and experimental routine

Ryegrass silage was chopped at harvest at two mean CL (31 mm = short or 44 mm = long (measured using a modified Penn State Separator and mean CL estimated by the procedure described by ASABE, 2007)) and was either fed alone (100:0 [DM basis]) or mixed with MS at a GS:MS ratio of 40:60 (DM basis). All diets were supplemented with concentrates at a 54:46 (DM basis) and fed as an iso-nitrogenous and iso-energetic total mixed ration (Table 4b.1). The dietary treatments were: 100:0 (DM basis) long chop grass:maize silage (**LG**); 100:0 (DM basis) short chop grass:maize silage (**SG**); 40:60 (DM basis) long chop grass:maize silage (**LM**); 40:60 (DM basis) short chop grass:maize silage (**SM**). Sixteen multiparous (2^{nd} or 3^{rd} parity) early lactating (60 days in milk) Holstein-Friesian cows producing 42 kg milk/d were used in a 4 × 4 Latin Square design with 4 periods of 28-d duration in a 2 × 2 factorial arrangement. Cows were fed *ad-libitum* daily at 10:00 h in 16 Hokofarm roughage intake feeders (RIC feeders, Marknesse, Netherlands) and orts were removed daily to measure the particle size distribution.

4b.2.2. Behaviour observation, sorting activity

Cow location in the yard, physical activity and jaw movements were visually recorded through instantaneous scan sampling at 5 min intervals for a 48 h period commencing at 05:30 h on day 18 of each period (Nielsen et al., 2000; Martin and Bateson, 2007). Cow's physical (walking, standing or lying) and eating activity (eating, ruminating or idling) was monitored for 22 h/d, while jaw activity was also recorded during the 2 h of milking (both am and pm). Eight people observed the cows and were trained for 1 h before the start of the study and an Inter-observer Reliability Pearson coefficient of 0.97 was attained. The observations complied with the recommendation of Martin and Bateson (2007), where each observation period required 2 observers with a duration of 4 h to minimise observer fatigue and enhance accuracy. The number of chews per bolus of all cows (50 full bouts/cow/period) were recorded manually using a clicker (Kononoff et al., 2002).

Diet particle distribution was measured at 0 and 24 h post-feeding for 5-d commencing at 20-d of each period using a modified Penn State Particle Separator containing additional sieves of 26.9 and 44 mm aperture size (Heinrichs, 2013; Chapter 2). Sorting activity was calculated as the function of actual intake of each fraction expressed as a percentage of the predicted intake of that fraction; a sorting value of 100% indicated no sorting, < 100% indicated selective refusal and > 100% was preferential consumption (Leonardi and Armentano, 2003).

4b.2.3. Statistical analysis

General descriptive statistics were used to test the normality of data using GenStat 18.1 (VSN International Ltd., Oxford, UK) and then analysed as a Latin Square design with a 2 × 2 factorial treatment structure. The following model was used to determine the main effects of chop length (C), forage ratio (F) and their interaction:

$$X = \mu + C_i + F_j + C \times F_{ij} + P_j + A_k + €_{ijk}$$

Where X is the observation, μ the overall mean, C_i is the effect of chop length, F_j is the effect of GS:MS ratio, $C \times F_{ij}$ is the interaction between C and F, P_j the effect of period, A_k the random effect of animal and $€_{ijk}$ the residual error. Results were reported as treatment means with standard error of difference (SED), with the level of significance set at <0.05 and a tendency stated at < 0.1.

4b.3. Results

The chemical and physical composition of the diets, and performance data of cows are presented in Chapter 4a and in Tables 4a.2 and 4a.3, and Table 4b.2. The chemical composition of the different fraction of the diets are presented in Table 4b.1.

Table 4b.1. Nutrient composition (g/kg DM) of dietary fractions of diets fed to cows that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM)

Fractions	DM (g/kg)	CP	Ash	OM	EE	NDF	ADF
LG							
>44 mm	234	128	100	900	20	448	311
26.9-44 mm	234	122	102	898	18	461	321
19-26.9 mm	232	123	103	897	16	456	316
8-19 mm	298	137	87	913	19	452	338
4-8 mm	523	160	68	932	24	141	100
<4 mm	474	397	94	906	54	378	158
SED	11.1	5.7	2.8	2.8	3.6	15.4	11.7
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SG							
26.9-44 mm	240	125	97	903	19	484	332
19-26.9 mm	243	136	98	902	24	478	313
8-19 mm	277	132	96	904	24	437	301
4-8 mm	407	157	72	928	24	196	139
<4 mm	415	396	94	906	57	359	180
SED	17.2	6.5	4.6	4.6	3.5	18.1	15.5
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SM							
26.9-44 mm	275	118	76	924	18	467	307
19-26.9 mm	276	122	80	920	19	438	291
8-19 mm	340	134	65	935	22	415	282
4-8 mm	474	149	56	944	26	184	117
<4 mm	466	302	68	932	37	333	156
SED	11.9	5.2	2.5	2.5	2.5	10.0	10.0
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LM							
26.9-44 mm	264	119	85	915	24	416	285
19-26.9 mm	276	128	82	918	23	407	272
8-19 mm	353	131	65	935	23	401	265
4-8 mm	523	144	53	947	25	144	98
<4 mm	479	301	71	929	31	291	148
SED	8.7	3.7	4.0	4.0	1.5	12.8	7.7
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

DM= dry matter, CP = crude protein, OM = organic matter; EE = ether extract, NDF= neutral detergent fibre, ADF = acid detergent fibre, SED = standard error of difference

Table 4b.2. Intake (kg/d) and performance of dairy cows fed diets that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM)

	Diets					*P*-value		
	LG	SG	LM	SM	SED	C	F	C × F
DM intake	20.0	20.5	22.8	24.0	0.56	0.035	<0.001	0.335
OM Intake	18.2	18.6	21.2	22.4	0.51	0.025	<0.001	0.275
N intake (g/d)	554	554	634	671	16.9	0.134	<0.001	0.133
NDF intake	8.09	7.89	7.77	8.28	0.245	0.374	0.846	0.049
fNDF intake	5.24	5.42	5.08	5.36	0.137	0.023	0.263	0.593
ADF intake	5.10	5.36	4.85	5.01	0.154	0.062	0.010	0.659
Milk yield (kg/d)	37.3	39.1	41.1	40.5	0.63	0.179	<0.001	0.011
Milk fat (g/kg)	40.1	38.5	39.5	38.6	0.93	0.090	0.560	0.418
Milk protein (g/kg)	30.9	30.7	32.3	32.4	0.28	0.738	<0.001	0.461

DM = dry matter, OM = organic matter, N = nitrogen, NDF = neutral detergent fibre, ADF = acid detergent fibre, fNDF = forage NDF, C = chop length; F = grass to maize silage ratio; C×F = interaction between C and F

4b.3.1. Cow position and movement

Cows fed diets containing MS spent 1.11 h/d longer (P = 0.013) in the cubicles and 1.32 h/d less (P < 0.001) in the feed passage compared to those fed grass silage only diets (Table 4b.3). Cows when fed the short CL diets occupied the feed bins for 0.64 h/d less (P = 0.029) time compared to when fed with the longer CL diets. There was a tendency for an interaction (P = 0.078) for standing time, where a long CL increased the standing time when fed the grass silage only diet but had little effect when fed the 40:60 GS:MS diets. Cows fed the short CL diets walked more (P = 0.031) than those fed long CL diets. Feeding a short CL grass silage tended (P = 0.089) to increase the lying time in cows when fed the grass silage only diets but had no effect when fed with the 40:60 GS:MS diets.

Table 4b.3. Hourly cow position and activity in the shed (time expressed as h/d) when fed diets that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM)

	Diets					*P* value		
	LG	SG	LM	SM	SED	C	F	C x F
Location								
Cubicle	14.03	14.37	15.41	15.22	0.599	0.857	0.013	0.540
Cub passage[1]	0.66	0.96	0.72	1.15	0.227	0.033	0.452	0.703
Feed bin	7.14	6.15	5.48	5.18	0.399	0.029	<0.001	0.235
Long passage	0.16	0.52	0.38	0.46	0.233	0.206	0.633	0.407
Activity								
Standing	11.12	9.99	10.17	10.38	0.521	0.222	0.453	0.078
Walking	0.46	0.55	0.40	0.52	0.066	0.031	0.330	0.828
Lying	10.42	11.46	11.43	11.10	0.549	0.370	0.405	0.089

C = chop length; F = forage ratio; C×F = interaction between C and F.
[1]Cubicle passage

4b.3.2. Eating and ruminating behaviour

When cows received the long CL diets they spent 0.63 h/d longer (P = 0.003) eating compared to when they received the short CL diets (Table 4b.4). Cows when fed the grass silage only diets spent 0.97 h/d longer (P < 0.001) eating compared to when fed the 40:60 GS:MS diets. Similarly, eating time (min/kg DMI and min/kg NDFI) was higher for cows fed grass silage only diets or long CL diets. Eating time when expressed per kg NDF intake was higher (P < 0.001) in cows when fed the long chop length and grass silage only diets. There was an interaction (P = 0.029) for total ruminating time, where a short CL diet increased ruminating time in cows fed the grass silage only diets but had little effect when fed the 40:60 GS:MS diets. An interaction for ruminating time (min/kg NDFI) was also observed, where a long CL diet decreased the ruminating time in cows fed the grass silage only diets but increased the time when fed the GS:MS diets. When expressed per kg forage NDF (fNDF) intake (fNDFI), a long CL diet increased ruminating time in cows when fed the GS:MS diets but had little effect when fed the GS only diets. Overall, forage ratio had a greater influence on ruminating time compared to the grass silage CL. Total drinking time was not affected by either CL or GS:MS ratio. However, when drinking time was expressed as %peNDF$_{19}$ or peNDF$_{26.9,}$ there was an interaction (P < 0.001) between CL and GS:MS, where a short CL increased the drinking time in cows when fed the grass silage only diets but increased the time substantially when fed the GS:MS diets. Idling time (total, or when expressed as per %peNDF$_{4\ or\ 8}$) was higher (P = 0.016) for cows when fed the GS:MS diets compared to when fed the grass silage only diets. When idling time was expressed as per %peNDF$_{19\ or\ 26.9}$ or X_m, cows fed the SM diets had a longer period of jaw rest compared to other three diets. Cows fed the short CL diets had 11 chews/bolus more (P < 0.001) compared to those fed the long CL diets. Feeding cows with 40:60 GS:MS diets resulted in 4 chews/bolus more (P = 0.011) compared to when fed GS only diets.

4b.3.3. Sorting activity

An interaction (P = 0.007) was observed for sorting of the >19 mm fraction, where a shorter CL resulted in refusals when fed GS alone, but there was no sorting when fed the GS:MS mix (Figure 4b.1). In contrast, cows when fed grass silage alone had a greater preferential consumption (P = 0.031) for the 4-8 mm fraction.

Table 4b.4. Eating and ruminating behaviour of cows fed diets that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM)

	Diets					*P* value		
	LG	SG	LM	SM	SED	C	F	C x F
Eating								
h/day	5.30	4.51	4.18	3.70	0.278	0.003	<0.001	0.463
min/kg DMI	15.9	13.3	11.2	9.41	0.881	0.002	<0.001	0.520
min/kg NDFI	39.8	34.8	32.6	27.4	2.43	0.006	<0.001	0.942
min/kg fNDFI	61.0	50.6	50.1	41.7	3.57	<0.001	<0.001	0.697
min/%peNDF$_4$	9.05	8.08	9.56	8.24	0.561	0.007	0.399	0.662
min/%peNDF$_8$	11.5	10.3	12.8	11.0	0.74	0.008	0.063	0.531
min/%peNDF$_{19}$	16.7	35.1	29.0	102	3.15	<0.001	<0.001	<0.001
min/%peNDF$_{26.9}$	22.5	48.6	31.4	218	7.89	<0.001	<0.001	<0.001
min/X_m (1 mm)	12.5	26.5	28.5	29.3	1.51	<0.001	<0.001	<0.001
Ruminating								
h/day	8.51	9.12	9.25	9.17	0.211	0.084	0.013	0.029
min/kg DMI	25.8	26.8	24.6	23.2	0.72	0.709	<0.001	0.026
min/kg NDFI	64.2	69.6	72.0	67.6	1.92	0.736	0.039	0.001
min/kg fNDFI	98.8	101	110	103	3.00	0.287	0.003	0.035
min/%peNDF$_4$	14.4	16.2	21.1	20.5	0.41	0.067	<0.001	<0.001
min/%peNDF$_8$	18.2	20.6	28.3	27.4	0.55	0.069	<0.001	<0.001
min/%peNDF$_{19}$	22.7	68.9	65.1	253	3.78	<0.001	<0.001	<0.001
min/%peNDF$_{26.9}$	28.4	94.0	72.4	540	12.41	<0.001	<0.001	<0.001
min/X_m (1 mm)	19.2	52.9	62.4	74.9	1.37	<0.001	<0.001	<0.001
min/milking[1]	53.2	46.6	45.6	41.3	5.02	0.134	0.080	0.750
Drinking								
h/day	0.22	0.26	0.23	0.24	0.032	0.194	0.768	0.336
min/kg DMI	0.65	0.77	0.61	0.59	0.092	0.442	0.100	0.321
min/kg NDFI	1.64	1.99	1.77	1.76	0.271	0.372	0.787	0.349
min/kg fNDFI	2.49	2.90	2.72	2.65	0.387	0.532	0.962	0.378
min/%peNDF$_4$	0.36	0.47	0.52	0.54	0.072	0.217	0.036	0.395
min/%peNDF$_8$	0.45	0.60	0.69	0.71	0.094	0.199	0.011	0.350
min/%peNDF$_{19}$	0.37	1.93	1.58	6.69	0.615	<0.001	<0.001	<0.001
min/%peNDF$_{26.9}$	0.28	2.49	1.77	14.3	1.313	<0.001	<0.001	<0.001
min/X_m (1 mm)	0.41	1.53	1.52	1.88	0.214	<0.001	<0.001	0.017
Idling								
h/day	7.98	8.10	8.34	8.90	0.322	0.146	0.016	0.348
min/kg DMI	24.0	23.9	22.2	22.4	1.20	0.937	0.063	0.852
min/kg NDFI	60.1	62.1	65.1	65.7	3.46	0.599	0.085	0.778
min/kg fNDFI	92.0	90.3	99.6	100	4.83	0.864	0.016	0.755
min/%peNDF$_4$	13.6	14.4	19.1	20.1	0.77	0.116	<0.001	0.877
min/%peNDF$_8$	17.1	18.3	25.6	26.8	1.01	0.097	<0.001	0.970
min/%peNDF$_{19}$	20.9	60.9	57.7	246	1.95	<0.001	<0.001	<0.001
min/%peNDF$_{26.9}$	25.2	83.1	63.2	526	12.22	<0.001	<0.001	<0.001
min/X_m (1 mm)	17.8	46.4	56.1	71.1	2.20	<0.001	<0.001	<0.001

Table 4b.4. Cont'

	Diets					*P* value		
	LG	SG	LM	SM	SED	C	F	C × F
Chews/bolus (n)								
Mean	54	65	59	69	2.3	<0.001	0.011	0.768
Mean Std[2]	66	76	63	70	3.0	<0.001	0.050	0.407
Minimum	32	41	37	40	4.3	0.052	0.470	0.410
Maximum	79	90	84	93	5.9	0.023	0.391	0.785

C = chop length; F = forage ratio; C × F = interaction between C and F; DMI = dry matter intake; NDFI = neutral detergent fibre intake; peNDF = physical effective fibre
[1]Jaw activity were recorded during both am and pm milking (2 h/d).
[2]No of chews/bolus were standardized to 24 kg/d DMI.

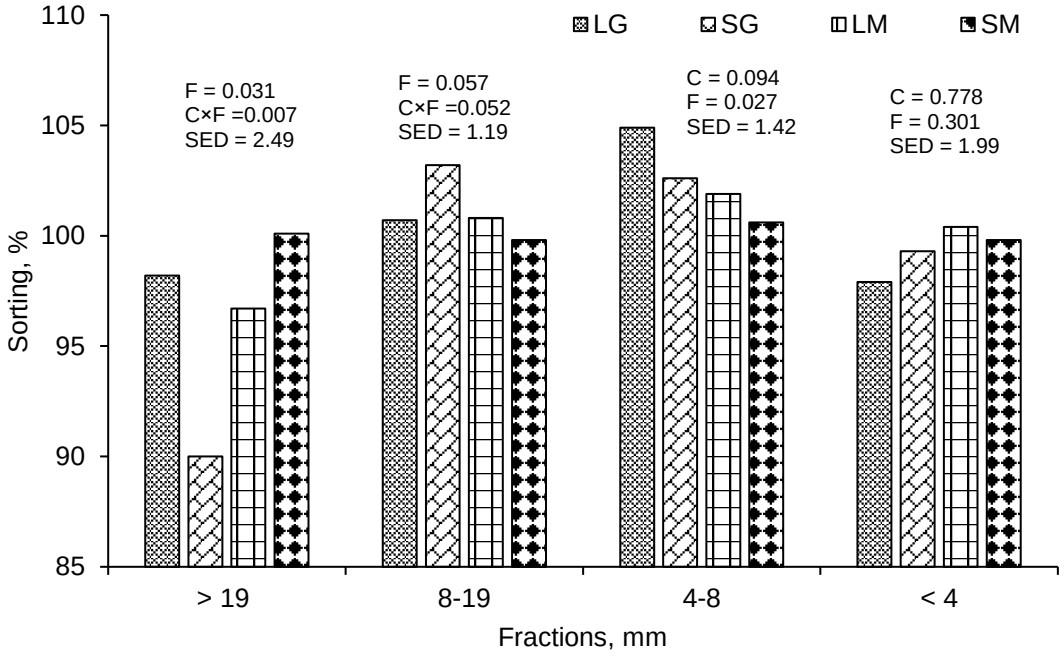

Figure 4b.1. Group level sorting activity of cows fed diets that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM). A sorting values of 100% indicated no sorting, <100% indicated selective refusals and >100% was preferential consumption**.**

4b.4. Discussion

4b.4.1. Cow positioning and movement

The location of cows within the shed was influenced by forage ratio and chop length. Cows fed the grass silage based diets spent 1.3 h/d longer in the feed passage

and over 1 h/d less in the cubicles compared to cows fed the both grass silage and maize silage diets. In addition, cows fed the long CL spent 0.6 h/d longer at the feed bins than cows fed the short CL, possibly due to the higher amount of time required to eat either long CL diets or grass silage only diets (Nasrollahi et al., 2016). Grant and Ferraretto (2018) stated that changing the content of dietary silage fibre, digestibility and particle size can influence eating time of lactating dairy cattle by over 1 h/d. Cows fed the grass silage and longer chop length took longer to consume their daily intake, increasing eating times and therefore the cows spent more time in the feed passage, closer to the food.

During the current study, on average, cows spent 10.4 h/d standing, 0.5 h/d walking and 11.1 h/d lying. Lying time was similar to the 11.5 h/d reported by Gomez and Cook (2010) for cows housed with mattress bedded cubicles. In the current study, cows spent less time lying than the normal duration (12-13 h/d) but there was no issue of lameness in any of the study cows. There was a tendency for an interaction for lying time, where a short CL increased (~1 h/d) lying time when fed the grass silage only diet but had little effect when fed the 40:60 GS:MS diets. Feeding cows with a short CL grass silage may therefore improve animal welfare by increasing lying time.

4b.4.2. Eating and ruminating behaviour

Feeding a longer particle size diet generally results in an increase in eating and rumination time in dairy cows (Beauchemin and Yang, 2005; Nasrollahi et al., 2016; Tafaj et al., 2007). For example, total rumination time increased by 100 min/d when the CL of hay increased from 6 to 30 mm (Zebeli et al., 2007). The findings of a meta-analysis and meta-regression reported a 19 min/d longer eating time, 1.1 min longer eating time/kg DMI, and a 44 min longer total chewing time when forage particle size was increased compared to a short forage particle size (Nasrollahi et al., 2016). The long CL grass silage used in the current study was representative of the average X_m of grass silage (43 mm) surveyed at the UK dairy herds (Chapter 3). The current findings of a longer eating time in cows when fed the long CL diets or grass silage only diets are in agreement with Tafaj et al. (2007). In comparison to the eating time, total rumination time in the current study was not increased when cows were fed the long CL grass silage, with a short CL diet increasing ruminating time (h/d, min/kg DMI, min/kg NDF intake [NDFI] or min/% peNDF) in cows fed the grass silage only diets, but had little effect when fed the GS:MS diets. Contrary to our findings, Kammes and Allen (2012) reported no effect of chop length of orchard grass silage on ruminating time. In the current study, cows when fed the both grass silage and maize silage based diets with a low X_m (8.2 mm) had a higher total rumination time compared to when fed the GS only diets despite the longer grass

silage X_m (18.65 mm). This may be explained by having less effective fibre in grass silage compared to maize silage, as grass silage is more fragile and has a shear strength value of 3.9 mJ/mm^2 compared to the 21.0 mJ/mm^2 in maize silage (McRandal and McNulty, 1980). When the effective fibre comes from maize silage, therefore cows may have to ruminate longer in order to breakdown the forage into fine particles compared to grass silage, as seen in the current study. Further studies are required to establish this by feeding cows with a similar peNDF content coming from different forage sources, as suggested previously by Tafaj et al. (2007). Farmers may therefore benefit by partially replacing grass silage with the maize silage in order to increase the rumination time in cows that may help to reduce SARA. Where 100% grass silage based diets are fed then a short CL grass silage (31 mm or less) should be adopted for feeding dairy cows.

4b.4.3. Sorting activity

Diet sorting is associated with either refusal of long CL or over consumption of the fine concentrate part of the diet, and may result in some cows receiving excess concentrates and others insufficient (Kononoff and Heinrichs, 2003; DeVries et al., 2007). Overall, mixing the grass silage with the maize silage in the current study reduced the sorting index compared to when diets were comprised solely of grass silage. In contrast to the findings of Alamouti et al. (2009), there was no effect of chop length on the particle size distribution of the TMR fractions post feeding. This may be due to feeding through feed bins that restricted the ability of the cows to push/sort the feed. Another possible explanation could be the comparatively high moisture content of the mixed rations in the current study that may have caused the adhesion of smaller particles to larger particles making it more difficult to sort (Beauchemin, 1991; Fish and DeVries, 2012; Leonardi et al., 2005).

4b.5. Conclusions

A longer grass silage CL increased eating time but decreased ruminating time when fed with the 40:60 GS:MS diets, and also resulted in more diet sorting. Cows fed grass silage spent less time ruminating than when they were fed maize silage. Cows tended to spend more time lying down when fed diets containing both grass silage and maize silage, or when fed a short CL, which may enhance their welfare by improving hoof health and locomotion.

CHAPTER 5: Effects of dietary ratios of neutral detergent fibre to starch and grass silage to maize silage on milk production, rumen function, digestion and serum haptoglobin in dairy cows

5.1. Introduction

The milk yield of dairy cows continues to increase, leading to increased energy and protein requirements (Eastridge, 2006; March et al., 2014). To meet these higher nutritional requirements, large proportions of cereal grains and other concentrate feeds are often included in dairy cow rations, supplying large quantities of readily degradable starch which may lead to negative effects, such as sub-acute ruminal acidosis (SARA; Kleen et al., 2003; Plaizier et al., 2008). In the UK dietary starch levels are generally lower than those encountered in North America (Eastridge, 2006), but the higher inclusion of wheat and barley that are rapidly degraded in the rumen (Offner et al. 2003; Endres and Espejo, 2010), increases the risk of SARA. Additionally, grass silage, which is often wet and acidic, is the main forage fed on many dairy farms in the UK (March et al., 2014; Chapter 3 and 4) and may also increase the risk of SARA at lower diet starch concentrations than when maize grain is fed. This cascade of SARA can result in an inflammation of the gut wall that disrupts the epithelium of the reticulo-rumen by altering the tight junctions of the epithelial lining (Zebeli and Metzler-Zebeli, 2012). Increases in endothelial permeability allows ruminal endotoxins to enter into the blood circulation and triggers the release of acute phase proteins such as haptoglobin as an innate immune response of SARA (Ametaj et al., 2010; Plaizier et al., 2012; Metzler-Zebeli et al., 2013).

The dietary inclusion of sufficient fibre can help to ensure optimum rumen function by maintaining an appropriate rumen pH, increasing particle retention time and improving diet digestibility in dairy cows (NRC, 2001). The dietary proportions of fibre and starch can also alter the rate of production and proportion of volatile fatty acid (VFA) in the rumen, which can have an impact on animal performance and milk quality (Zebeli et al., 2010). The composition of rumen-fermentable carbohydrates and physically effective fiber (peNDF), and their interaction should therefore be considered when formulating diets (Allen, 1997; Armentano and Pereira, 1997; Mertens, 1997), and the NDF to starch ratio has been proposed as a key indicator to evaluate the effect of carbohydrate composition on nutrient digestibility and milk production (Beckman and Weiss, 2005).

A previous study reported that feeding a short compared to a longer chop length grass silage had little effect on the reticulo-rumen pH in dairy cows, but altered intake and milk performance when fed alone or in combination with grass silage (Chapter 4a, b).

However, the effects of different dietary NDF to starch levels in diets based on a short chop grass silage or grass/maize silage mixtures on rumen metabolism and performance under UK conditions are unclear. It was hypothesized that diets containing a high level of starch would reduce rumen pH and fibre digestion, while diets containing a higher content of NDF will decrease rumen passage rate and DM intake. Therefore, the objective was to determine the effect of dietary ratio of grass to maize silage (GS:MS) and NDF to starch on rumen pH, digestibility, rumen function and passage kinetics, eating behaviour, serum haptoglobin concentration and milk production and composition in lactating dairy cows.

5.2. Materials and methods

5.2.1. Forages and diets

A first cut perennial ryegrass silage (*Lolium perenne*) was mown and harvested using a self-propelling precision forage harvester in 2017 and ensiled with an additive (Axphast Gold, Biotal, Worcestershire, UK) at the rate of two litres/tonne. Maize silage (*Zea mays*) was harvested in 2017 and ensiled in a concrete-walled clamp without additive. The mean particle size (X_m) of the maize silage and ryegrass silage were 10.2 and 23.6 mm, respectively (measured using the Penn State Separator as described in Chapter 2). Four total mixed ration (TMR) diets with a forage:concentrate ratio of 50:50 (DM basis) were formulated to have two ratios of GS:MS; either 82:18 GS:MS (G) or 18:82 GS:MS (M) on a DM basis. Concentrates for the diets were formulated with either a high or low NDF:starch ratio using soyhulls as a primary NDF source, and cracked wheat and maize as starch sources (Table 5.1). The two GS:MS and two NDF:starch ratios were used in a 2 × 2 factorial design resulting in 4 diets consisting of high grass silage with a high NDF content (82:18 GS:MS, 414 g/kg NDF and 90 g/kg starch; GSF), high grass silage with a high starch content (82:18 GS:MS, 309 g/kg NDF and 220 g/kg starch; GSS), high maize silage with a high NDF content (18:82 GS:MS, 345 g/kg NDF and 214 g/kg starch; MSF), and high maize silage with a high starch content (18:82 GS:MS, 258 g/kg NDF and 319 g/kg starch; MSS) on a DM basis (Table 5.1). Diets were formulated to contain similar CP concentration (170 g/kg DM), provide similar amounts of metabolisable protein, and meet expected nutrient requirements of the cows used in the study (Thomas, 2004). The formulated diet NDF to starch ratio was highest in GSF at 4.6 and lowest for MSS at 0.8.

Table 5.1. Dietary formulation (kg/kg DM) and predicted composition (g/kg DM) of diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (GSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS).

Ingredients	GSF	GSS	MSF	MSS
Grass silage	0.410	0.410	0.090	0.090
Maize silage	0.090	0.090	0.410	0.410
Cracked wheat	0.056	0.170	0.080	0.140
Maize meal	-	0.072	-	0.090
Soyhulls	0.212	0.030	0.150	-
Soybean meal	0.052	0.040	0.120	0.120
Sopralin[1]	0.080	0.088	-	-
Rapeseed meal	0.050	0.050	0.100	0.100
Molasses	0.020	0.020	0.020	0.020
Limestone	0.005	0.005	0.005	0.005
Salt	0.005	0.005	0.005	0.005
Hi-mag mineral[2]	0.010	0.010	0.010	0.010
Megalac[3]	0.010	0.010	0.010	0.010
Predicted composition[4]				
ME (MJ/kg DM)	11.6	11.9	12.1	12.4
MPE[5]	113	114	116	118
MPN[6]	127	127	122	122
NDF	414	309	345	258
Starch	90	220	214	319
NDF:starch[7]	4.6	1.4	1.6	0.8

[1]A rumen bypass soybean meal
[2]Mineral/vitamins premix supplied calcium (230 g/kg), sodium (95 g/kg), magnesium (40 g/kg), selenium (30 mg/kg), phosphorous (20 g/kg), zinc (5.2 g/kg), manganese (2.2 g/kg), copper (1.2 g/kg), and vitamin A (400,000 IU/kg), vitamin D (80,000 IU/kg), and vitamin E (2,000 IU/kg)
[3]A rumen protected source of fat (Volac, Royston, UK)
[4]Feed into Milk by Thomas, 2004, diets were formulated to produce 37 kg/d milk
[5]MPE, MP-rumen energy limited
[6]MPN, MP-rumen nitrogen limited
[7]NDF to starch ratio

5.2.2. Animals, feeding and experimental routine

Four early lactation (61 ± 0.1 DIM) Holstein dairy cows (in their 2nd parity and producing 44.2 kg milk/d (± 0.05)) were fitted with a rumen cannula ((#1C Bar Diamond rumen cannula, PO Box 60, 29575 Bar Diamond Lane, Parma, Idaho, USA) at the end of their previous lactation and randomly assigned to one of 4 dietary treatments within a 4 × 4 Latin Square Design, with 4 periods each of 28-d duration. One cow was removed from the study in period 2 due to poor health, and another intact cow of similar yield and parity was added. The experiment was conducted under the authority of the UK Animal (Scientific Procedures) Act (1986; amended 2013). The first week of each period was used for incremental diet change, week 2 for adaptation to the diet, with weeks 3 and 4 designated as sampling weeks. Diets were prepared daily using a Calan Data Ranger

(American Calan, New Hampshire, USA). During the first two weeks of each period, cows were housed in a cubicle yard with individual feeding through Calan gates (American Calan, New Hampshire, USA). Cows were fed 4 times/d (1000, 1600, 2200 and 0500 h) throughout the experiment, and refusals were removed daily at 0930 h. Whilst in the cubicle yard cows were milked twice daily at 0600 and 1600 h in a 50-point rotary parlour (Dairy Master, Worcestershire, UK). At the start of week 3, cows were moved to individual metabolism stalls and followed a similar feeding and milking routine using facilities described previously (Thomson et al., 2017).

5.2.3. Intake and milk yield and composition

Measurements of DMI, milk yield and milk composition were taken for 6-d during the final week of each period. Fresh feed was offered daily to provide *ad libitum* intake with 10% refusals. Daily TMR samples were composited for the final week of each period and stored at -20℃ for subsequent analysis. Forage samples were collected daily to determine DM content and to allow the adjustment of the fresh weight inclusion of the diet components. Consecutive milk samples were collected for 6-d during the final week of each period and analysed for fat, protein, casein, lactose, urea, milk FA and somatic cell count (SCC) according to the procedure described in Chapter 2. The live body weight (BW) of cows was recorded at the start of the study and at end of each period. Fresh water was available continuously.

5.2.4. Rumen degradability and passage kinetics

On d-15 of each period, the in situ dacron bag method was used to estimate the degradability of grass silage NDF (GS-NDF; Åkerlind et al., 2011). Duplicate samples of grass silage (5 ± 0.13 g DM) were incubated in the rumen of each cow at 0, 2, 4, 8, 16, 24, 48 and 96 h intervals per period according to procedure described previously by Tayyab et al. (2016). Particle passage kinetics was estimated using the chromium-mordant technique on GS-NDF (Cr-NDF) according to Udén et al. (1980) as described in Chapter 2. The Cr-NDF was inserted directly in the rumen via the rumen cannula or fed to the intact cow by mixing with the diet at 0800 h on d-21 of each period. Faeces were collected at -1 (background concentration of marker), 3, 6, 9, 12, 15, 18, 21, 24, 28, 32, 36, 40, 44, 48, ,52, 56, 64, 72, 80, 88, 96, 108, 120, 132 and 144 h to estimate particle passage kinetics (Hammond et al., 2014).

5.2.5. Eating and rumination behaviour

Continuous recordings of the eating and ruminating behaviour of each cow were made for a 4-d period commencing on d-15 of each period using jaw movement

recorders (Rutter et al., 1997). Recordings commenced daily at 1000 h and continued for 23.5 h; data were downloaded daily during the remaining 30 min period. Jaw movement recording was analysed with propietary software (Rutter, 2000) to identify periods of eating and ruminating.

5.2.6. Particle size determination and sorting activity

Offered diets and refusals for particle size determination were sampled for 5-d during the final week of each period and stored at -20℃ for subsequent analysis. Samples were defrosted at room temperature for 6 h, pooled across each period and assessed in triplicate using the modified Penn State Particle Separator (PSPS; Chapter 2) to determine particle size distribution (DM basis). The PSPS had sieves holes that measured 33, 19, 8 and 4 mm diameter, and a bottom pan. The X_m of the diets and forages was calculated using the method described by ASABE (2007). The physical effectiveness factor (pef) was determined as the DM proportion of particles longer than 4 or 8 mm (Lammer et al., 1996; Thomson et al., 2017). The physically effective fibre content (peNDF) was calculated by multiplying the NDF content of the diet by its physical effectiveness factor (Mertens, 1997). Sorting activity was calculated as the actual intake of each fraction expressed as a percentage of the predicted intake of each fraction, where a sorting value of < 100% indicated selective refusals, > 100% preferential consumption, and 100% no sorting (Leonardi and Armentano, 2003).

5.2.7. Diet digestion and nitrogen excretion

During the last 5-d of each period, a total collection of faeces and urine was performed by using a harness and chute fitted on each cow (Figure 5.1). Faeces were collected via a chute into a tray that was emptied at regular intervals into a large bucket. Urine was collected via a collection cup glued over the vulva of the cow and tube that emptied into a 25 L container containing 1200 ml of 10N sulphuric acid to maintain urine pH < 2.0. The urine collection container was agitated several times during the day to ensure mixing of the acid and urine. Sub-samples of mixed 24 h collections were bulked as a proportion of the daily excretion to account for daily differences in excreta weight (5% for faeces, 1.25% for urine). At the end of each sampling week the bulked samples were mixed and subsamples stored at -20℃ for subsequent analysis. Water intake was also recorded for 6-d during the final week of each period.

5.2.8. Rumen pH, ammonia and volatile fatty acids

On 22-d of the each period spot samples of rumen liquor were taken prior to feeding and then at 0.5, 1.5, 3 and 6 h post feeding. Samples were used to determine

pH and VFA and ammonia concentration (Thomson et al., 2017). Approximately 80 ml of rumen fluid was collected into a beaker by inserting a fixed probe through the seal of the rumen cannula bung to a fixed depth in the ventral sac of the rumen. Following the measurement of pH the sample was transferred into 50 ml polypropylene centrifuge tubes and stored at -20℃. An indwelling pH probe (Sentix 41‑3 probe, WTW Trifthof, Weilheim, Upper Bavaria) was also used to monitor rumen pH in the ventral sac for a 3-d period commencing at 1000 h on 22-d (Thomson et al., 2017). The pH probe was calibrated in standard solution of pH 4 and 7 prior to insertion and after removal and data was recorded at 15 min intervals. Data were discarded if values were drifted >5%.

Figure 5.1. Faeces and urine collection by using a harness and chute fitted on cow (Author's own).

5.2.9. Blood sampling

Blood samples were collected by coccygeal venepuncture on d-26 of the final week of each period at 0930 and 1530 h into vacutainers (BD Vacutainer, Plymouth, UK). Samples were held at room temperature for 3 h prior to centrifuging at 3,000 g for 20 min and the serum stored at -20℃. Serum samples were analysed for the acute phase protein haptoglobin (HP) as described previously (Khafipour et al., 2009; Section 2.10).

5.2.10. Chemical analysis

The DM content (at 60℃ for 48 h, Section 2.1) of forages and diets was determined and then samples were milled through a 1 mm screen hammer mill

(Crompton Control Series 2000, Wakefield West Yorkshire UK). The ash, ether extract and CP was analysed as described in Section 2.2 to 2.4. The NDF (using sodium sulphite and heat-stable α-amylase; Sigma, Gillingham, UK) and ADF were analysed according to the procedure described in Section 2.5 and 2.6, and expressed exclusive of residual ash (intra-assay CV of 1.3% and 0.6%, respectively). The starch content of the diets was determined using the method described in Chapter 2. Milk samples were analysed for milk fat, milk protein, casein, milk lactose, urea, SCC, milk FA content using a mid-infrared spectroscopy on a Combi Foss machine (National Milk Laboratories, Wiltshire, UK). Serum samples were analysed for haptoglobin (HP) using an ELISA assay (Abcam, Cambridge, UK; intra-assay CV 9.1%). All spectrophotometric measurements were undertaken using a BioTeck microplate reader (BioTeck Instruments Ltd, Potton, UK) at 450 nm absorbance. Rumen VFA concentrations were determined using a GC (3400, Varian Inc.) using the procedures described in Chapter 2 and rumen ammonia concentrations were determined by colorimetric procedure (Sutton et al., 2003; Chapter 2). Faecal chromium concentration was analysed using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, NexION® 2000, PerkinElmer, Seer Green, UK) as described by Cope et al. (2009), with an intra-assay CV of 6.6% (Section 2.15).

5.2.11. Statistical analysis

Fat corrected (40 g/kg) milk yield was calculated as described previously (Tyrrell and Reid, 1965). Rumen degradability profiles were fitted assuming an exponential degradation curve including a lag time using SigmaPlot (Systat Software Inc.) according to the procedure described by Ørskov and McDonald (1979). Rumen degradable NDF was estimated at an effective rumen degradability (ED) of 5 or 8%/h rumen fractional passage rate (including lag time) (Åkerlind et al., 2011). Rumen retention time was calculated according to the procedure described by Dhanoa et al. (1985) and presented in Chapter 2.

Data was analysed as a Latin Square Design using GenStat 17.1 (VSN International Ltd., Oxford, UK), with main effects of NDF:starch ratio (**C**), GS:MS ratio (**F**), and their interaction using the following model:

$$Y = \mu + C_i + F_j + C \times F_{ij} + P_j + A_k + €_{ijk},$$

Where Y is the observation, μ the overall mean, C_j is the NDF:starch ratio effect, F_i is the GS:MS ratio effect, $C \times F_{ij}$ is the interaction between C and F, P_j the fixed effect of period, A_k the random animal effect and $€_{ijk}$ the residual error. Data for rumen pH and VFA was analysed as repeated measurement ANOVA in a 3 × 4 Youden Square Design. Rumen

pH and acute phase protein data were analysed as repeated measures ANOVA. Results were presented as means ± SED, with a significance level of <0.05 and a tendency set at <0.1.

5.3. Results

5.3.1. Forages, diet's chemical and physical composition

All diets had a similar CP content of approximately 174 g/kg DM (Table 5.2). The forage NDF content of the 82:18 GS:MS diets were higher compared to the 18:82 GS:MS diets. In contrast, the starch content of the 18:82 GS:MS diets was higher than the 82:18 GS:MS diets. The 82:18 GS:MS diets had a higher proportion of DM retained on the > 33 and 19 – 33 mm screens compared to the 18:82 GS:MS diets, while the 18:82 GS:MS diets had a greater proportion of particles retained on the 4 – 8 and 8 – 19 mm screens. The concentrate source also influenced the particle size distribution, with the high fibre diets having a higher proportion of DM retained on the 4 – 8 mm screen, with lower amounts retained on the < 4 mm screen compared to the high starch concentrate diets. The X_m of the 82:18 GS:MS diets was higher than the 18:82 GS:MS diets (7.55 and 5.96 mm, respectively). Both forage ratio and NDF:starch ratio had an effect on the physically effective fibre content ($peNDF_{>4}$) with GSF diet having the highest (25.1%) and MSS diet the lowest (15.2%) concentration.

5.3.2. Intake, production and milk composition

Cows when fed the 18:82 GS:MS diets consumed 1.34 kg/d more ($P < 0.05$) DM compared to when offered the 82:18 GS:MS diets (Table 5.3). Similarly, there was an increase of 2.46 milk/d ($P < 0.04$) in cows when fed the 18:82 GS:MS diets. Milk fat concentration was 2.88 g/kg higher ($P < 0.01$) in cows fed the 82:18 GS:MS diets compared to when fed the 18:82 GS:MS diets, while cows when fed the high starch diets produced more fat (+1.8 g/kg; $P < 0.04$) compared to when they received the high NDF diets. Both milk protein and casein protein concentration and milk protein yield were higher ($P < 0.01$) in cows when fed the 18:82 GS:MS diets. Milk fat to protein ratio (F:P) was also higher ($P < 0.01$) in cows when fed the 82:18 GS:MS diets compared to the 18:82 GS:MS diets. The total concentrations of SFA, UFA, C16:0 and C18:0 were higher ($P < 0.04$) in milk from cows when fed the 82:18 GS:MS diets compared to when fed the 18:82 GS:MS diets. The high starch diets resulted in 0.147 g/100g FA higher total milk SFA concentration compared to when cows were fed the high NDF diets, due in part to higher C16:0 concentration.

Table 5.2. Chemical (g/kg DM) and physical composition (% DM retained above screen) of diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (GSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS)

	Treatments					P value		
	GSF	GSS	MSF	MSS	SED	C	F	C × F
DM, g/kg	450	444	455	449				
OM	912	916	927	931				
Ash	88	84	73	69				
CP	175	173	174	173				
Ether extract	20	25	24	22				
NDF	399	295	347	266				
ADF	253	168	208	144				
Forage NDF	248	248	196	196				
Starch	117	236	215	323				
NDF:Starch	3.44	1.26	1.70	0.84				
fNDF:Starch	2.13	1.05	0.94	0.61				
Particle size distribution								
>33 mm	6.39	5.94	0.39	0.43	0.810	0.940	<0.001	0.432
19-33 mm	21.66	21.78	13.01	13.78	1.625	0.898	0.001	0.819
8-19 mm	20.40	21.06	29.82	30.96	1.010	0.150	<0.001	0.474
4-8 mm	14.51	9.64	16.01	11.72	0.401	<0.001	0.002	0.225
<4 mm	37.04	41.57	40.78	43.10	1.718	0.039	0.078	0.384
$X_{m,}$ mm	7.40	7.69	6.08	5.85	0.549	0.947	0.010	0.542
SD_{xm}	3.15	3.16	2.71	2.79	0.061	0.371	<0.001	0.395
$pef_{>4}$, %	62.96	58.43	59.11	56.90	1.718	0.039	0.078	0.384
$pef_{>8}$, %	48.45	48.79	43.31	45.17	1.791	0.423	0.018	0.572
$peNDF_{>4}$, %	25.07	17.27	20.46	15.16	0.851	<0.001	0.003	0.094
$peNDF_{>8}$, %	19.28	14.43	14.95	12.04	0.767	<0.001	0.002	0.133

F = grass to maize silage ratio, C = NDF to starch ratio, F × C = interaction between F and C, fNDF = forage NDF, X_m = geometric mean particle size; SD_{xm} = SD of X_m; *pef* = physical effectiveness factor; peNDF = physically effective fibre

Table 5.3. Production performance of cows fed diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (GSF) or a low grass:maize silage ratio (18:82) with a high starch content (MS)

	Treatments					*P* value		
	GSF	GSS	MSF	MSS	SED	C	F	C × F
DMI, kg/d	23.1	23.1	24.9	24.1	0.67	0.436	0.047	0.450
Milk yield, kg/d	40.9	40.6	44.5	41.9	1.15	0.161	0.038	0.239
4% FCM, kg/d	40.7	41.4	40.7	40.4	0.99	0.753	0.531	0.504
Feed efficacy[1]	1.76	1.76	1.79	1.75	0.027	0.259	0.665	0.352
Fat, g/kg	39.7	41.2	36.5	38.7	0.79	0.033	0.007	0.584
Fat, kg/d	1.63	1.66	1.63	1.62	0.04	0.753	0.531	0.504
Protein, g/kg	30.3	30.8	31.5	32.0	0.34	0.107	0.007	0.837
Protein, kg/d	1.23	1.24	1.40	1.34	0.046	0.476	0.015	0.308
F:P ratio	1.32	1.33	1.16	1.22	0.026	0.092	0.002	0.303
Lactose, g/kg	46.9	46.9	46.8	46.8	0.36	0.920	0.796	0.935
Lactose, kg/d	1.92	1.91	2.08	1.96	0.044	0.098	0.023	0.165
SCC, $\times 10^3$ cells/ml	28	10	21	59	30.4	0.655	0.386	0.261
Casein, g/kg	2.41	2.46	2.52	2.55	0.025	0.073	0.004	0.701
Urea, g/kg	0.024	0.024	0.024	0.024	0.0026	0.958	0.913	0.976
LW, kg	664	669	667	671	5.13	0.260	0.537	0.819
LWC, kg/d	-1.4	5.5	11.9	7.0	10.71	0.903	0.384	0.477
Water intake, kg/d	95.5	83.0	86.5	82.5	5.47	0.100	0.287	0.337
Milk FA, g/100g milk								
∑MUFA	0.93	0.93	0.87	0.90	0.029	0.366	0.087	0.424
∑PUFA	0.15	0.14	0.15	0.15	0.006	0.794	0.214	0.329
∑SFA	2.69	2.82	2.47	2.63	0.058	0.023	0.008	0.820
∑UFA	1.09	1.09	1.00	1.05	0.031	0.352	0.034	0.358
C16:0	1.15	1.23	1.03	1.12	0.022	0.006	0.002	0.793
C18:0	0.35	0.35	0.31	0.32	0.011	0.498	0.010	0.633
C18:1	0.80	0.81	0.75	0.78	0.031	0.403	0.146	0.548
N[2]	4	3	3	4				

F = grass to maize silage ratio, C = NDF to starch ratio, F × C = interaction between F and C, SCC = somatic cell count, FCM = fat corrected milk, F:P = Fat to protein ratio, LW = final live weight, LWC = LW change over the 28-d period, FA = fatty acids, ∑ = total sum

[1]Feed efficiency = kg milk/ kg DMI

[2]n = number of observations

5.3.3. Diet digestibility and grass fibre degradation and passage kinetics

Digestibility of OM was higher ($P < 0.05$) and there was a tendency ($P = 0.06$) for a higher DM digestibility in cows fed the high starch diets compared to when fed the high NDF diets (Table 5.4). Cows fed the 18:82 GS:MS or high NDF diets excreted more ($P < 0.01$) DM and OM compared to when fed the 82:18 GS:MS or high starch diets. The NDF and ADF intake was higher ($P < 0.01$) in cows fed the high NDF diets, and there was a tendency ($P < 0.07$) for a higher NDF intake and a higher ADF intake ($P < 0.02$)

for cows fed the 82:18 GS:MS diets compared to when fed the 18:82 GS:MS diets. Cows fed the 82:18 GS:MS diets had higher ($P < 0.01$) NDF and ADF digestibility compared to when fed the 18:82 GS:MS diets. Similarly, cows when fed the high NDF diets had higher ($P < 0.04$) NDF and ADF digestibility than when fed the high starch diets.

Table 5.4. Diet digestion in cows fed diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (GSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS).

	Treatments					P-value		
	GSF	GSS	MSF	MSS	SED	C	F	C × F
DM, kg/d								
Intake	22.97	22.80	24.87	23.68	0.908	0.350	0.096	0.471
Output	6.24	5.69	6.99	6.21	0.160	0.004	0.005	0.368
Digest, kg/kg	0.728	0.750	0.719	0.737	0.0108	0.056	0.226	0.764
OM, kg/d								
Intake	20.94	20.93	23.05	22.05	0.866	0.455	0.058	0.467
Output	5.42	4.88	6.14	5.46	0.159	0.006	0.004	0.565
Digest, kg/kg	0.740	0.767	0.734	0.752	0.0107	0.044	0.222	0.614
NDF, kg/d								
Intake	9.14	6.84	8.65	6.31	0.281	<0.001	0.062	0.927
Output	3.07	2.65	3.79	3.09	0.068	<0.001	<0.001	0.044
Digest, kg/kg	0.663	0.607	0.558	0.501	0.0246	0.031	0.004	1.000
ADF, kg/d								
Intake	5.80	3.82	5.16	3.42	0.174	<0.001	0.013	0.389
Output	2.08	1.71	2.43	1.87	0.048	<0.001	0.002	0.049
Digest, kg/kg	0.641	0.544	0.523	0.444	0.0255	0.008	0.004	0.632

F = grass to maize silage ratio, C = NDF to starch ratio, F × C = interaction between F and C, DM = dry matter, Digest = digestibility, OM = organic matter, NDF = neutral detergent fibre, ADF = acid detergent fibre

There was no effect of either forage ratio or NDF:starch ratio on the overall *in situ* degradation kinetics of grass silage NDF, although the initial disappearance rate was faster for 82:18 GS:MS compared to 18:82 GS:MS (Table 5.5). However, the Cr-NDF escaped the rumen (k1) at a faster rate ($P < 0.01$) when cows were fed the 18:82 GS:MS diets compared to the 82:18 GS:MS diets, but concentrate composition had no effect. Similarly, rumen mean retention time (R-MRT) and total-tract retention time (TT-MRT) was higher ($P < 0.04$) in cows when receiving the 82:18 GS:MS diets.

Table 5.5. *In situ* rumen degradation (% dry matter) and passage kinetics of grass silage NDF in cows feeding diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (GSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS).

	Treatments					P value		
	GSF	GSS	MSF	MSS	SED	C	F	C × F
2 h	8.2	7.4	6.9	6.1	0.69	0.223	0.077	0.966
4 h	13.4	11.7	10.2	8.9	1.29	0.201	0.048	0.840
8 h	23.2	16.8	16.3	18.6	0.52	0.012	0.006	0.001
16 h	42.7	27.3	36.6	33.9	8.30	0.223	0.970	0.358
24 h	58.1	47.2	51.1	56.1	4.08	0.378	0.773	0.071
48 h	78.0	73.0	68.4	73.3	5.62	0.992	0.332	0.303
96 h	88.9	85.9	83.9	85.3	2.12	0.620	0.159	0.231
a, %	10.4	9.5	9.1	9.1	0.66	0.357	0.156	0.377
b, %	81.2	87.1	82.6	81.5	4.59	0.521	0.564	0.362
c, %/h	0.038	0.026	0.031	0.034	0.0051	0.297	0.823	0.130
lag time	2.84	3.76	3.41	3.45	0.543	0.303	0.763	0.332
ED5, %	37.6	31.6	32.4	33.6	2.55	0.281	0.429	0.141
ED8, %	29.0	23.7	24.5	25.4	2.21	0.258	0.440	0.146
Rumen passage kinetics, h								
k1, /h	0.0252	0.0263	0.0344	0.0370	0.00236	0.329	0.004	0.642
k2, /h	0.1212	0.1175	0.1216	0.1167	0.01196	0.637	0.978	0.947
Tp	39.58	39.25	38.92	40.52	2.721	0.757	0.883	0.642
TT	18.23	17.74	19.58	19.75	1.902	0.912	0.280	0.819
R-MRT	41.3	36.4	27.2	28.2	3.30	0.444	0.009	0.280
TT-MRT	67.8	62.8	55.2	57.1	4.20	0.632	0.037	0.310
cT	203.3	188.4	165.6	171.3	12.60	0.632	0.037	0.310

F = grass to maize silage ratio, C = NDF to starch ratio, F × C = interaction between F and C, a = soluble fraction, b = potentially degradable fraction, c = rate of degradation, ED5 = effective degradability at 5%/h passage rate, ED5 = effective degradability at 8%/h passage rate, k1 = emptying rate of rumen, k2 = emptying rate of intestines, Tp = time to peak marker flow, TT = transit time, R-MRT = rumen mean retention time, TT-MRT = total-tract mean retention time, cT = clearance time

5.3.4. Nitrogen balance

There was a tendency ($P < 0.1$) for a higher N intake for cows when fed the 18:82 GS:MS diets compared to the 82:18 GS:MS diets, due to the higher DMI for the 18:82 GS:MS diets (Table 5.6). Faecal N output was higher ($P < 0.03$) in cows when fed the 82:18 GS:MS diets compared to when fed the 18:82 GS:MS diets, such that N digestibility was higher ($P < 0.01$) in cows when they received the 18:82 GS:MS diets compared to the 82:18 GS:MS diets. An interaction was found between GS:MS ratio and NDF:starch ratio, where a high dietary starch content decreased urinary-N output when cows were fed the 82:18 GS:MS diets, but had no effect on urine N output when the 18:82 GS:MS diets were fed. Milk N excretion increased ($P < 0.02$) in cows when fed the 18:82 GS:MS diets compared to the 82:18 GS:MS diets, while there was no effect of

concentrate source. Milk N content as a % of N intake was also higher ($P < 0.05$) in cows when fed the 18:82 GS:MS diets compared to the 82:18 GS:MS diets.

Table 5.6. Nitrogen balance in cows fed diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (GSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS).

N, g/d	Treatments					P-value		
	GSF	GSS	MSF	MSS	SED	C	F	C × F
Intake	643	630	691	656	23.7	0.229	0.092	0.546
Faecal output	225	217	211	191	7.8	0.063	0.023	0.317
Digested	418	413	480	465	20.2	0.535	0.016	0.757
Digestibility, g/g	0.650	0.656	0.695	0.709	0.0109	0.276	0.003	0.620
Faecal-N of intake N, %	35.0	34.4	30.5	29.1	1.09	0.276	0.003	0.620
Urine	162	112	151	167	15.1	0.178	0.109	0.035
Urine-N of manure N, %	41.7	34.1	41.4	46.6	2.85	0.589	0.039	0.034
Urine-N of intake N, %	25.3	17.7	21.5	25.5	3.12	0.464	0.406	0.058
Milk N	197	199	224	214	7.4	0.476	0.015	0.308
Milk-N of intake N, %	30.6	31.6	32.5	32.9	0.77	0.257	0.045	0.634

F = grass to maize silage ratio, C = NDF to starch ratio, C = concentrate source, F × C = interaction between F and C

5.3.5. Rumen pH, ammonia, volatile fatty acids and serum haptoglobin

There was no effect of GS:MS ratio or NDF:starch ratio on mean, minimum or maximum rumen pH (Table 5.7). However, cows fed the 18:82 GS:MS diets spent 187 min/d more ($P < 0.01$) time with rumen pH below 5.8 compared to when they were fed the 82:18 GS:MS diets. In contrast, cows when fed the 82:18 GS:MS diets spent a longer time at a rumen pH of 6.2-6.5. There was a tendency ($P = 0.07$) for a longer time spent at rumen pH of 6.5-6.8 in cows when receiving the high starch diets compared to the high NDF diets. Hourly rumen pH was affected by both F and C, where cows when fed the 82:18 GS:MS diets had a 0.1 unit higher ($P < 0.01$) rumen pH throughout the day, whilst the high starch diets increased rumen pH by 0.02 unit pH compared to the high NDF diets (Figure 5.2). Manual rumen pH values followed the hourly rumen pH values measured by indwelling pH probe (Figure 5.3). Rumen ammonia concentrations increased post feeding at 1000 h and reached a peak at 1130 h, with cows fed the 18:82 GS:MS diets having a 31.1 mg/L higher ($P < 0.01$) ammonia concentration compared to when fed the 82:18 GS:MS diets (Figure 5.4). The high NDF content diets increased (+ 20 mM; $P = 0.01$) the rumen acetate concentration in cows compared to when fed the high starch diets (Table 5.8). The concentration of propionate was 9 mM higher ($P <$

0.01) in cows when fed the 18:82 GS:MS diets compared to when fed the 82:18 GS:MS diets. Similarly, the acetate to propionate ratio (A:P) was higher in cows when fed either the 82:18 GS:MS diets (+ 0.79) or high NDF diets (+ 0.24) compared to when fed the 18:82 GS:MS diets or high starch diets, respectively. The concentration of HP was 5.3 ng/ml higher in cows when fed the high starch diets compared to when they received the high NDF diets (Figure 5.5). There was no effect of time, F or their interaction on HP concentration.

Table 5.7. Rumen pH of cows fed diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (MSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS).

Parameter	Treatments					P value		
	GSF	GSS	MSF	MSS	SED	C	F	C × F
Mean pH	6.19	6.20	6.08	6.11	0.055	0.607	0.087	0.796
Min pH	5.72	5.84	5.71	5.69	0.112	0.552	0.380	0.461
Max pH	6.47	6.58	6.59	6.61	0.151	0.574	0.561	0.692
T <5.5 pH[1]	20	71	35	16	43.6	0.337	0.560	0.642
T <5.8 pH	60	103	262	275	37.8	0.373	0.006	0.603
T 5.8-6.0 pH	134	193	283	285	52.9	0.478	0.049	0.497
T 6.0-6.2 pH	486	278	420	224	53.0	0.013	0.208	0.877
T 6.2-6.5 pH	661	541	345	404	55.9	0.493	0.010	0.110
T 6.5-6.8 pH	69	227	79	179	53.0	0.071	0.712	0.585
T >6.8 pH	4	20	27	33	14.7	0.370	0.185	0.670

F = grass to maize silage ratio, C = NDF to starch ratio, F × C = interaction between F and C
[1]Time (min/d) spent under different pH levels during a day.

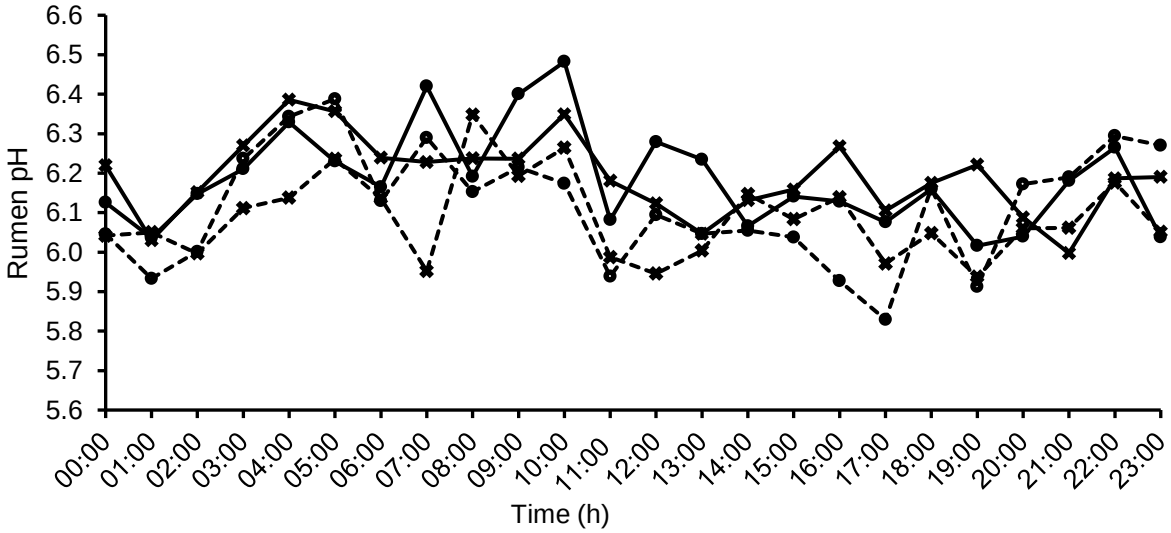

Figure 5.2. Hourly rumen pH of cows fed diets containing high grass:maize silage ratio (82:18) with high NDF content (GSF; --✖--), high grass:maize silage ratio (82:18) with high starch content (GSS; --●--), low grass:maize silage ratio (18:82) with high NDF content (MSF; --✖--) and low grass:maize silage ratio (18:82) with high starch content (MSS; --●--). (SED= 0.137, Time; $P < 0.001$, F; $P < 0.001$, C; $P < 0.040$).

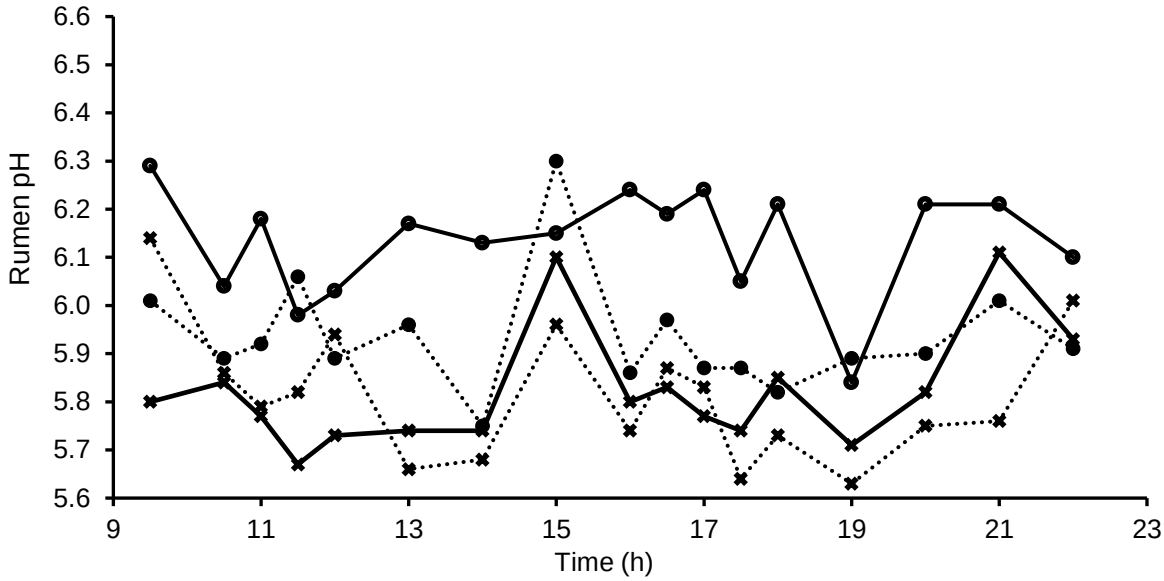

Figure 5.3. Rumen fluid pH for manual samples in cows fed diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF; –✖–), high grass:maize silage ratio (82:18) with a high starch content (GSS; –●–), low grass:maize silage ratio (18:82) with a high NDF content (MSF; --✖--) or a low grass:maize silage ratio (18:82) with a high starch content (MSS; --●--). (SED = 0.175, F; *P* = 0.028, C; *P* = 0.001, F × C; *P* = 0.034).

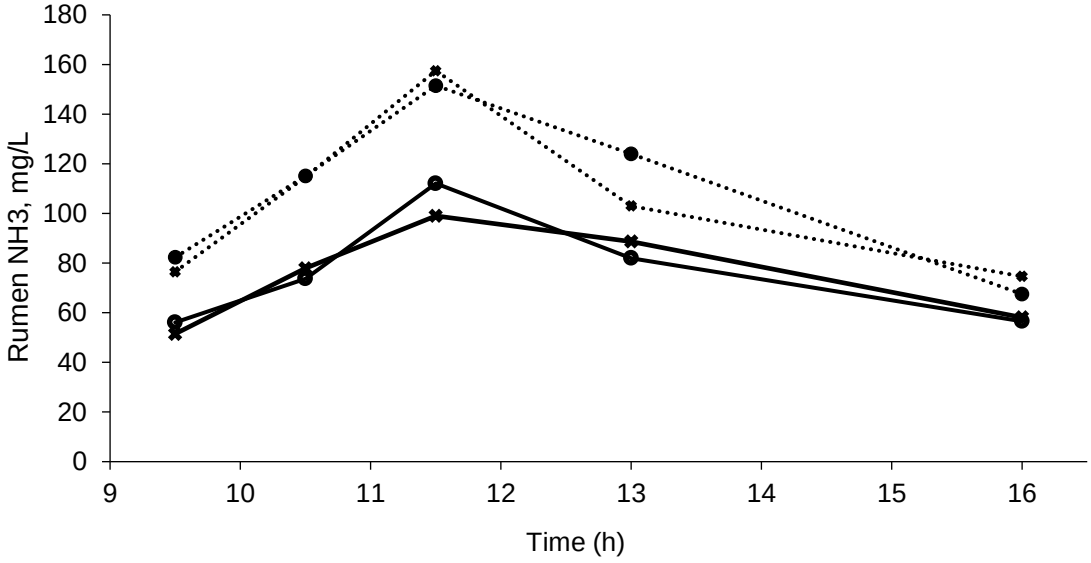

Figure 5.4. Rumen ammonia concentrations in cows when fed diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF; –✖–), high grass:maize silage ratio (82:18) with a high starch content (GSS; –●–), low grass:maize silage ratio (18:82) with a high NDF content (MSF; --✖--) or a low grass:maize silage ratio (18:82) with a high starch content (MSS; --●--) (SED = 19.30, Time; *P* <0.001, F; *P* = 0.003).

Table 5.8. Rumen volatile fatty acid content (mM) in cows when fed diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (MSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS).

VFA	Treatments					P value			
	GSF	GSS	MSF	MSS	SED	Time	C	F	C × F
Acetate									
0930 h	120.9	92.4	88.6	110.1	22.31	0.056	0.012	0.110	0.130
1030 h	131.2	117.8	107.1	104.8					
1130 h	178.8	138.7	112.2	108.9					
1300 h	143.5	105.2	148.7	105.1					
1600 h	122.5	88.1	122.7	109.9					
Propionate									
0930 h	34.6	28.5	33.9	51.7	6.88	0.013	0.677	<0.001	0.104
1030 h	35.1	37.2	39.0	40.6					
1130 h	52.1	46.8	45.8	47.7					
1300 h	40.4	33.0	57.0	48.5					
1600 h	35.8	28.7	48.1	49.5					
A:P ratio									
0930 h	3.41	3.41	2.83	2.23	0.173	0.033	<0.001	<0.001	0.432
1030 h	3.62	3.28	2.84	2.80					
1130 h	3.37	3.05	2.60	2.37					
1300 h	3.50	3.30	2.67	2.35					
1600 h	3.40	3.27	2.65	2.39					
Butyrate									
0930 h	25.9	21.3	20.2	26.2	4.40	0.013	0.079	0.304	0.307
1030 h	26.6	26.8	23.0	23.0					
1130 h	36.2	31.3	27.9	27.3					
1300 h	29.8	24.0	32.1	24.5					
1600 h	26.5	20.9	26.7	23.3					
Iso-Butyrate									
0930 h	1.05	0.97	0.85	1.17	0.185	0.013	0.770	0.898	0.014
1030 h	1.17	1.11	0.91	1.27					
1130 h	1.52	1.36	1.03	1.29					
1300 h	1.27	1.05	1.34	1.24					
1600 h	1.05	0.86	1.08	1.13					
Valerate									
0930 h	2.85	2.38	2.53	3.53	0.536	0.007	0.113	0.142	0.179
1030 h	3.07	2.90	2.68	2.92					
1130 h	3.89	3.66	3.44	3.43					
1300 h	3.63	2.78	4.50	3.47					
1600 h	3.16	2.23	3.56	3.14					
Iso-valerate									
0930 h	2.45	1.91	1.76	2.23	0.423	0.001	0.028	0.516	0.038
1030 h	2.46	2.19	1.98	2.36					
1130 h	3.51	2.90	2.50	2.62					
1300 h	3.05	2.07	3.14	2.43					
1600 h	2.35	1.62	2.46	2.11					
Caproate									
0930 h	2.22	1.41	1.08	1.32	0.362	0.011	<0.001	<0.001	0.032
1030 h	2.21	1.82	1.22	1.23					
1130 h	2.62	2.24	1.63	1.60					
1300 h	2.68	1.62	2.20	1.58					
1600 h	2.18	1.31	1.68	1.27					

F = grass to maize silage ratio, C = NDF to starch ratio, F × C = interaction between F and C

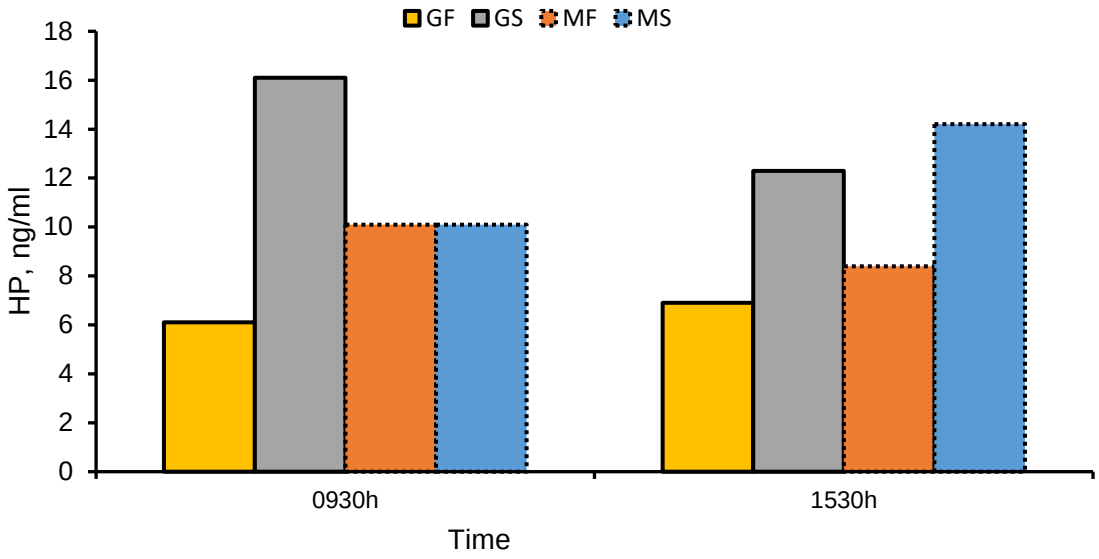

Figure 5.5. Concentration of serum haptoglobin (HP) in cows when fed diets containing high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (MSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS) (SED= 4.04, C; *P* = 0.023).

5.3.6. Dietary behaviour and sorting activity

There was no difference in eating time expressed as total (min/d), min/kg DMI, min/kg NDFI, and min/% peNDF between the dietary treatments (Table 5.9). Total rumination time tended (*P* < 0.06) be higher in cows when fed the high NDF diets compared to when fed the high starch diets. Cows receiving the 82:18 GS:MS diets had a 2.2 min/kg DMI longer (*P* < 0.02) rumination time compared to when they received the 18:82 GS:MS diets. When rumination time was calculated per kg NDF intake or per % peNDF basis, cows when fed the high starch diets had a longer rumination time compared to fed the high NDF diets. There was no difference between treatments in sorting activity of the different dietary fractions, with only an interaction being observed for the > 33 mm fraction (Table 5.10), but the DM proportion of this fraction in the 18:82 GS:MS diets was small (Table 5.2).

Table 5.9. Eating behaviour in cows when fed diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (MSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS).

Parameter	Treatments				SED	P value		
	GSF	GSS	MSF	MSS		C	F	C × F
Eating								
min/d	313	294	285	253	40.0	0.419	0.285	0.821
min/kg DMI	13.4	12.6	11.7	10.5	1.66	0.423	0.175	0.863
min/kg NDFI	33.8	41.8	34.1	39.0	4.57	0.115	0.713	0.663
min/kg fNDFI	55.2	55.9	61.2	52.7	6.51	0.438	0.767	0.361
min/% peNDF$_{>4}$	12.5	16.3	14.1	16.9	1.75	0.057	0.422	0.680
min/% peNDF$_{>8}$	16.2	19.7	19.2	21.3	2.15	0.136	0.204	0.660
Ruminating								
min/d	561	515	522	500	18.6	0.060	0.108	0.395
min/kg DMI	24.1	22.2	21.5	20.7	0.75	0.061	0.019	0.329
min/kg NDFI	60.4	75.3	61.3	77.3	3.97	0.005	0.623	0.858
min/kg fNDFI	97.8	96.0	113	105	5.19	0.228	0.023	0.422
min/% peNDF$_{>4}$	22.4	29.5	25.4	33.4	2.10	0.007	0.079	0.772
min/% peNDF$_{>8}$	29.1	35.5	34.8	42.1	2.84	0.027	0.038	0.835

F = grass to maize silage ratio, C = NDF to starch ratio, F × C = interaction between F and C

Table 5.10. Diet sorting[1] (%) in cows when fed diets containing a high grass:maize silage ratio (82:18) with a high NDF content (GSF), high grass:maize silage ratio (82:18) with a high starch content (GSS), low grass:maize silage ratio (18:82) with a high NDF content (MSF) or a low grass:maize silage ratio (18:82) with a high starch content (MSS).

Fraction	Treatments				SED	P value		
	GSF	GSS	MSF	MSS		C	F	C × F
>33 mm	98.3	103.7	103.1	99.5	1.67	0.516	0.816	0.018
19-33 mm	98.1	96.2	100.3	102.8	3.49	0.908	0.152	0.428
8-19 mm	99.6	100.0	100.6	98.3	0.77	0.155	0.561	0.074
4-8 mm	98.4	95.8	97.7	96.0	2.86	0.346	0.909	0.832
<4 mm	101.8	100.6	100.3	101.4	0.82	0.909	0.582	0.119

F = grass to maize silage ratio, C = NDF to starch ratio, F × C = interaction between F and C
[1]A sorting value of < 100% indicated selective refusals, > 100% preferential consumption, and = 100% was no sorting.

5.4. Discussion

5.4.1. Forages and diet composition

The current study is part of a larger project where the particle size and peNDF of forages and diets fed on the UK dairy herds were characterised (Chapter 3 and 4). The particle size of the grass silage used in the current study was within the shortest 2% of the mean values fed on UK dairy herds reported in Chapter 3. However, the particle size of the MS used in the current study was similar to the mean value fed on UK dairy herds

(Chapter 3). The high NDF diets were supplemented with soyhulls to increase the NDF content, however the forage NDF content remained the same for both the 82:18 GS:MS and 18:82 GS:MS diets. Diets containing 82:18 GS:MS had a higher proportion of particles retained on the larger pore size screens of the PSPS resulting in a longer X_m. The high starch content diet had a lower $peNDF_{>4}$ content compared to the high NDF diet or 82:18 GS:MS diet.

5.4.2. Production performance

The DMI was higher for cows when fed the 18:82 GS:MS diets compared to the 82:18 GS:MS diets, a finding in agreement with Hart et al. (2015) and Chapter 4a where DMI was increased when a proportion of the GS in the diet was replaced by MS. This may also partly be due to the shorter X_m of the 18:82 GS:MS diets compared to the 82:18 GS:MS diets that likely reduced rumen fill, potentially limiting DMI (Zebeli et al., 2012a; Nasrollahi et al., 2015). The higher DMI in cows when fed the 18:82 GS:MS diets resulted in a higher milk yield compared to the 82:18 GS:MS diets. Feeding dairy cows with diets containing a high fibre content is usually associated with a higher milk fat content (Mertens, 1997). However, milk composition is less responsive to dietary particle size in early to mid-lactation cows because of negative energy balance and mobilisation of body fat reserves resulting in an increase in milk fat content (Zebeli et al., 2006a). Contrary to previous findings, in the current study, feeding cows with a high dietary starch content increased milk fat concentration compared to when cows were fed the high NDF diets. The reason for this high milk fat concentration is unclear, but may have been due to the higher rumination time/$\%peNDF_{4or8}$ in cows when fed the high starch concentrate diets, resulting in a higher rumen pH, as well as the higher rumen degradation rate of soyhulls (Ipharraguerre and Clark, 2003). Additionally, feeding excessive dietary peNDF (> 14-18%) has not been reported to increase milk fat content (Zebeli et al., 2012a).

5.4.3. Digestibility, nitrogen excretion, rumen degradation and passage kinetics

The digestibility of DM and OM were not affected by forage type, however the high starch diets had higher digestibility coefficients. The higher starch content may have provided a greater energy supply to the rumen microbes to degrade and digest the diet compared to the high NDF diets as seen by the trend for a higher DM and OM digestibility in cows when fed high starch diets in previous studies (Caton and Dhuyvetter, 1997). The more likely reason for the increase in OM digestibility is that the starch which replaced NDF in the high starch concentrate is more digestible compared to NDF (NRC, 2001). The degradability of NDF was depressed in cows fed the high starch diets, a finding in agreement with Ipharraguerre and Clark (2003) who reported a lower total-tract

NDF digestibility when starch replaced soyhulls in the diet of dairy cows. Replacing a fibrous component of the diet by starch usually reduces the total-tract digestibility of fibre (NDF or ADF) in cows (Putnam and Loosli, 1959; Tyrrell and Moe, 1972; Valadares et al., 2000).

Nitrogen digestibility, milk N output and milk-N % of total N intake was higher in cows when fed the 18:82 GS:MS diets, a finding in agreement with previous findings (O'Mara et al., 1998; Sinclair et al., 2015; Chapter 4a). This was likely due a higher metabolisable energy supply coming from maize silage, a higher starch content and predicted MPE (MP-rumen energy limited) content of the 18:82 GS:MS diets (Table 5.1), alongside the resulting increase in DMI. The values for milk N output and milk-N as a % of total N intake were somewhat higher than reported in previous studies (Powell et al., 2010; Moorby et al., 2016; Nevens et al., 2006; Reynolds et al., 2014), reflecting the higher milk protein yield of cows in the present study. All cows had a positive N balance in the current study, a finding in agreement with Moorby et al. (2009) where cows when fed the grass-based diets had a positive N balance.

A shorter particle size diet resulted in a higher passage rate through the gastrointestinal tract of dairy cows compared to a longer particle size (Tafaj et al., 2001). Rumen passage rate is influenced by various factors including diet composition, amount and source of starch (wheat vs. maize starch) as concentrate, and fibre concentrations (Tafaj et al., 2007). However, in the current study, different concentrate sources did not affect the passage rate of grass-NDF, but the high grass silage diet resulted in a higher rumen-mean retention time (R-MRT) compared to the high maize silage based diets. The high R-MRT could explain the lower DMI in cows fed the 82:18 GS:MS diets due to the negative effect of rumen fill on intake (Zebeli et al., 2007). Previous studies have found no relationship between forage particle size and digesta passage rate through the rumen (Beauchemin and Yang, 2005; Tafaj et al., 2007). This lack of an effect of particle size on passage rate may be due to particle size reduction by chewing and mastication that may potentially increase the rate of finer particles escaping from the rumen (Beauchemin and Yang, 2005).

5.4.4. Rumen pH, volatile fatty acids, ammonia and serum haptoglobin

Rumen pH primarily depends on dietary composition, forage source, amount of concentrates, fermentability of concentrates and amount of fibre in the diet, but can be influenced by other factors such as rate of VFA absorption across the rumen epithelium (Nasrollahi et al., 2016; Zebeli et al., 2012a). On a low forage diet (<50 F:C), rumen pH has been shown to decrease with decreasing mean particle size, but there was no effect

when the forage proportion was high (Nasrollahi et al., 2016). To avoid SARA, Zebeli et al. (2012) suggested a high F:C ratio (56:44 DM basis) in the diet, but in the current study forages were fed at 50% (DM basis) and with a high starch concentrate diet (MS diet) that was formulated to induce SARA. Tafaj et al. (2007) reported a strong positive association (R^2 = 0.41) between NDF content and rumen pH, but in the current study feeding a high starch diet increased rumen pH by 0.2 pH units compared to the high fibre diets. This may be explained by the use of maize meal as a starch source that is more resistant to rumen degradation compared to wheat-based starch (Moharrery et al., 2014). Subacute ruminal acidosis has been defined as cows spending 5-6 h/d (300-360 min/d) under rumen pH level of 5.8 (Zebeli et al., 2008). In the current study, no cow experienced SARA according to this criteria, however, when cows were fed the 18:82 GS:MS diets they spent an average of 269 min/d under pH 5.8 compared to when fed the 82:18 GS:MS diets where they spent 82 min/d, irrespective of concentrate composition. Feeding a high starch diet (320 g/kg DM) to dairy cows decreased the acetate concentration and increased the propionate concentration in the rumen compared to when fed a low starch diet (Oba and Allen, 2003), a finding in agreement with the current findings. The higher acetate to propionate ratio (A:P) in the current study was also in agreement with Beckman and Weiss (2005), where a high NDF:Starch diet (1.27) increased A:P by 0.33 in the rumen compared to a low NDF:Starch (0.74) diet. The higher ammonia concentration in cows fed the 18:82 GS:MS diets was likely due to a higher inclusion of soybean meal, rapeseed meal and lack of sopralin in the diet compared to the 82:18 GS:MS diets. The serum concentration of HP in the current study was higher in cows when fed the high starch diets compared to when they received the high NDF diets, a finding in agreement with Khafipour et al. (2009) where cows fed a high grain based diets had increased serum HP concentration (+475.6 µg/ml) compared to those fed a high NDF diet with a low starch concentration. However, the serum HP concentration was lower in the current study compared to previous studies, which may be due to the higher starch concentration of the diet fed to induce SARA in the previous study by Khafipour et al. (2009).

5.4.5. Dietary behaviour and sorting activity

The lack of an effect of GS:MS ratio and NDF:starch ratio on eating time in the current study could be due to the comparatively low X_m (< 8 mm) and $peNDF_{>8}$ content (< 20%) of the diets fed. Feeding a longer dietary particle size diet generally results in an increase in eating and rumination time in dairy cows (Beauchemin and Yang, 2005; Tafaj et al., 2007). For example, increasing forage X_m in the diet from 6.7 to 10 mm resulted in an increase in eating time (+19 min/d) and ruminating time (+ 28 min/d)

(Nasrollahi et al., 2016). The GSF diet had the highest NDF content at 399 g/kg DM, but 38% of the NDF content was contributed by soyhulls that are a highly degradable source of fibre in the rumen and may not be as effective as forage NDF in promoting rumination (Ipharraguerre and Clark, 2003). Inclusion of a high starch content in the current study increased the rumination time when expressed per kg NDFI or per %peNDF compared to the high NDF diets, which may have resulted in a higher rumen pH (Figure 5.2 and 5.3). Sorting activity is often associated with an excessive consumption of starch rich concentrates in the diet and a lower fibre intake, which can decrease rumen pH and induce SARA (Leonardi and Armentano, 2003). Since the mean particle size of the diets in the present study was short compared to the average particle size (14 mm) of dairy rations in the UK as reported previously in Chapter 3, there was little effect of diet sorting across all diets. Additionally, diets of individual cows were mixed separately and a homogeneous mixing procedure was adopted during the current study that may have helped in reducing sorting (Heinrichs et al., 1999) and may not reflect the feeding conditions in commercial practice.

5.5. Conclusions

A short chop length grass silage when fed at a low GS:MS ratio increased intake, milk yield, rumen passage rate, nitrogen digestibility and milk nitrogen use efficiency, and rumen ammonia content, but decreased milk fat content, fibre digestibility, rumen pH, acetate to propionate ratio and rumination time in dairy cows compared to when fed at a high GS:MS ratio. Feeding dairy cows with a high starch content diet increased milk fat content, organic matter digestibility, rumination time (min/peNDF), rumen pH and haptoglobin concentration, but decreased fibre digestibility, and acetate to propionate ratio compared to high fibre diets. Concentrate composition had no effect on grass silage degradability or rumen passage rate. Feeding dietary starch levels well in excess of that currently undertaken in the UK and in diets based on a short particle length grass silage when fed at a low or high maize inclusion rate can be achieved if starch source, ration composition, effective mixing and high feeding frequency are undertaken.

CHAPTER 6: General discussion and conclusions

6.1. General discussion

This thesis has described the particle size distribution and peNDF content in UK forages (grass and maize silage) and partial and total mixed rations, and their effects on rumen function, performance and health of dairy cows fed concentrates with a range of carbohydrate composition. In order to characterize the particle size distribution of forages and rations in the UK, fifty commercial dairy herds feeding a range of grass silage and maize silage based rations were sampled during the winter of 2015/2016 (Chapter 3). Overall, the particle size distribution of maize silage in the UK was similar to the current guidelines for maize silage based on North America rations, and consequently, there is little requirement for separate recommendations. Out of the 50 herds used in this study, the minimum % DM of grass silage retained on the >19 mm sieve was 49%, considerably higher than the 10-20% guidelines for lucerne haylage in the USA (Heinrichs, 2013). The mean particle size distribution of the mixed rations surveyed in Chapter 3 differed from the guidelines based on North American rations (Heinrichs, 2013), with the long (>19 mm) particle size fraction being 38%, approximately 50% higher than that reported by Sova et al. (2013), and approximately 4 times higher than that reported by Heinrichs (2013). The difference in particle size distribution of mixed rations in the current study reflected the high inclusion of grass silage that contained a very long particle size (>19 mm = 80% DM, X_m = 42.6 mm). It was also determined in Chapter 3 that there were 42% of herds that had a well-mixed ration, 26% had a moderately mixed and 32% had a poorly mixed ration. Similarly, out of the 50 herds, 82% had either selective refusal or did not show preferential consumption for the >19 mm fraction of mixed rations which may be associated with the inclusion of long particles of grass silage. There was no sorting activity observed for the <8 mm fraction in 46% of the herds. There was a positive relationship (R^2 = 0.33) between X_m and mean milk fat concentration (g/kg) across all herds, illustrating the importance of this measurement to practical dairy cow nutrition. In order to more accurately describe forage particle size and functional fibre under UK conditions, Penn State particle separator was modified with the additional 26.9, 44 and 60 mm pore size sieves. However, as a very small proportion of particles was retained on the 19-26.9 mm screen, therefore a larger screen of 33 mm was adopted for Chapter 5.

After characterising the particle size distribution of forages and rations, a controlled study (Chapter 4 ab) was conducted to determine the effect of chop length of grass silage when fed at different ratios of GS:MS on the intake, performance, reticular pH, diet digestibility,

metabolism and eating behaviour in Holstein-Friesian dairy cows. The particle size of the long chop length grass silage and maize silage used in Chapter 4ab were similar to the mean values fed on UK dairy farms reported in Chapter 3 (43 and 11 mm, respectively), whereas the short chop length GS was within the shortest 5% of the grass silage surveyed at 31 mm. The increase in DM intake when cows were fed the 40:60 GS:MS compared to the 100:0 GS:MS based diets is in agreement with previous studies that have investigated the effect of including maize silage (Hart et al., 2015; O'Mara et al., 1998). Feeding cows diets containing a short chop length GS increased DM intake in Chapter 4a, possibly due to less time required for chewing prior to swallowing, a finding in accordance with previous studies that have investigated the effect of chop length (maize silage or lucerne) on DM intake in dairy cows (Nasrollahi et al., 2015). There was an interaction between chop length and forage ratio on milk yield in Chapter 4a, with a short chop length grass silage increasing yield in cows when grass silage was the sole forage, but not when grass silage was fed along with maize silage. Milk fat production was not affected by chop length in Chapter 4a, possibly due to a sufficient dietary $peNDF_{>4}$ content of all four diets (minimum of 26%), as it has been suggested that milk fat concentration is only influenced by chop length when dietary peNDF levels are lower than the recommended level of 18-22% DM (Zebeli et al., 2012a). Similar to previous studies (Yang and Beauchemin, 2007), the highest reticular pH was recorded prior to feeding, with a nadir reached at approximately 9 h after fresh feed delivery. Cows when fed the 40:60 GS:MS compared to the 100:0 grass silage based diets had a lower mean and minimum reticular pH, which may be associated with the higher concentration of starch and lower concentration of $peNDF_{>8}$ in the MS diets (130 vs 199 g starch/kg DM and 27.1 vs 19.1% $peNDF_{>8}$, for the grass silage and maize silage based diets respectively). In contrast, chop length had no effect on reticular pH, a finding in agreement with Tafaj et al. (2007). Chop length did however influence eating time in Chapter 4b, with cows spending more time eating the long than the short chop diets, a finding in agreement with Kammes and Allen (2012) who reported a tendency for a longer daily eating time when cows were offered a long versus short chop length orchard grass silage. Lying time in Chapter 4b was similar to the 11.5 h/d reported by Gomez and Cook (2010) for cows housed with mattress bedded cubicles. There was a tendency for an interaction for lying time, where a short CL increased (~1 h/d) the lying time when fed the grass silage only diet but had little effect when fed the 40:60 GS:MS diets. Feeding cows with a short CL GS may therefore improve animal welfare by increasing lying time. When the effective fibre comes from maize silage, cows may have to ruminate longer in order to breakdown the forage into fine particles compared to grass silage as seen in Chapter 4b. Additionally, when cows were fed the grass silage only diets this resulted in

diet sorting compared to when fed the 40:60 GS:MS diets. Alternatively, the grass silage based diets may have resulted in a more suitable rumen microbial environment for the capture of degraded nitrogen, as seen by the higher reticular pH. Overall, the inclusion of maize silage in the diet altered the FA profiles of the milk more than the grass silage chop length, although chops were comparatively small.

The final study (Chapter 5) was undertaken to evaluate the effect of concentrate supplementation (either a high NDF or a high starch content) at two different forage ratios of GS:MS (82:18 or 18:82 DM basis) on production performance, diet digestibility, rumen pH, rumen function and passage kinetics, eating behaviour and systematic inflammatory response in dairy cows. Cows were fed with either a high 82:18 GS:MS diet or a low 18:82 GS:MS forage ratio that were supplemented with either a high NDF or high starch content concentrate. The diet containing the 18:82 GS:MS with high a starch content concentrate was formulated to challenge rumen pH and potentially result in acidosis. The DM intake was higher for cows when fed the 18:82 GS:MS diets compared to the 82:18 GS:MS diets which may also be due the short mean particle size of the 18:82 GS:MS diets that reduced rumen fill, potentially increasing DM intake (Zebeli et al., 2012; Nasrollahi et al., 2015). The higher DM intake in cows when fed the 18:82 GS:MS diets was associated with a higher quantity of milk production compared to when fed the 82:18 GS:MS diets. Contrary to previous findings, in Chapter 5, feeding cows with a high starch concentrate increased milk fat concentration compared to when fed with the high NDF concentrate. Reasons for this high fat content are unclear, but may have been due to the higher rumination time/%peNDF$_{4or8}$ in cows when fed the high starch concentrate diets as suggested by the higher rumen pH. The high dietary starch concentration increased the digestibility coefficients of DM and OM, most probably due to the higher rumen digestibility of starch. The NDF degradability was depressed when the high starch concentrates were fed, a finding in agreement with Ipharraguerre and Clark (2003) who reported a lower total-tract NDF digestibility when starch replaced soyhulls in the diet of dairy cows. Nitrogen digestibility and milk N output was higher for cows when fed with the 18:82 GS:MS diets, a finding in agreement with previous studies (O'Mara et al., 1998; Sinclair et al., 2015; Chapter 4). This may have been due a higher energy supply from the starch in the maize silage which could have increased microbial protein and metabolisable protein supply (Sinclair et al., 2014). Different concentrate composition had no effect the passage rate of grass-NDF but the 82:18 GS:MS diets resulted in a higher rumen-mean retention time (R-MRT) compared to the 18:82 GS:MS diets. The high R-MRT may explain the lower DM intake on the 82:18 GS:MS diets due to a negative effect on rumen fill (Zebeli et al., 2007). Feeding a high starch concentrate

increased rumen pH by 0.2 pH units compared to the high fibre diets. This may possibly be explained by the use of maize meal as a starch source that is more resistant and slowly degraded in the rumen compared to wheat-based starch (Moharrery et al., 2014). Subacute ruminal acidosis has been defined as cows spending 5-6 h/d (300-360 min/d) under a rumen pH level of 5.8 (Zebeli et al., 2008). In Chapter 5, not a single cow experienced SARA according to this criteria, although cows fed the 18:82 GS:MS diets spent on average 269 min/d under pH 5.8 compared to when fed the 82:18 GS:MS diets (82 min/d) irrespective of concentrate composition. The plasma concentration of HP in the current study was higher for cows fed with the high starch diets compared to those that received the high NDF diets (Khafipour et al., 2009). This was associated with longer period of time under rumen pH 5.8 and may have resulted in inflammation of rumen wall that causes an increase in rumen-endothelial permeability allowing ruminal endotoxins (local inflammatory proteins) to enter into blood circulation and trigger the release of acute phase proteins as an innate immune response of SARA (Ametaj et al., 2010; Plaizier et al., 2012; Metzler-Zebeli et al., 2013). Inclusion of a high starch concentrate in Chapter 5 also increased the rumination time when expressed per kg NDF intake or per %peNDF compared to high NDF diets, which may have resulted in the higher rumen pH. The mean particle size of the diets in the Chapter 5 was short compared to the average particle size (14 mm) of dairy rations in the UK (Chapter 3), and there was no issue of diet sorting across all diets. Diets of individual cows were mixed separately and a homogeneous mixing procedure adopted during the current study that may have helped in reducing the sorting (Heinrichs et al., 1999).

Previous studies have reported a relationship between peNDF and milk performance (Tafaj et al., 2007; Zebeli et al., 2012). Combining the data from the survey (Chapter 3) and the two dairy cow studies (Chapter 4ab and 5) allows this to be investigated using UK based diets. Analysis of this data revealed that there was also a negative relationship between mean particle size or $peNDF_{>4}$ and ECM ($R^2 = 0.21$ and $R^2 = 0.24$; $P = 0.005$, respectively) although these were not as strong as with $peNDF_{>8}$ ($R^2 = 0.47$; $P < 0.001$) (Figures 6.1, 6.2, 6.3). Overall, the shorter particle size diets resulted in a higher milk yield, and dairy cows in the UK should be fed diets with less than 15 mm particle size according to data presented in Figure 6.1. Feeding cows with a longer particle size will reduce DM intake, milk yield and increase diet sorting (Nasrollahi et al., 2016), an agreement with the current study (Figure 6.4). The $peNDF_{>4}$ content of the TMR should be between 15-30% in order to avoid any reduction in DM intake (Figure 6.4) and milk yield without having negative effect on rumen pH and cow health (Figures 6.2, 6.3).

Similarly, the peNDF$_{>8}$ content of the TMR should be between 15-30% in order to avoid any milk production losses in dairy cows (Figure 6.2). This proposed range of peNDF$_{>8}$ content for UK dairy rations is higher compared to the 18-22% range suggested by the Zebeli et al. (2012). The positive relationship between mean particle size and milk fat concentration (Figure 6.5), and the negative relationship with milk yield is in agreement with De Brabander et al. (1999).

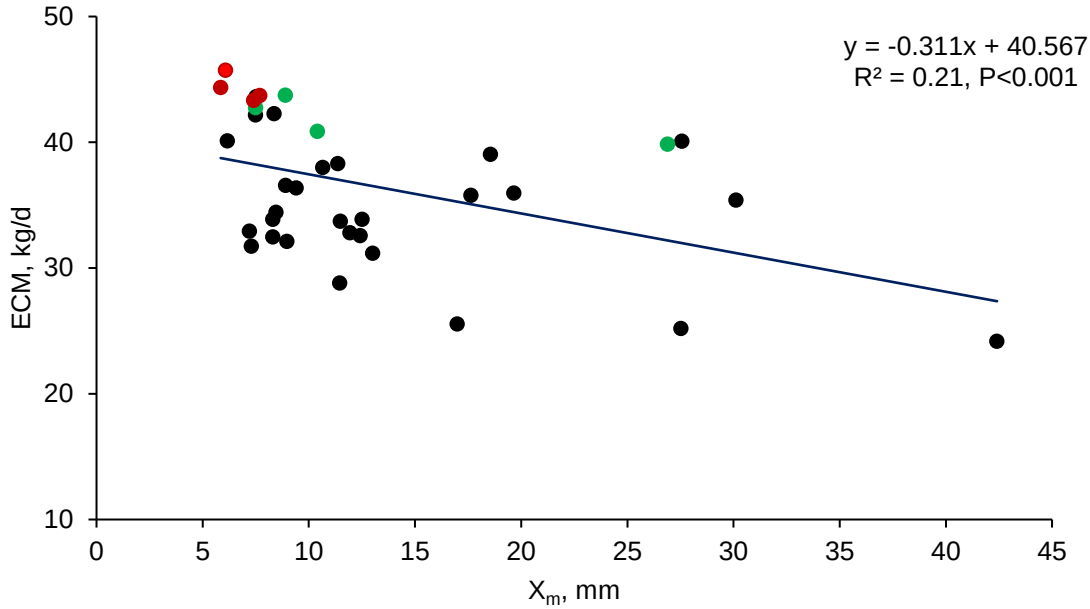

Figure 6.1. Relationship between mean particle size (X_m, mm) of the TMR (●=Chapter 3 [n=28]; ●=Chapter 4 [n=4]; ●=Chapter 5 [n=4]) and energy corrected milk (kg/d).

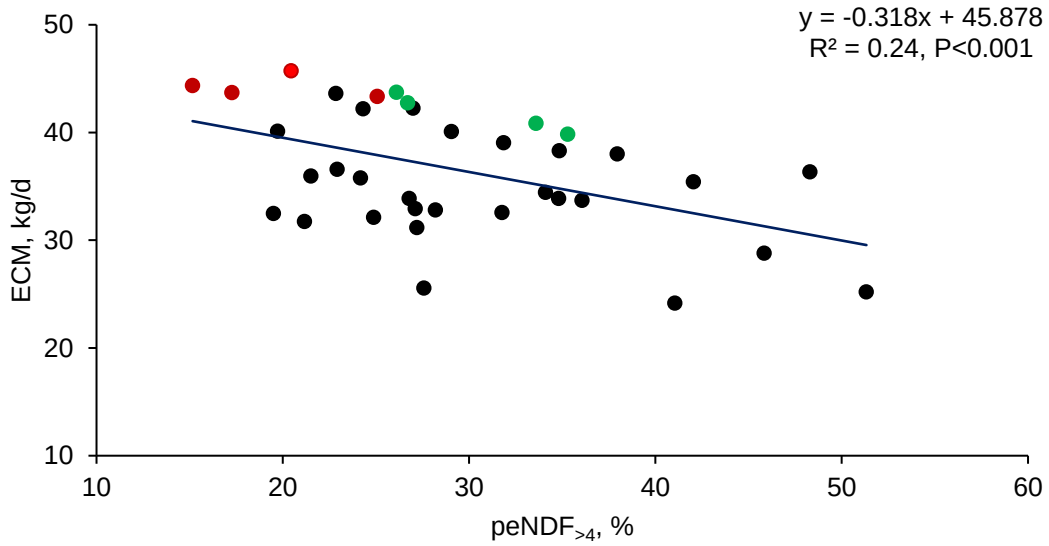

Figure 6.2. Relationship between physically effective fibre (peNDF$_{>4}$, %) of the TMR (●=Chapter 3 [n=28]; ●=Chapter 4 [n=4]; ●=Chapter 5 [n=4]) and energy corrected milk (kg/d).

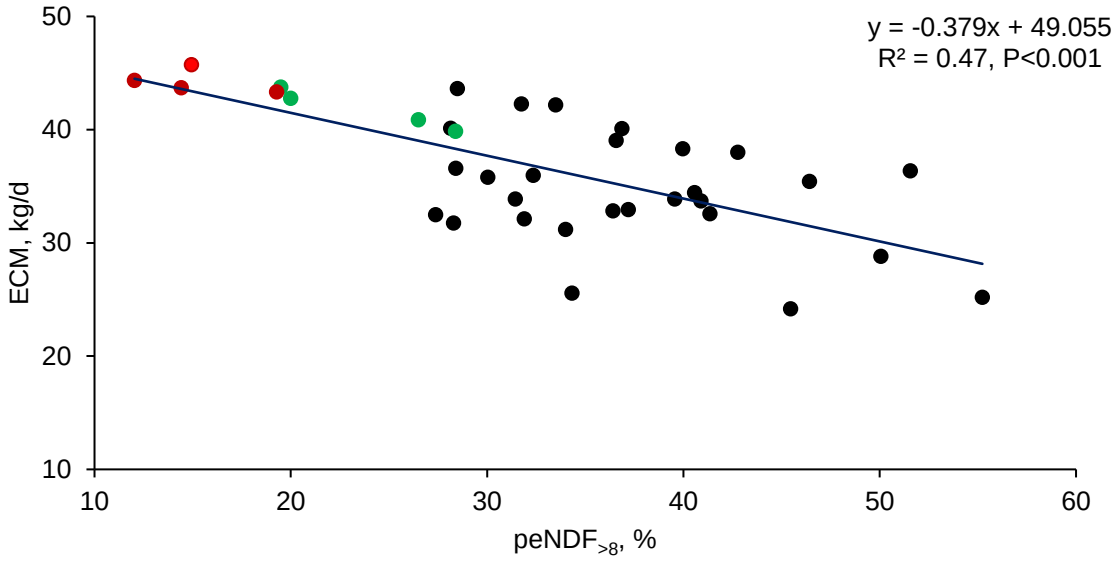

Figure 6.3. Relationship between physically effective fibre (peNDF$_{>8}$, %) of the TMR (●=Chapter 3 [n=28]; ●=Chapter 4 [n=4]; ●=Chapter 5 [n=4]) and energy corrected milk (kg/d).

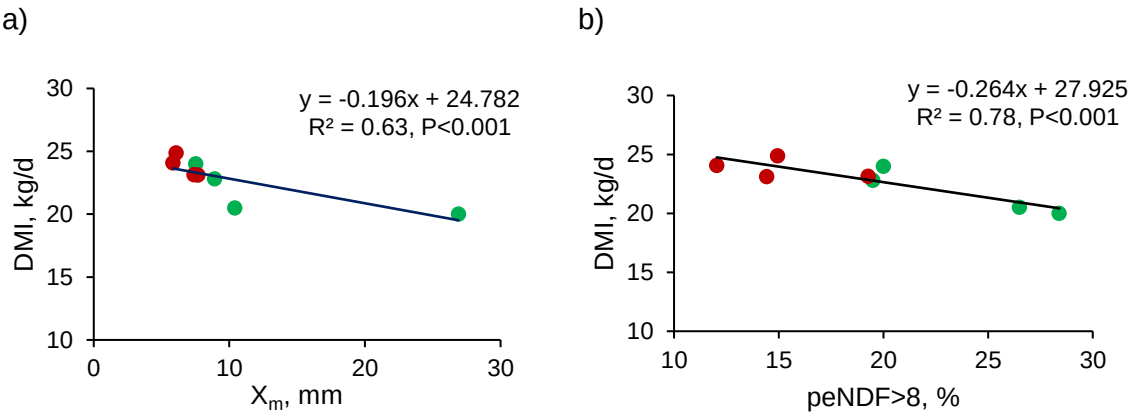

Figure 6.4. Relationship between a) geometric mean particle size (X_m) or b) physically effective fibre (peNDF$_{>8}$) of the TMR (●=Chapter 4 [n=4]; ●=Chapter 5 [n=4]) and dry matter intake (kg/d).

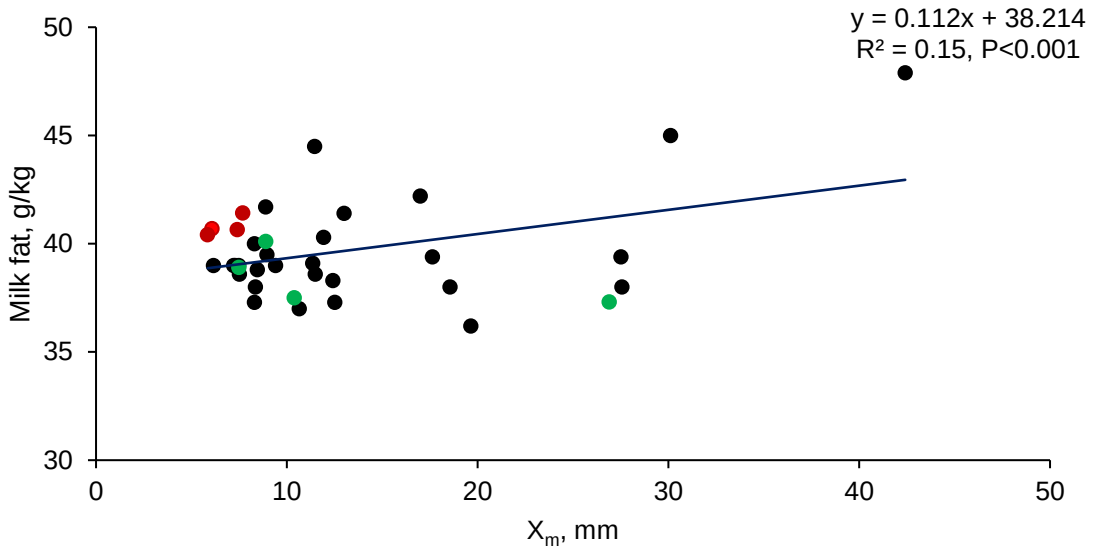

Figure 6.5. Relationship between mean particle size (X_m, mm) of the TMR (●=Chapter 3 [n=28]; ●=Chapter 4 [n=4]; ●=Chapter 5 [n=4]) and milk fat (g/kg).

There was a strong relationship between $peNDF_{>4}$, $peNDF_{>8}$ of the mixed rations and reticulo-rumen pH (R^2 = 0.79 and R^2 = 0.80; P < 0.001, respectively) although this was not strong with X_m (R^2 = 0.41) (Figure 6.6). However, these relationships became weaker when the reticular pH data of Chapter 4 was corrected by -0.2 unit pH to convert it in to rumen pH as suggested by Neubauer et al. (2018). In the current thesis, no cow experienced SARA according to criteria defined by Zebeli et al. (2008; 5-6 h/d under rumen pH level of 5.8), despite feeding the short chop length grass silage or excessive use of starch.

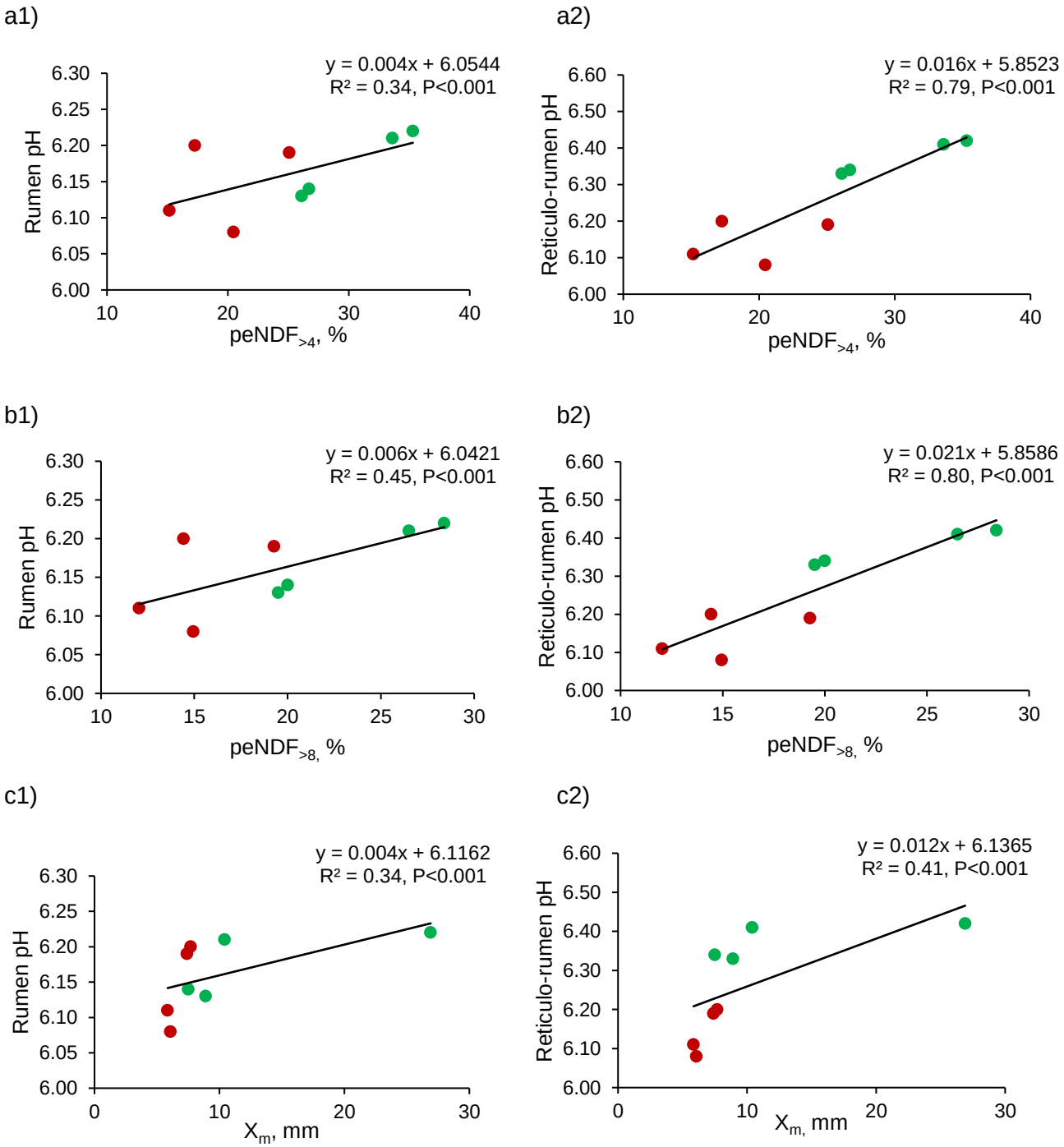

Figure 6.6. Relationship between physically effective fibre (peNDF; peNDF>4 [a] or peNDF>8 [b]), mean particle size (c) of the total mixed rations (●=Chapter 4 [n=4]; ●=Chapter 5 [n=4]) and rumen pH (1) or reticulo-rumen pH (2). For rumen pH data; the reticular pH was adjusted by 0.2 pH according to Neubauer et al. (2018).

6.2. General conclusions

The particle size distribution of grass silage and mixed rations based on grass silage in UK dairy herds was found to be considerably higher than current guidelines that are based on North American forages and rations. This suggests that the particle size of UK dairy rations is either too long, or that new guidelines or methods of particle size evaluation, for grass silage and GS/MS based mixed rations in the UK and Northern Europe are required. The poor consistency of mixing and high degree of selection recorded on the majority of herds is of concern, and the high use of concentrates by 50% of the herds in the current study is a potential risk of sub-acute rumen acidosis.

The short chop length grass silage used in the Chapters 4a and b was within the shortest 5% of that fed in the UK but had no effect on reticular pH compared to an average chop length grass silage, but increased intake and milk performance when fed as the sole forage. Milk performance will therefore benefit from replacing a proportion of grass silage with maize silage in a mixed ration when fed to high producing dairy cows, irrespective of the chop length of the grass silage, but with a reduction in reticulo-rumen pH and fibre digestion.

A longer grass silage particle size increased eating time but decreased ruminating time when fed with the 40:60 GS:MS diets and also resulted in more diet sorting. Cows fed grass silage spend less time ruminating than when they are fed maize silage. Cows also tend to spend more time lying down (due to less eating time) when fed diets containing both grass silage and maize silage, or when fed a short chop length, which may enhance their welfare by improving hoof health and locomotion.

A shorter chop length grass silage when fed at a low GS:MS ratio can increase intake, milk, rumen passage rate, nitrogen digestibility and nitrogen efficiency, rumen ammonia content but decrease milk fat, fibre digestibility, rumen pH, acetate to propionate ratio and rumination time in dairy cows. Feeding dairy cows with a high starch content diet can increase organic matter digestibility, rumination time (min/peNDF), rumen pH and haptoglobin concentration but decrease fibre digestibility, and acetate to propionate ratio compared to high fibre diets. However, concentrate composition may have little effect on *in situ* grass silage degradability and rumen passage rate.

6.3. Future prospects

Further studies of grass silage chop length (shorter than 23.6 mm particle size) at different DM content and concentrate levels and compositions fed in different feeding

system (total mixed rations vs partial mixed rations) are required. It is also pertinent to determine how short a grass silage chop length can be fed to dairy cows without negative effects on production and rumen health. Additionally, studies are required to investigate the effect of mixer wagon protocols and their influence on particle size of grass silage based diets. Further studies are also required to establish the difference in rumination activity in cows fed grass silage vs maize silage based diets. Cows could be fed with similar amounts of NDF coming from different forages to characterise their physical effectiveness factor in order to further improve the peNDF concept.

6.4. Proposed particle size distribution guidelines

Without further studies to determine the effect of particle size distribution on rumen health and cow performance, it is not possible to provide definite guidelines. It is clear from the current series of studies that a considerably shorter grass silage than the current mean from the UK can be fed with little effect on rumen pH and an improvement in performance, even at higher dietary starch levels. Care should be exercised however if wet, acidic grass silage is fed as this may confound the effect of starch level, form and forage particle length. Despite these reservations, tentative recommendations on the particle size distribution for TMR/PMR can be made. Based on the findings of this thesis, the suggested guidelines for particle size distribution and mean particle size of UK dairy rations (both total and partial mixed rations) are presented in Table 6.1. Compared to the particle size recommendations for North American dairy rations (Table 3.9), the > 19 mm fraction of the UK rations are approximately 50% more, and the fine fraction (< 8 mm) is ~20% less.

Table 6.1. Tentative proposed guidelines for particle size distribution, mean particle size and physically effective fibre of TMR or PMR.

	Particle size distribution (% DM basis)	
	TMR	PMR
Fractions		
> 33 mm	5.0-10.0	10.0-20.0
19 - 33 mm	15.0-25.0	25.0-40.0
8 - 19 mm	30.5-45.0	20.0-37.0
4 - 8 mm	10.0-18.0	7.0-11.0
< 4 mm	15.0-25.0	5.0-15.0
Mean particle size, mm	6.0-15.0	15.0-38.5
$peNDF_{>4}$, %	15-30	15-30
$peNDF_{>8}$, %	15-35	15-35

CHAPTER 7: General references

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